Tuesday, August 2, 2016

Poster Session: 9:30 AM - 5:00 PM

Clinical Studies/Outcomes

A-095

Vitamin D status of women with risk factors for gestational diabetes mellitus in Abuja, Nigeria

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Objective: Gestational diabetes mellitus is associated with maternal and perinatal morbidity and mortality. Studies have suggested that vitamin D plays a role in pancreatic beta-cell function and insulin sensitivity and has been associated with type 2 DM. Despite conflicting reports on the association between GDM and vitamin D insufficiency, vitamin D supplementation in pregnancy is practised in some developed countries. The study was aimed at determining the relationship between vitamin D status and GDM in this environment. **Methodology:** This was a case-control study involving pregnant women at 24 - 28 weeks gestation referred for oral glucose tolerance testing at the Department of Chemical Pathology of a tertiary healthcare facility over a period of 10 months (February - November, 2014). A standard 75 g OGTT was administered on subjects after 8 - 10 hours of previous overnight fast. Cases were defined based on the current WHO (2013) criteria for the diagnosis of GDM requiring a fasting-, 1 hour- or 2 hours-plasma glucose value ≥ 5.1 mmol/L, ≥ 10.0 mmol/L or ≥ 8.5 mmol/L respectively, while controls were subjects whose corresponding plasma glucose levels fell below the above stated values. One hundred cases and a hundred controls that met the eligibility criteria were recruited into the study. Socio-demographic and clinical characteristics were obtained via semi-structured interviewer-administered questionnaire/data collection form. Plasma glucose, calcium, phosphate, albumin were measured with Cobas c311 (Roche Diagnostics, GmbH) analyzer. Plasma parathyroid hormone (PTH) and 25-hydroxycholecalciferol were assayed on the Cobas e411 (Roche Diagnostics, GmbH) analyzer. Univariate, bivariate and multivariate analyses were performed using Statistical Package for Social Sciences version 20 (SPSS, Chicago, USA). **Results:** The overall mean age was 31.73 ± 4.32 years and pre-pregnancy BMI was 28.02 ± 5.12 kg/m^2. The mean age of cases was higher than that of controls with a difference of 1.95 years (95% CI: 0.227 - 3.627 years; *p*-value = 0.023). Mean pre-pregnancy BMI was higher in cases with a difference of 3.79kg/m^2 (95% CI: 1.87 - 5.70; *P*-value < 0.001). The overall mean values of plasma 25-hydroxycholecalciferol, PTH, corrected total calcium and phosphate were 28.77 ± 12.42 ng/mL, 7.69 ± 8.36 pg/mL, 2.12 ± 0.15 mmol/L, and 1.00 ± 0.25 mmol/L respectively. Overall, 58% of the subjects had plasma 25-hydroxycholecalciferol levels below 30 ng/mL (defined as vitamin D insufficiency). The proportion of cases with vitamin D insufficiency was 62%, while the proportion of controls with vitamin D insufficiency was 54%. Odds ratio for GDM was 1.39 (95% CI: 0.79 - 2.44) and *p*-value = 0.3159. Adjusted odds ratio for GDM, after logistic regression using age, pre-pregnancy BMI, history of DM in first-degree relative, average time spent outdoors daily, skin exposure, use of vitamin supplements, and fish diet as possible confounding variables was 0.984 (95% CI: 0.944 - 1.025) and *p*-value = 0.438. **Conclusion:** The results indicated that was no association between vitamin D insufficiency and gestational diabetes mellitus. However, the high proportion of vitamin D insufficiency among the study population requires appropriate attention and possible intervention.

A-096

Method Comparison Improvements: A Case Study Using Serum Free Light Chain

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BACKGROUND: Assay validation is a required process before any new test is approved for clinical testing. Patient sample comparisons between an existing and a new assay are used to determine method accuracy. Kappa and lambda free light chains (FLC) are important for diagnosis and prognosis of malignant plasma cell proliferation disorders. Historically, FLC were performed at our institution on an IMMAGE 800 analyzer (Beckman/Coulter) which was replaced by a SPAPLUS instrument (The Binding Site Company). The original method comparison indicated a high bias for kappa FLC which was deemed to be clinically significant. Due to this fact, it was decided to test all patients using both methods for a period of time to determine if a new baseline was required for our patient population. **METHODS:** Two months of serum kappa and lambda FLC samples ordered in our laboratory were assayed prospectively using IMMAGE 800 and SPAPLUS instruments**.** Both methods employ the same test principle, which depends on antigen-antibody interactions that form insoluble immune complexes. A beam of light passes through the sample and light scattering is monitored. Data analysis was performed using Excel (Microsoft) and/or EP Evaluator Release 10 (Data Innovations). EP Evaluator uses a complex iterative algorithm to identify outliers and defines outliers as points whose distance from the regression line exceeds 10 times the standard error of estimate (SEE). The SEE is computed from the data set with the outliers removed. **RESULTS:** Primary analysis of the original kappa FLC validation (N=95) using Excel revealed a high bias ~56%. A secondary analysis of the original validation data using EP evaluator including outliers yielded a slope of 0.778, an intercept of 22.4 mg/L with a bias of 9.3% for kappa FLC while excluding outliers (N=84) the slope was 2.333, the intercept was -13.0 mg/L and the bias was 57.0%. Analysis of post-validation kappa FLC samples including outliers (N=1560) determined a slope (0.842), intercept (18 mg/L) and bias (-7%) excluding outliers (N=1517) the slope (1.055),

intercept (10.5 mg/L), and bias (14.7%). Similar results were seen for lambda FLC. **DISCUSSION:** One of the first things noticed was when outlier accounted for ~12% of the original data set. All the samples that were removed were above 100 mg/L and yielded a similar bias as the Excel data. The marked difference between the original and post-validation data sets would suggest a flaw in the collection parameters for the original sample set. Although the original specimen number (N=95) exceeded our requirements for comparison (N=40), the sample distribution was significantly skewed towards the lower end of the reference interval. The subsequent data set with the increased number of patient specimens that covered the whole reference interval produced a much reduced bias. Indicating a proper comparison required not only an adequate number of samples but also an appropriate distribution of samples due to statistical sampling errors. Erroneous or incomplete validation processes have both monetary and clinical consequences that need to be critically evaluated. A consistent statistical method should be used and any deviation must have prior approval from the technical director.

A-097

MicroRNA-218 is a prognostic indicator in colorectal cancer and enhances 5-fluorouracil-induced apoptosis by targeting BIRC5

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Background: One major reason for the failure of advanced colorectal cancer (CRC) treatment is the resistance to fluoropyrimidine(FU)-based chemotherapy. The enhanced ability of tumor cells to undergo anti-apoptosis process is the main contributor to drug resistance. Previous studies have found that miR-218 was significantly down-regulated in cancer patients and had a role in cancer progression. However, the functional significance of miR-218 in CRC chemoresistance remains unknown. To further explore the possible mechanisms and promote chemosensitivity of CRC treatment, we evaluated the prognostic effect of miR-218 in patients received 5-FU-based treatment and investigated the pro-apoptotic role of miR-218 *in vitro*.

Methods: Paired resected surgical specimens from primary tumor and adjacent non-tumor sites were selected and utilized from 63 CRC patients received 5-FU-based treatment with histological diagnosis. All patients received standard follow-up with computed tomography of abdomen after operation. Reverse transcription quantitative PCR (RT-qPCR) was performed to quantify miRNAs and mRNAs expression. The apoptosis assay was performed by using the flow cytometry after staining with annexin V FITC and propidium iodide (PI). Besides, apoptotic cells were also analyzed using the One Step TUNEL Apoptosis Assay Kit to get a more visualized image. Dual-Luciferase reporter assay was carried out to find the target genes. Western blot analysis was used to detect the thymidylate synthasev (TS) and BIRC 5 protein expression.

Results: The expression of miR-218 was downregulated in HT29 and HCT116 cell lines and significantly decreased in tumor tissues compared with paired normal tissues (*P*<0.001). High miR-218 expression was associated with positive response to first-line 5-FU treatment in CRC patients. A Kaplan-Meier survival analysis indicated that patients with high miR-218 expression was associated with long OS (*P*=0.0002) and PFS (*P*=0.002). Flow cytometry showed that miR-218 increased apoptosis in both CRC cell lines and the TUNEL assay indicated that cells transfected with miR-218 showed significantly elevated fluorescence level of DNA cleavage. Cell proliferation was significantly inhibited in miR-218-transfected CRC cells. At day 5, the cell proliferation of miR-218-transfected HT29, HCT116 cells were reduced by 23% and 55% respectively. We also found that *BIRC5* mRNA and protein expression was

significantly reduced in cell lines transfected with miR-218 when compared with negative control. Moreover, luciferase assay showed that cells transfected with miR-218 significantly inhibited the luciferase activity while miR-218 did not inhibit the luciferase activity of the reporter vector containing mutated 3'UTR of *BIRC5* mRNA. We also found that miR-218 decreased the IC_{50} value of 5-FU in both cell lines. MiR-218 suppressed the expression of 5-FU-targeted TS protein, which explained the potential regulatory mechanism of miR-218 effects on enhancement of 5-FU-induced apoptosis.

Conclusion: The present work has identified miR-218 as a non-coding RNA related to the response to 5-FU based treatment in CRC patients and revealed that miR-218 promotes apoptosis by suppressing *BIRC5* protein expression in CRC. miR-218 also induces inhibition of cell proliferation and enhances 5-FU cytotoxcity *in vitro*. Thus, restoration of miR-218 levels could be a potential novel strategy to enhance chemosensitivity to 5-FU based treatment.

A-100

Combined assessment of plasma D-dimer concentration and estimated glomerular filtration rate for predicting the risk of all-cause and cardiovascular mortality in outpatients with stable coronary artery disease

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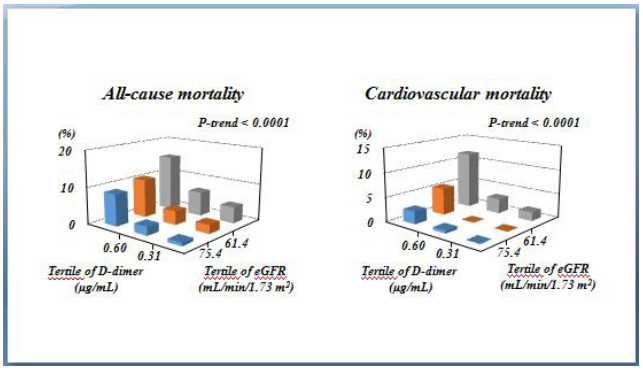
Background: Renal dysfunction is an important risk factor for cardiovascular mortality in patients with coronary artery disease (CAD); it may accelerate hypercoagulability in them. Whether the combined assessment of D-dimer level and glomerular filtration rate estimated by creatinine-based equations (eGFR) is useful for predicting mortality in outpatients with stable CAD was prospectively investigated. **Methods:** Plasma D-dimer level and eGFR were measured in 1526 outpatients (median age: 66 years; male, 77%) with angiographically documented significant coronary artery stenosis (> 50%) and/or a history of myocardial infarction, of whom 42% had a history of old myocardial infarction; 46%, history of coronary revascularization; and 32%, diabetes. **Results:** D-dimer levels significantly correlated with eGFR ($r = -0.26$; $p < 0.0001$). During a mean follow-up period of 41 months, 97 (6.4%) all-cause mortality cases were recoded, including 48 cardiovascular mortality cases. The patients who died were older (median: 73 vs. 66 years; $p < 0.0001$) and exhibited higher D-dimer (0.90 vs. 0.42 μg/mL; $p < 0.0001$) and high-sensitive C-reactive protein levels (2.0 vs. 1.9 mg/L; $p = 0.02$) but had lower left ventricular ejection fraction (52% vs. 57%; $p = 0.003$) and eGFR (59.2 vs. 68.5 mL/min/1.73 m^2; $p < 0.0001$) than those who survived. Multivariate Cox regression analysis, including 9 clinical, biochemical, and echocardiographic variables, identified D-dimer (relative risk: 2.38 per 10-fold increment; $p = 0.0002$) and eGFR (relative risk: 0.86 per 10 mL/min/1.73 m^2 increment; $p = 0.02$) as independent predictors of all-cause mortality. Similar results were obtained for cardiovascular mortality. The combination of D-dimer and eGFR tertiles was significantly associated with all-cause and cardiovascular mortality rates (Figure). **Conclusions:** The combined assessment of D-dimer and eGFR may improve the prediction of mortality in outpatients with stable CAD.



A-101

Multicenter Evaluation Supports Accuracy of the Beckman Coulter Gram-Negative Identification Product with Improved Database for Clinically Significant Bacteria

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Background: Bacterial identification is essential for determining effective antimicrobial therapies against infections. The MicroScan Gram-negative Identification (NID) organism database was revised and includes an additional 39 new taxa - 22 fermentative and 17 non-fermentative Gram-negatives - along with updated nomenclature. A multicenter study was performed to evaluate the accuracy of the updated NID database.

Methods: MicroScan NID panels were evaluated at two sites with 609 fresh clinical isolates comprised of 55 fermentative and non-fermentative Gram-negative taxa. MicroScan panels were inoculated using both the turbidity and PROMPT® System inoculation methods. Reference testing was performed following manufacturer's instructions and sequencing of 16S rDNA was included for discrepant isolates. All NID panels were incubated in a WalkAway instrument, and the NID panel identifications were generated using the updated NID organism database. Percent correct and incorrect results were used to assess accuracy of the updated NID panel. Thirteen isolates could only be identified to genus-level using the reference methods available.

Results: A correct identification was obtained for 98.6% (414/420) of the fermentative Gram-negative taxa. A correct identification was obtained for 94.7% (179/189) of the non-fermentative Gram-negative taxa. Therefore, an overall correct species-level identification was obtained for 97.4% (593/609) of the isolates. Only 3.1% of all isolates (19/593) required additional tests to confirm a low-probability correct identification, including 1 *Alcaligenes faecalis*, 5 *Achromobacter xylosoxidans/denitrificans*, 1 *Citrobacter freundii*, 2 *Chryseomonas indologenes*, 1 *Enterobacter aerogenes*, 2 *Escherichia coli*, 1 *Klebsiella oxytoca*, 1 *K. pneumoniae*, 1 *Morganella morganii*, 2 *Pseudomonas aeruginosa*, and 2 *Proteus mirabilis*. Furthermore, the clinically significant species *A. baumannii/haemolyticus*, *E. coli*, *P. aeruginosa*, and *P. mirabilis* were correct at 100%, 97.4%, 97.8%, and 96.4% respectively. Incorrect species-level identifications were obtained for 2.5% (15/609) of the isolates, including 1 *A. xylosoxidans,* 1 *E. hormaechei*, 2 *Achromobacter* species, 4 *Acinetobacter* species, 3 *Enterobacter* species, 1 *Pantoea* species, and 3 *Pseudomonas* species. The 13 isolates with genus-level reference identifications were counted as incorrect species-level identifications. A very rare biotype was obtained for 0.2% (1/609) - a single *E. aerogenes* isolate.

Conclusions: The results of this evaluation with fresh clinical isolates show that the MicroScan Dried Gram-negative ID panel with an updated database provides accurate identification results for clinically important Gram-negative bacteria.

A-102

Multicenter Evaluation of Clindamycin MIC Results for Staphylococci Using MicroScan Dried Gram Positive MIC Panels

R. M. Humphries[1], J. A. Hindler[1], P. C. Schreckenberger[2], J. Tjhio[3], C. R. Polage[4], M. P. Weinstein[5], M. Evans[6], R. K. Brookman[6], J. Y. Chau[6], D. Carpenter[6]. *[1]UCLA David Geffen School of Medicine, Los Angeles, CA, [2]Loyola University Medical Center, Maywood, CA, [3]Loyola University Medical Center, Maywood, IL, [4]UC Davis Medical Center, Sacramento, CA, [5]Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, [6]Beckman Coulter Microbiology, West Sacramento, CA*

Background: Clindamycin has activity against Staphylococci both *in vitro* and in clinical infections. A multicenter study was performed to evaluate the accuracy of a revised formulation of clindamycin on a MicroScan Dried Gram Positive MIC (MSDGP) Panel when compared to frozen CLSI broth microdilution reference panels.

Methods: For efficacy, MSDGP panels were evaluated at four sites by comparing MICs obtained to MICs using a CLSI broth microdilution reference panel. A total of 784 *Staphylococcus* spp. clinical isolates were tested using the turbidity and Prompt™* methods of inoculation. For reproducibility, a subset of 10 organisms was

tested on MSDGP panels at each site. MSDGP panels were incubated at 35 ±2°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGP panels were at 16-20 hours. Frozen reference panels, prepared according to ISO/CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ±2°C and read visually. Frozen reference panels were read and reported at 18 hours for all organisms. CLSI M100-S25 breakpoints (µg/ml) were used for MIC interpretation (i.e., ≤ 0.5 S, 1-2 I, and ≥ 4 R).

Results: When compared to frozen reference panel results, essential and categorical agreements for isolates tested in Efficacy are as follows:

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors %		Major Errors %		Minor Errors %	
	T	P	T	P	T	P	T	P	T	P
Visual	98.7 (774/784)	97.7 (766/784)	99.1 (777/784)	98.9 (775/784)	0.6 (1/168)	0.6 (1/168)	0.5 (3/608)	0.2 (1/608)	0.4 (3/784)	0.9 (7/784)
Walk Away	98.2 (770/784)	98.1 (769/784)	98.9 (775/784)	98.5 (772/784)	0.0 (0/168)	0.0 (0/168)	0.3 (2/608)	0.5 (3/608)	0.9 (7/784)	1.1 (9/784)
auto SCAN-4	96.7 (758/784)	96.6 (757/784)	98.9 (775/784)	98.6 (771/784)	0.6 (1/168)	0.6 (1/168)	0.7 (4/608)	0.5 (3/608)	0.5 (4/784)	1.1 (9/784)
T = Turbidity inoculation method, P = Prompt™* inoculation method										

Reproducibility among the four sites were greater than 95% for all read methods for both the turbidity and Prompt™* inoculation methods.

Conclusion: The MicroScan Dried Gram Positive MIC panel showed excellent correlation with MICs obtained using a CLSI broth microdilution reference panel for susceptibility testing of a revised formulation of clindamycin and *Staphylococcocus* spp.

* PROMPT™ is a registered trademark of 3M.

A-103

Multicenter Evaluation of Vancomycin MIC Results at 18 hours for Staphylococci and Enterococci Using MicroScan Dried Overnight Performance Evaluation Device Panel

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Background: With increasing rates of antibiotic resistance, MIC determination is important to initiate effective antimicrobial therapy. Vancomycin has been shown to be active against most strains of Enterococci and Staphylococci, both *in vitro* and in clinical infections. A multicenter study was performed to evaluate the accuracy of a revised formulation of vancomycin on a MicroScan Dried Gram Positive MIC (MSDGP) Panel when compared to CLSI broth microdilution reference panels.

Methods: For efficacy, MSDCP panels were evaluated at four sites with 947 clinical isolates. For reproducibility, a subset of 11 organisms was tested on MSDGP panels at each site. MSDGP panels were inoculated using both turbidity and Prompt™* inoculation methods. Frozen reference panels, prepared according to ISO/CLSI methodology, were inoculated using the turbidity inoculation method. All panels were incubated at 35 ±2°C and visually read. Frozen reference panels were read and reported at 24 hours for all organisms. Read times for the MSDGP panels were 18 hours for all species. FDA/CLSI breakpoints (µg/ml) were used for interpretation of MIC results.

Results: When compared to frozen reference panel results, essential and categorical agreements for isolates tested in Efficacy are as follows:

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors %		Major Errors %		Minor Errors %	
	T	P	T	P	T	P	T	P	T	P
Visual	99.4 (941/947)	96.2 (911/947)	99.5 (942/947)	99.2 (939/947)	0.0 (0/62)	0.0 (0/62)	0.0 (0/882)	0.1 (1/882)	0.5 (5/947)	0.7 (7/947)
Walk Away	99.5 (942/947)	95.8 (907/947)	99.6 (943/947)	99.2 (939/947)	0.0 (0/62)	0.0 (0/62)	0.0 (0/882)	0.0 (0/882)	0.4 (4/947)	0.8 (8/947)
auto SCAN-4	99.0 (938/947)	96.2 (911/947)	99.4 (941/947)	99.3 (940/947)	0.0 (0/62)	0.0 (0/62)	0.1 (1/882)	0.0 (0/882)	0.5 (5/947)	0.7 (7/947)
T = Turbidity inoculation method, P = Prompt™* inoculation method										

Reproducibility among the four sites were greater than 95% for all read methods for both the turbidity and Prompt™* inoculation methods.

Conclusion: This multicenter study showed that vancomycin MIC results read at 18 hours for *Enterococcus* and *Staphylococcus* species obtained with the MSDGP panel with a revised vancomycin formulation correlate well with MICs obtained using frozen reference panels read at 24 hours.

* PROMPT is a registered trademark of 3M.

A-104

Correlation of Anti-Pneumococcal Capsular Polysaccharide IgM, IgG and IgA specific antibodies in adult blood donors.

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Background: Post vaccination serum IgG antibody measurements are used to assess immune system competence, recovery and are included in guidelines for the assessment of antibody deficiencies. Recently, the measurement of PCP IgM and IgA has been reported in patients with common variable immunodeficiency (CVID). At present, the measurement of antigen-specific IgM and IgA antibodies is not routinely performed for the assessment of immunocompetence or risk of infection. We hypothesise that the simultaneous measurement IgM and IgA in addition to PCP IgG may give the clinician a more detailed antibody profile for the assessment of immunocompetence.

Objectives: Anti-pneumococcal capsular polysaccharide (PCP) IgM, IgG and IgA ELISAs have been developed (VaccZyme™ PCP ELISA, The Binding Site Group Limited, UK) to aid assessment of the adaptive immune system. The relationship between the concentrations of PCP IgM, IgG, and IgA was investigated.

Methods: The concentrations of PCP IgM, IgG, and IgA were measured in serum samples obtained from 231 adult blood donors (125 males and 106 females) aged 18-90 years. Only subjects who were free of recurrent infections or inflammation and whose C-reactive protein concentrations were <10mg/L were included in the analysis.

Results: Concentrations of each isotype were not normally distributed. The median concentration for PCP IgM was 54 U/mL (range 37-75 U/mL), IgG 40 mg/L (range 26-79 mg/L) and IgA 21 U/mL (range 13-44 U/mL). The median PCP IgM titres decreased with age and there was a significantly lower median PCP IgM titre in patients aged 81-90 years compared to those aged 18-80 years (27 *vs* 55 U/mL, p=0.0017). By contrast, there was a significantly higher median serum PCP IgG titre in the 61-90 years (61-70 years: 98 mg/L; p=0.0004, 71-80 years:110 mg/L; p<0.0001 and 81-90 years:67 mg/L; p=0.018) compared to those aged 18-60 years (median 35 mg/L). For PCP IgA, there was a significantly higher median titre in the 51-60 years (28 U/mL; p=0.036), 61-70 years (35 U/mL; p=0.0078), 71-80 years (43 U/mL; p=0.0065), and 81-90 years (55 U/mL; p=0.0034), compared to those aged 18-50 years (20 U/mL). The correlation between PCP IgG and IgA was more significant than between IgM and IgA and between IgM and IgG. Correlation of PCP IgA and IgM concentrations identified four immunophenotypes: those with i) high PCP IgM and IgA, ii) high PCP IgM only, iii) high PCP IgA only and iv) low PCP IgM and IgA. A significant number of individuals with a PCP IgG concentration >50mg/L had low PCP IgA (10-94%) and IgM (7-53%) concentrations.

Conclusion: The additional measurement of PCP IgA and PCP IgM, alongside PCP IgG, in individuals investigated for a compromised immune system may provide a more detailed antibody profile.

A-105

A Comparison of Two Compact Nucleic Acid Extractors, The Qiagen EZ1 Advanced XL And The Roche Magnapur Compact.

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Background: Nucleic acid extraction and purification is a fundamental and important prequel to successful and accurate downstream analytical processes. While numerous methods/manufacturers are available, contemporary considerations such as complexity of operation, breadth of application, cost and environmental impact become increasingly significant in choice consideration.

Via open tender process we shortlisted and evaluated two instruments fitting our modus operandi. Performance of extractors were evaluated based on outcomes of two viral hepatitis quantitation assays, influenza swabs in transport media, human genomic DNA and stool for *Clostridium difficile* testing.

Materials and methods: Plasma, stool, whole blood and where applicable, External Quality Assurance samples, with previously tested outcomes were used as evaluation reference samples. EZ1 Advanced XL (EZ1) (Qiagen, Germany) and MagnaPure Compact (MPC) (Roche, USA) were compared against current method, Qiagen QiaSymphony SP.

Outcomes were evaluated using regression analysis or binary classification testing for specificity and sensitivity against current/reference extraction methods.

Results: 41 anonymised stool samples were processed according to respective instrument protocols then analysed for *C.difficile tcdC* gene using Lightmix *C.difficile* kit (TibMolbiol, Germany). Specificities and sensitivities were 86% and 75% for MPC and 92% and 100% for EZ1 respectively. In addition, 2 samples negative for *C.difficile* processed by MPC displayed absence of Internal Control.

Both extractors showed equivalent quality when 32 UTM samples with some spiked with Influenza A virus were fed through respective instruments. Upon analysis, mean cycle threshold value was 29.88 and 30.50 for MPC and EZ1 respectively, indicating both instruments had extracted equal amounts of target RNA, based on 'M' protein assay developed by the Chinese National Influenza Center.

For extraction of human genomic DNA, concentration, yield and A260/280ratio for EZ1 were 44.3ng/uL, 9.2ug and 1.9 compared to MPC which yielded 62.5ng/uL, 12.5ug and 2.0 and Qiagen QiaSymphony SP, which yielded 43.5ng/uL, 8.7ug and 1.8.

MPC was unable to process samples to be tested by Roche Hepatitis B and C assays as the start and elution volumes were not compatible with kit Quantitation Standard concentration. MPC thus did not qualify for this segment of evaluation.

Conclusion: Both extractors use established magnetic bead capture techniques on contemporary compact instruments. User interaction is minimal, intuitive and requires only rudimentary technical skill. Extraction times are fairly quick with largely high quality eluate. Complex starting materials such as stool require pre-processing and may have significant influence on downstream testing outcomes.

The two instruments showed marginal difference in human genomic DNA extraction and Influenza A detection, but MPC was challenged with stool samples not only in specificity and sensitivity but also in number of definitive results. 2 stool samples which were successfully resulted on EZ1 and our current method were negative on MPC for target and internal control, indicating likely inhibition. However, the most decisive outcome was the fact that we were unable to incorporate, into a single extraction run, Hepatitis viral load assays because the start and eluate volumes were not compatible with the kit reagents. As such, it was clear that EZ1 was more suited to the operational and diagnostic needs of our laboratory.

A-106

Both Proinflammatory (IL-6) and Anti-Inflammatory (TGF-beta1) Cytokines Were Elevated in Clinicaly Stable Schizophrenia Patients

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Background: Pro-inflammatory cytokines, microglial cells, astrocytes, and invading immune cells mediate inflammation in the CNS. Inflammatory mechanisms should be well regulated, as excessive response can be a source of injury for the host cells. Besides several hypotheses, overactivation of inflammatory mechanisms is proposed for the etiology of schizophrenia. Increase in some proinflammatory cytokines have been shown in the blood and CSF of these patients and the therapeutic benefit of anti-inflammatory medications in some studies. However, there were a lot of controversies. In this study, we aimed to measure the serum levels of some pro-inflammatory (IL-6, TNF-alpha) and anti-inflammatory (TGF-beta1) cytokines and reveal their effects on disease mechanism.

Methods: The study was conducted in Marmara University Pendik E&R Hospital Department of Psychiatry and Biochemistry Laboratory. The study was approved by the ethical committee of Marmara University School of Medicine and informed consent was obtained from each case. Clinically stable patients with diagnosis of schizophrenia (n=30) and 29 healthy controls were enrolled. Broad neuropsychological test battery was conducted to assess cognitive functions. Patients with neurologic diseases, known acute or chronic inflammatory or allergic diseases, those using anti-iflammatory or immunosuppressive drugs were excluded. Serum IL-6, TNF-alpha, TGF-beta1 levels were measured with ELISA.

Results: Serum IL-6 and TGF-beta1 levels were found to be significantly higher in the patient group when compared to the controls. However, TNF-alpha levels were not significantly different (Table 1). Multiple logistic regression revealed a positive association between the disease state, IL-6 and TGF-beta1 (for IL-6 OR=2.52, P=0.031; for TGF-beta1 OR=1,00, P=0.027).

Conclusion: We concluded that inflammatory response in clinically stable schizophrenia patients is increased compared to controls. To clarify the significance of inflammation, further research both on serum cytokine levels in different schizophrenia patient groups and the relation between cognitive functions and symptom clusters in homogeneous patient groups on a broader scale is needed.

	Patient	Control	P
IL-6 (pg/mL)	1.2 (0.8-2.0)	0.9 (0.5-1.2)	0.048
TNF-alpha (pg/mL)	6.0 (4.0-7.5)	5.9 (3.9-8.1)	0.726
TGF-beta1 (pg/mL)	1891 (1185-7944)	1320 (984-1723)	0.012

A-107

A Queuing Model Analysis to Evaluate the Impact of High-sensitive Troponin I on Emergency Department Management Metrics

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Objectives: Emergency departments (ED) are important entry points to hospital care. Access to treatment and patient flow is however frequently challenged by ED crowding that is a key issue for many hospitals. Crowding could negatively impact clinical endpoints as well as efficiency of health care processes. Different approaches have been made in order to alleviate crowding issues and improve patient flow. A new diagnostic assay (high-sensitive troponin I, hsTnI) has been recommended to facilitate faster clinical decisions for patients presenting with chest pain (CPP) in the ED suggestive of having myocardial infarction. The objective of the study was to develop a model and evaluate the impact of an accelerated hsTnI driven chest pain triaging pathway on ED patient flow and ED performance metrics from a hospital perspective. **Methods:** An economic model based on a queuing theory has been developed and applied to data from a major emergency department in Rijadh. The management of CPP is based on serial measurements of troponin at defined time points. The standard scenario running a contemporary troponin (cTnI) with a standard protocol time of 6 hours was tested against the use of a high-sensitive troponin (hsTnI) supported scenario with a 3 hours protocol time as recently recommended by clinical guidelines. In order to measure the impact of a shorter protocol time on ED metrics (Waiting time, ED time, risk for waiting, diversion rate, required beds per day, total costs of ED stay) both, a deterministic base case analysis and a probabilistic analysis (5,000 iterations) were performed to test robustness and reflect variability and uncertainty. **Results:** Deterministic and probabilistic analysis showed significant improvements in all ED performance indicators for the hsTnI scenario: ED waiting time (> -7hrs), ED time (> -10hrs), required beds for CPP (-9.9 beds per day). In addition, results suggested that the hsTnI scenario would be less affected by variation in demand and service. Despite increased test costs for high-sensitive troponin, total costs could be expected to be significantly reduced from an average of 726€ (95%CI 724-728) per CPP in the cTnI scenario to 429€ (428-430) per CPP in the hsTnI scenario. **Conclusions:** The management of CPPs requires a substantial amount of ED resources and time. This economic model suggests that a switch to hsTnI with shorter protocol times for risk stratification would significantly improve ED flow metrics. It would stabilize the CPP pathway which would become more predictable and manageable. It would release resources that could be devoted to other ED patients, thus alleviating ED crowding issues and generating benefits for the overall hospital system.

A-108

Autoantibodies against CD74 - A new diagnostic marker for Spondyloarthritis

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Background: Spondyloarthritis (SpA) is a common debilitating inflammatory disorder. Pathogenesis of axial SpA (axSpA) including ankylosing spondylitis (AS) is still largely unclear. Diagnosis is difficult, since abnormalities in conventional X-rays develop with a latency of several years and only HLA-B27 is used as laboratory marker. The presence of radiographic sacroiliitis is essential for SpA diagnosis. To prevent destructive effects early diagnosis and intervention in SpA patients may be important. To evaluate antibodies to the human leukocyte antigen class II-associated invariant chain peptide (anti-CD74) as a diagnostic marker of SpA.

Methods: Sera of 117 patients with axial SpA and 38 non-SpA patients were analyzed for IgA and IgG antibodies against CD74 by ELISA. HLA-B27 status was available in 112 patients. All donors provided informed consent for the study approved by the local ethics committee (project number 4928).

Results: Anti-CD74 antibodies were detected in 85.1% of SpA patients but only in 5% of non-SpA patients ($p \leq 0.0001$). Detection of both IgG and IgA anti-CD74 antibodies for diagnosing SpA revealed a sensitivity of 77% and a specificity of 90%. Remarkably, IgA autoantibodies against CD74 alone had a sensitivity of 67% and a specificity of 95%. IgA anti-CD74 antibodies were even more frequent in SpA patients with short disease duration and significantly correlate with more advanced radiological sacroiliitis and reduced spinal mobility.

Conclusion: Anti-CD74 IgA antibodies were strongly associated with SpA. Antibodies against CD74 could provide an important additional tool for diagnosis of SpA.

A-109

Industrial food additive microbial transglutaminase is immunogenic in children with celiac disease

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Background: Microbial transglutaminase (mTg) is capable of cross-linking numerous molecules. It is a family member of human tissue transglutaminase (tTg), involved in CD. Despite declarations of mTg safety, direct evidence for immunogenicity of the enzyme is lacking.

Methods: he serological activity of mTg, tTg, gliadin complexed mTg (mTg neo-epitope) and gliadin complexed tTg (tTg neo-epitope) were studied in: 95 pediatric celiac patients (CD), 99 normal children (NC) and 79 normal adults (NA). Sera were tested by ELISAs, detecting IgA, IgG or both IgA and IgG: *AESKULISA®* tTg (tTg), *AESKULISA®* tTg New Generation (tTg neo-epitope (tTg-neo)), microbial transglutaminase (mTg) and mTg neo-epitope (mTg-neo). Marsh criteria were used for the degree of intestinal injury.

Results: Comparing pediatric CD patients with the 2 normal groups: mTg-neo IgA, IgG and IgA+IgG antibody activities exceed the comparable mTg ones ($p<0.0001$). All mTg-neo and tTg-neo levels were higher ($p<0.001$). tTg IgA and IgG+IgA were higher than mTg IgA and IgA+IgG ($p<0.0001$). The levels of tTg-neo IgA/IgG were higher than tTg IgA/IgG ($p<0.0001$). The sequential antibody activities reflecting best the increased intestinal damage were: tTg-neo IgG ≥ mTg-neo IgG > mTg-neo IgA+IgG > tTg-neo IgA. Taken together, mTg-neo IgG and tTg-neo IgG correlated best with intestinal pathology ($r^2=0.989$, $r^2=0.989$, $p<0.0001$, $p<0.0001$, respectively).

Conclusion: mTg is immunogenic in children with CD and by complexing to gliadin its immunogenicity is enhanced. Anti-neo-epitope mTg antibodies correlate with intestinal damage to the same degree as anti-tTg. Further studies are needed to explore the pathogenic potential of anti-mTg antibodies in CD.

A-110

Correlation between age and semen quality in healthy Brazilian men

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Although the effect of maternal age on fertility is well known, it is unclear whether paternal age also affects fertility. This retrospective study sought to characterize the association between age and semen quality, a well-known point of fertility status. Samples data of 54 men (36±9.0years old, range 20 - 58 yo) cryopreserved between 2010 and 2015 without known fertility problems and considered healthy by initial trials and attested by an urologist were evaluated at Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil). The cohort was divided in two groups, group 1 (G1 n=28) 20-36 yo and group 2 (G2, n=26) 37-58 yo. Cellularity (concentration of sperm cells) and motility were assessed by Makler chamber and vitality was measured by eosin Y. The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests. The comparison between the G1 and G2 was evaluated by Student's t-test for parametric data or by Mann-Whitney test for non-parametric analysis. Further, the correlation between age and the different parameters of sperm was evaluated by Spearman coefficient. Data was shown as median ± SDand a p value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6. No correlation was observed between age and cellularity ($r = 0.2658$, $p = 0.0521$), motility ($r = 0.1679$, $p = 0.2249$) and viability ($r = 0,1704$, $p = 0,2179$). Evaluating the population in separated groups based on the median of the age of the cohort (36 ± 9.0 yo), G1 showed semen with less cellularity (4.93 ± 11.0 x 107 per mL) compared to the older group G2 (7.63 ± 14.6 x 107 per mL), but the difference was not statistical significant ($p = 0.0965$). Spermatozoa motility was measured according with World Health Organization (WHO) classification, being the frequency of motile spermatozoa classified as "a" (sperm with progressive motility) plus "b" (non-linear motility). G1 showed 52% of motility and G2 60% ($p = 0.1297$), and the viability showed similar difference between G1 (54%) and G2 (58%) ($p = 0.2646$). Therefore, no statistical significant and impact were observed between age and sperm quality in our cohort. Further studies with a large cohort are needed to confirm these findings.

A-111

Quality evaluation of umbilical cord blood cryopreserved from vaginal and cesarean deliveries

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Umbilical cord blood (UCB) is largely employed as an alternative source of stem cells in the treatment of hemato-oncological diseases. In this study, we determined the impact of the modeof delivery, maternal factors and laboratory parameters of hematopoietic potential, such as viability, cell recovery after processing and percentage of $CD34^+$ cells. Data of 170 cryopreserved UCB samples from live births of 34 - 41 weeks collected between 2013 and 2015 from cesarean and vaginal deliveries were evaluatedand processed by Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil). The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests and the comparison between cesarean and vaginal deliveries data were measured by Student's *t*-test for parametric data or by Mann-Whitney test for non-parametric analysis. Data was shown as median ± SD and a *p* value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6. Maternal and neonatal parameters include age of the mother, gestational weeks, mode of delivery and baby's gender and laboratory parameters include cord blood volume, frequency of $CD34^+$cells, viability and recovery after processing. Amongst the 170 UCB samples evaluated here, 85 (50%) correspond from cesarean deliveryin which 46% of the babies correspond to female gender. The median age of the mother of this group was 36.0 ± 4.0 years old (yo) and gestational age was 38 ± 0.9 weeks. On the other hand, 85 UCB samples (50%) were from vaginal birth and 59% of the neonates were female. The median age of the mother was 34.0 ± 4.0 yo and gestational age was 39.0 ± 1.0 weeks. The total of umbilical cord blood volume collected was higher in cesarean delivery (80 ± 33.3 mL) compared to vaginal delivery (69 ± 27.8 mL, $p = 0.0417$). No difference was observed between cesarean and vaginal delivery in the follow parameters: percentage of cell viability (97.7 and 96.8, respectively, $p = 0.1639$),percentage of recovery (92.4 and 91.4, respectively, $p = 0.4843$) and percentage of $CD34^+$cells ($9.2 \times 10^{-6}$and 8.9×10^{-6},

respectively, $p = 0.5434$). Thus, the study concludes that cesarean delivery provide a higher volume of UCB but no difference in samples quality was observed between cesarean and vaginal deliveries inthe cohort here evaluated.

A-112

Semen quality of men infected by *Mycoplasma hominis*

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This study was undertaken to determine the prevalence of *Mycoplasma hominis* infection among men and to study the effects of these infection on semen quality. A total of cryopreserved samples data from 54 men (36.0 ± 9.0 years old(yo), range 20 - 58yo), being 6 (11%) infected with *M.hominis* and 48 (89%) negative for sexually transmitted diseases and considered healthy by initial trials and attested by an urologist were evaluated before and after sample freezing at Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil). *M.hominis*was detected by polymerase chain reaction and sperm quality was assessed by cellularityand motility through Makler chamber and viability by eosin Y. The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests.The comparison between infected and uninfected groupswas evaluated by Student's *t*-test for parametric data or by Mann-Whitney test for non-parametric analysis. Data was shown as median ± SDand a p value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6. The men positive for *M.hominis*had semen sampleswith lower spermatozoa viability than those uninfected and showed statistically significant ($p = 0.0447$). In addition, the percentage of reduction of sperm motility after freezing were higher in samples of men infected (38%) compared to those uninfected (27%, $p = 0.0279$).No statistically difference was observed in sperm cellularity from infected men ($5.4 \pm 3.4 \times 10^7$) from those uninfected ($6.9 \pm 0.1 \times 10^7$,$p = 0.2418$). Therefore, these results showed that *M. hominis* infection interfere on semen quality negatively, but not in cellularity of the sperm.

A-113

The effects of cryopreservation on sperm cellularity, motility and vitality

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The effects of cryoinjury were determined simultaneously on cellularity, motility and viability of ejaculated human sperm. This retrospective study included 54 men (36yo± 9.0, range 20 - 58yo) without known fertility problems and considered healthy by initial trials and attested by anurologist at Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil).Semen cellularity and spermatozoa motility were measured byMakler Counting Chamber andviability was assessed by eosin Y. Data are presented as a percentage of the difference between median of the values obtained before and after freezing. The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests and the comparison between data before and after freezing was measured by Student's *t*-test for parametric data or by Mann Whitney for non-parametric analysis. Data was shown as median ± SDand a p value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6.Freeze-thawingcaused 42% (p= 0.0005) reduction in cellularity. Spermatozoa motility was measuredaccording with World Health Organization (WHO) classification, being thefrequency of motile spermatozoa classified as "*a*" (sperm with progressive motility) plus "*b*"(non-linear motility). Motility and vitality of the sperm showed similar reduction after freezing (31%, p = 0.0002 and 32%, p< 0.0001, respectively). Semen cellularity, motility and vitality are equally susceptible to cryopreservation-induced damage. To avoid critically reducing sperm quality after thawing, it is necessary criticism at the time of cryopreservation, ensuring high rates of motility and viability or high cellularity of the sample.

A-114

Association between umbilical cord blood volume and CD34 positive cells.

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Umbilical cord blood (UCB) has been recently considered as an alternative source of hematopoietic progenitor cells for clinical application. Some of the parameters commonly used to evaluate an UCB unitand predict transplant outcomes have been $CD34^+$cells concentration, which is a hematopoietic stem cells marker, and total cord blood volume collected. Thus, the aim of the study was to find the correlation between umbilical cord blood volume and $CD34^+$ cells concentration derived from cord blood. For this study, 2787 UCB samples that were processed and cryopreserved at Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil) were evaluated. $CD34^+$ cells were detected by Flow Cytometry at Hermes Pardini Institute (Vespasiano-MG/Brazil) and were expressed in percentage, considering the ratio between the number of $CD34^+$ cells and the total number of collected cells x 100. The total cord blood volume was noted at the moment that the bag arrivedin the technical area. The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests. The correlation between umbilical cord blood volume and $CD34^+$ cells was evaluated by Spearman correlation coefficient. Data was shown as median ± SDand a p value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6. Here, only cesarean deliveries were evaluatedandthe median of gestational age was 39 weeks (39 ± 1.5 weeks). The median of mother age was 38 years old(38 ± 27) and the cohort was composed of 1431 UCB samples of male gender (51%) and 1356 (49%) of female. The median of the total cord blood volume was 85 mL (85 ± 30 mL, range 22 - 227 mL) and the percentage of $CD34^+$cells were 9.9×10^{-6}% (± 0.18). We found that $CD34^+$ cells concentration was higher in greater volume of collected cord blood ($r = 0.4343$, $p < 0.0001$). Our study concludes that higher volume of cord blood should be preferred for processing and stem cell infusion.

A-115

Bone marrow cryopreservation: recovery due to the freezing solution concentrations and storage temperatures

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The transfusion of autologous cryopreserved bone marrow (BM) cells is a well-established procedure in the management of both hematological and non-hematological malignant diseases. The good quality of cryopreserved BM depends on storage temperature and cryopreservant concentration (dimethyl sulfoxide - DMSO).Thus, the aim of this study was to evaluate the effect of different of DMSO concentrations and temperatures of storage on cryopreservation of BM among distinct storage time points. For this study, 40 mL of BM that would be discarded from medical procedures was collected from posterior iliac crest by a hematologist at Criovida - Hermes Pardini Institute (Belo Horizonte/Brazil). BM was processed by SEPAX® with a single-use kit CS-900. The material was concentrated to 30 mL with 75% of recovery. Cells were cryopreserved using 2.5% or 5% of DMSO for -80°C storage and 10% or 20% of DMSO for cryopreservation at -196°C (nitrogen liquid). Cryopreserved BM cells were evaluated 1, 30 and 90 days after freezing. The cell quality was assessed by CD34 positive cells and the percentage of viable cells using 7-AAD as a vital exclusion marker by flow cytometry at Hermes Pardini Institute (Vespasiano-MG/Brazil).The results obtained show that frequency of $CD34^+$ cells and cell viability had, in all conditions, better results stored at -196°C compared to -80°C (Table 1). However, DMSO concentration was decisive to the number of $CD34^+$ cells.The value was proportional to DMSO concentration. On the other hand, the viability was higher at 10% DMSO concentration condition, even better than 20%, probably by the cell toxicity of the cryopreservant. Therefore, cryopreservation of BM at -196°C with 10% of DMSO could be more effective to ensure the quality of the cells, however, more samples are required to confirm this result.

Evaluation of bone marrow among distinct storage and DMSO concentrations time points							
		1 day		30 days		90 days	
Storage Temperature	[DMSO]	CD34+ (%)	Viability (%)	CD34+ (%)	Viability (%)	CD34+ (%)	Viability (%)
-80°C	2,5%	0,64	62,9	0,30	61,3	0,06	56,0
	5%	1,00	62,5	0,29	59,5	0,33	56,0
-196°C	10%	1,31	99,8	1,31	70,8	0,24	62,0
	20%	1,57	61,8	1,21	56,0	0,54	49,1

A-116

Evaluation of serum biomarker assays for mild traumatic brain injury in a cohort of mixed martial arts fighters

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Background: Identifying mild traumatic brain injury (mTBI) in sports is a continuous clinical challenge. Assessments are dominated by self-reported symptoms and cognitive function tests, but lack good sensitivity and specificity to detect mTBI injury. Biomarker tests may improve discrimination between injured and non-injured. There is increasing participation in mixed martial arts (MMA), a sport similar to boxing in which participants have increased exposure to head injury. The **objective** of our clinical study was to assess pre- and post-fight serum concentrations of two mTBI biomarkers, S100 and neuron-specific enolase (NSE), in a cohort of otherwise healthy amateur MMA fighters (mean age 25 y.o., range 16-46). All fighters were eligible to participate and completed informed consent approved by the university's IRB.

Methods: *S100 and NSE measurements*: Blood samples were collected in a serum-separator tube on 58 fighters (97% male) both before (pre-) and within 30 minutes after the fight ended (post-). Blood was centrifuged after clot formation following standard protocol, and serum was frozen and kept at minus 80° C until analysis. S100 and NSE tests were performed on a Roche Diagnostics Modular E170 instrument using manufacturer-supplied reagents, calibrators, and controls. Both tests are sandwich immunoassays with chemiluminescent signal detection proportional to protein concentration of S100 (ug/L) or NSE (ng/mL). All subject test results fell within the analytical measurement range of the assays. We confirmed agreement with manufacturer reported precision values with repeated tests of low, mid, and high level samples. *Statistics:* Descriptive statistics and paired Wilcoxon-rank sum test were analyzed using Minitab software. ROC curve analysis was performed using MedCalc software.

Results: For this initial analysis, we adopted serum concentration cut-offs provided by the manufacturer of S100 > 0.105 ug/L or NSE > 16.3 ng/mL to indicate evidence of mTBI. For S100 test, 86% had evidence of mTBI within 30 minutes post-fight, whereas only 19% had pre-fight results greater than the cut-off value. All but 3 fighters (95%) had increased S100 post-fight *versus* pre-fight, while 90% had increased NSE post-fight. Median test value differences in fighters were statistically significant between pre- and post-samples for S100 (0.056 *vs* 0.199 ug/L) and NSE (12.1 *vs* 17.1 ng/mL), both $p<0.001$. Importantly, observed post-fight increases are unlikely explained by chance alone since changes were > 3 SD at these test levels (based on precision experiments). ROC curve discriminate analyses were excellent for both post-fight S100 levels (AUC = 0.921) and NSE levels (AUC = 0.900) in this cohort.

Conclusions: Increased levels of S100 and NSE serum markers were observed in the majority of subjects post-fight, suggesting mTBI injury may be common following MMA fights. In particular, S100 protein levels above cut-off value were found in 86% of subjects within 30 minutes post-fight. Moreover, increased S100 and NSE levels after the fight compared with baseline were observed in the majority of fighters. To our knowledge, this is the first study demonstrating early increases in serum levels of S100 and NSE biomarkers in a group of mixed martial arts fighters.

A-117

Gene deletion is associated with Second Toe Signal phenotype in Neurofibromatosis Type 1

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The early diagnosis of Neurofibromatosis types 1 (NF1), based on consensus criteria, is useful for themanagement of clinical aspects and genetic counseling. Additional specific congenital lesions might assist in the early diagnosis of NF1. Our group previously reported, through questionnaire (12%) and photographic register (5.8%), the prevalence of a not yet described NF1 phenotype component: bilateral superposition of the second toe over the first and the third toes, which we referred to as the "Second Toe Signal" (STS) (Figure 1). Regarding the fact that the most severe NF1 phenotypes are associated with microdeletions (the former whole-gene deletions), we assessed the association between STS and microdeletions. Multiplex Ligation-dependent Probe Amplification (MLPA) was performed for 21 NF1 patients presenting at least three NIH diagnostic criteria. The kits used were P081 e P082 - version C1. The samples' results were analyzed by Coffalyser v.140721.1958 software. Statistical analysiswas performedwith the open source calculator OpenEpi (version 3, www.openepi.com), using the Fisher exact test. Results: We found three microdeletions, including the regions of the flanking probes of the NF1 gene. All three microdeletion subjects have STS,one patient has STS but not microdeletion and the 17 others have neither STS nor microdeletion (P=0.006). These three microdeletions patients with STS present the generally accepted microdeletion clinical phenotype. As we increase our sample size, we suggest that STS, particularly regarding its presence at birth, is a useful clinical sign of NF1 microdeletion.

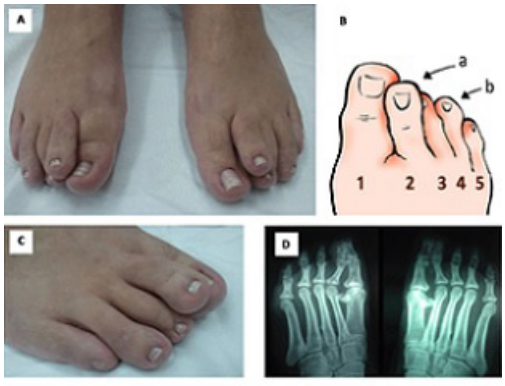

Figure 1 – Pictures (A, C) of one NF1 volunteer (three NIH criteria ate least) with bilateral superposition of the up of second toe (diagram B, a) and enlargement of the forth (diagram B, b) and feet XR (D). The patient presents the NF1 gene microdeletion phenotype and gene deletion at MLPA.

A-118

Cerebrospinal Fluid Angiotensin-Converting Enzyme Activity Levels of Patients With Alzheimer's and Parkinson's Disease

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Background: Angiotensin-converting enzyme (ACE) is an endopeptidase expressed by endothelial, epithelial and neuronal cells. It is found as both in membrane-bound and soluble forms. ACE is also one of the enzymes degrading amyloid-β (Aβ) in central nervous system. Accumulation of Aβ has an important role in the pathogenesis of Alzheimer's disease (AD). The angiotensin-converting enzyme may also be involved in the pathogenesis of Parkinson's disease (PD). There have been several studies investigating the association between several ACE gene polymorphisms and

PD risk. The aim of this study is to measure activity level of ACE in cerebrospinal fluid (CSF) samples of AD and PD patients and compare with the results of control group.

Method: 20 patients with AD, 17 patients with PD and 25 control cases. Control cases had no neurodegenerative disease but had diagnostic lumbar puncture for headache or peripheral nervous system disorders. All subjects were recruited from Hacettepe University Hospitals Neurology Clinics in the period of September 2012-June 2014. Participants were assessed by their age and CSF ACE activity. ACE activity was measured by spectrophotometric method from all CSF samples and calculated according to the total protein content of samples. Groups were assessed with Mann-Whitney U test. SPSS 21.0 was used for statistical analysis.

Results: Mean ages of AD, PD and control groups were 67.5±1.96, 59.76±2.71 and 50.68±3.4, respectively. The mean age of control group was lower than both AD and PD groups ($p<0.05$). CSF ACE enzyme activities of AD, PD and control groups were measured as 0.30±0.06, 0.33±0.07 0.53±0.11 U/L, respectively. CSF ACE specific enzyme activities of AD, PD and control groups were measured as 0.75±0.24, 0.82±0.29 and 1.29±0.47 U/g protein, respectively. According to CSF ACE enzyme activity and specific enzyme activity levels, there was statistically significant difference between AD and control group ($p<0.05$). However, the differences of enzyme activities and specific enzyme activities between PD and control group were not found to be statistically significant ($p>0.05$).

Conclusion: In this study, it is shown that CSF ACE activity was found to be significantly decreased in AD patients than control whereas the decrease in PD patients' CSF ACE activity was not significant. Further new studies with large-scale patient groups should be planned to assess CSF ACE as a biomarker for neurodegenerative diseases thoroughly.

Keywords: Alzheimer's Disease, Parkinson's Disease, Angiotensin-converting enzyme, Cerebrospinal fluid

A-121

Graft-derived cell-free DNA - a promising Rejection Marker in Liver transplantation - Results from a prospective Multicenter Trial

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Background: There is a need for cost-effective, non-invasive biomarkers of graft integrity with a short turn-around time that can be used with therapeutic drug monitoring (TDM) to personalize post-transplant immunosuppression. Graft-derived cell-free DNA (GcfDNA) has shown promise as such a biomarker for the detection of graft injury.

Methods: This is the first report on a prospective multicenter trial that monitored plasma GcfDNA in 106 adult liver transplant (LTx) recipients followed over at least one year post transplant. A total of 128 Patients were recruited at 3 German transplant centers (Charité/Berlin, UKE/Hamburg-Eppendorf, UMG/Göttingen), of which 22 were lost to follow up or censored based on exclusion criteria. cfDNA was extracted from ≥ 1 ml EDTA plasma, obtained in Cell-free DNA-BCT tubes. GcfDNA was determined as described elsewhere (Clin Chem 2013; 59: 1732-1741). The turn-around time for an initial sample is about two days and one working day for any consecutive sample.

Results: The GcfDNA percentage was highly elevated (>50% of total cfDNA) on the first day after transplantation, evaluated in a subset of 23 patients; presumably due to ischemia/reperfusion damage. The median GcfDNA percentage decreased in stable patients with no signs of graft injury within the first week to a baseline level below 10%, where it remained throughout the one year observation period. In otherwise stable patients with positive HCV virus detection (N=17, n=60 samples), GcfDNA values were generally only slightly elevated (median: 7.2%, 95% CI 4.9%-13.0%) compared to stable, HCV negative patients (N=87, median: 3.3%, 95% CI 3.0%-3.7%). In patients (N=20), with samples (n=34) drawn during biopsy-proven acute rejection periods, values (median: 30.7% CI 25.4%-45.0%) were about 10-fold higher than median values observed in samples (n=279) from stable patients without rejection (N=87). Interestingly, in five otherwise clinically stable patients with samples available 7-15 days prior to a diagnosis of biopsy proven acute rejection, also elevated GcfDNA values (>20%) were already observed (median: 38.7%, range: 21.1%-51.3%) at that time. Aminotransferases, γ-glutamyltransferase, glutamate dehydrogenase, and bilirubin showed low overall correlations with GcfDNA (correlation coefficients (r) ranged from 0.32 to 0.59) and these conventional liver function tests (LFTs) had greater overlap in acute rejection, HCV positive, and stable patients. The diagnostic sensitivity and specificity of GcfDNA ≥10% was 91.2% (CI 76.3%-98.1%) and 92.5% (CI 88.7%-95.3%) respectively, comparing stable HCV negative samples (n=279) versus biopsy-proven acute rejection samples (n=34). The AUC under the ROC curve was overall highest for GcfDNA (0.97, 95% CI:0.93-1.0), compared to LFTs; of which ALT was best with an AUC of 0.93 (CI: 0.85-1.0).

Conclusion: Plasma GcfDNA determinations allowed for better discrimination of liver transplant patients with acute rejection or allograft injury, compared to conventional LFTs and may be helpful to personalize post-LTx immunosuppression.

A-122

Presepsin Predicts Acute Kidney Injury and Mortality in Cardiac Surgery Patients

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Background

Presepsin (sCD14-ST) represents a 13 kDa fragment of sCD14 which is released after conversion of CD14++ monocytes into CD14+/16+ monocytes upon monocyte activation. Presepsin has been shown powerful prognostic validity in inflammatory related conditions like sepsis and SIRS and association with disease severity, multi organ dysfunction syndrome and outcome.

Objective

Preoperative mortality risk assessment should support optimizing peri- and postoperative care of patients. We thought to evaluate the prognostic value of presepsin for outcome prediction in patients undergoing elective cardiac surgery in comparison with EuroSCORE 2 and cardiac, inflammatory, and renal diagnostic markers (NT-proBNP, CRP, procalcitonin (PCT), Leucocytes, Cystatin C).

Methods

We included 856 consecutive patients having cardiac surgery and measured preoperative plasma concentration of presepsin, NT-pro-BNP, PCT, leucocytes, and cystatin C as well as postoperative presepsin levels. Presepsin was determined by using the PATHFAST Presepsin assay (LSI Medience corporation,Tokyo). The diagnostic markers NT-proBNP, CRP, PCT, Leucocytes, and Cystatin C were measured in the central laboratory by using routine clinical chemistry methods. Outcome measures were in-hospital mortality, 6- month mortality and occurence of acute kidney injury (AKI) during hospitalization. Areas-under-the-curves (AUCs) were compared using the tests of DeLong and Clarke-Pearson. Logistic regression analysis was used to calculate univariable and multivariable odds ratios.

Results

Patients with in-hospital mortality (n=27, *3.2%*) and 6-month mortality (n=49, *6.1%)* had higher preoperative presepsin levels than survivors: 1166±1453 pg/mL vs. 258±391 pg/mL; $p<0.001$ and 913±1215 pg/mL vs. 231±194 pg/mL; $p<0.001$, respectively. C-statistics showed elevated presepsin level to accurately predict occurrence of in-hospital mortality (AUC 0.88) and 6-month mortality (AUC 0.87) whereas the EuroSCORE 2 showed

significantly less predictive power (AUC values 0.74 and 0.76) as well as NT-pro-BNP (AUCs 0.77 and 0.79), PCT (AUCs 0.59 and 0.56), leucocytes (AUCs 0.58 and 0.63), and cystatin C (AUCs 0.76 and 0.74).

222 patients (25.9%) who developed AKI (AKI classification: 1 (n=122), 2 (n=54), 3 (n=46)) revealed higher mortality and higher presepsin values compared to patients without AKI (in-hospital mortality: 8% vs 1.4%; preoperative presepsin: 441±585 vs 233±436 pg/mL; $p<0.001$; postoperative presepsin: 927±926 vs 426±583 pg/mL; $p<0.001$). ROC analysis of postoperative presepsin showed the highest discriminatory power for risk prediction of AKI occurence (AUC 0.78) and renal replacement therapy (AUC 0.88) during hospitalization in comparison with the other diagnostic markers and EuroSCORE 2. Even after adjustment for confounding factors (i.e. EuroSCORE 2, age, glomerular filtration rate, and operation duration) presepsin remained an independent risk predictor.

Conclusion

Preoperative plasma presepsin level is an predictor of post-operative mortality and AKI in elective cardiac surgery patients, and is a stronger predictor than several other commonly used factors. Presepsin has proven as an independent risk indicator to predict outcome and may be used for risk stratification in patients scheduled for surgery already preoperatively.

A-124

Clinical Value of Bioactive Hepcidin-25, Soluble Transferrin Receptor and Their Ratio in Predialysis Patients Chronic Kidney Disease: Correlation with the Response to Intravenous Ferric Carboxymaltose

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Background: Anemia of inflammation, also known as anemia of chronic disease, is a complicating factor of a broad spectrum of inflammatory disorders, including chronic renal failure, autoimmune diseases, infections and certain cancers. Hepcidin is elevated in these conditions because of the increased production of cytokines (e.g. interleukin-6, BMP2), although in chronic kidney disease (CKD), decreased kidney clearance of hepcidin may also contribute. No reliable biomarker exists to predict responsiveness to intravenous (IV) iron (Fe) in iron deficient patients with CKD. We aimed to investigate the clinical value of bioactive Hepcidin-25 and soluble Transferrin Receptor (sTfR) levels in predialysis patients.

Patients and Methods: In this prospective study 78 stable stage III-IV CKD predialysis patients with (responders) and without (non-responders) adequate erythropoiesis after IV administration of ferric-carboxymaltose (FCM). Patients were divided in two groups according to their response to IV administration of ferric-carboxymaltose (FCM). Group R (responders) included 40 patients who had true iron deficiency and increased their Hg concentration by > 1g/dl from baseline. Group NR (non-responders) included 38 patients, who failed to respond. Along with measurements of common hematologic and blood chemistry parameters, determinations of sTfR (immunonephelometric technique, BN Prospec Nephelometer-Siemens Healthcare Diagnostics, Liederbach, Germany) and bioactive Hepcidin-25 (ELISA, DRG Instruments GmbH, Marburg, Germany) were performed.

Results: The main results of the study showed that: Hepcidin-25 levels (mean±SEM) were lower in the responders 1.4±1.2ng/mL (range from 0.5-33.6ng/mL) compared to non-responders 3.4±1.7ng/mL (range from 0.4-38.6ng/mL), (p=0.03), while sTfR and sTfR/Hepcidin-25 ratio were higher (p<0.01 and p=0.002 respectively). Diagnostic efficacy was analyzed by ROC analysis. Cut off point of 1.49 for Hepcidin-25 had sensitivity 84% and specificity 48%, while cut off point of 1.21 for sTfR/Hepcidin-25 ratio had sensitivity 82% and specificity 52% to predict correctly response to iron supplementation therapy. Furthermore, log sTfR/Hepcidin-25 correlated significantly with hs-CRP (r=-0.462, p=0.005) and IL-6 (r=-0.335, p<0.04) in non-responders, while such correlations were not found in responders (p>0.05).

Conclusions: These results suggest that lower Hepcidin-25, as well as higher sTfR and sTfR/Hepcidin-25 ratio were significant predictors of favorable hemoglobin response within a month after IV administration of FCM in patients with CKD. Further experiments and clinical studies in other groups of patients are needed to better elucidate the role of Hepcidin-25 and sTfR/Hepcidin-25 ratio as predictors of response to intravenous iron administration.

A-129

Do samples without HbA have dectected HbA1c?

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Background: Hemoglobin (Hb)A1c is a widely used biochemical marker for the management of diabetes mellitus and can be quantified by many methods. Some methods were reported least interference from those factors. However, those methods may present incorrect HbA1c results. The current study evaluated the five different HbA1c systems for five patients with no Hemoglobin A

Methods: Fresh no variants blood samples and 5 patients without Hemoglobin A were screened by capillary electrophoresis technique (Capillarys 2 Flex Piercing, Sebia), and identified by genetic sequence analysis. Those samples are analyzed for HbA1c by ion exchange HPLC(Variant II, Variant IITurbo 2.0, Bio-Rad), boronate affinity HPLC(Ultra2,Trinity Biotech), CE(Capillarys 2 Flex Piercing, Sebia), and Tinaquant immunoassay(Moddular PPI 800, Roche). The Bio-Rad Variant II(Bio-Rad, USA) has certified as NGSP Level I Laboratory and was used as Comparative method. The results of normal fresh samples for each method were used to eliminate any inherent calibration bias.

Results: The HbA1c values of normal samples obtained from VII-T 2.0, Ultra2,C2FP and PPI system were well correlated with VII system. Bias of each method to VII system were between -6% and 6% which met the standard of NGSP. But the samples without Hemoglobin A fraction that do not contain HbA1c analyte were unexpectedly obtained HbA1c results from different systems (Table 1). The VII and VII-T 2.0 randomly detected HbA1c of 2 and 3 samples without HbA; the C2FP system only detect HbA1c of the fifth patient with abnormal HbA2 value sign; the Ultra2 system and PPI system reported the HbA1c values of all samples

Conclusion: The evaluated HbA1c methods are affected to different extents by samples without HbA. Only VII,VII-T 2.0 and CE technique partially provides the abnormal sign in its profiles. The laboratories must be carefully selecting HbA1c analyzing methods and reporting HbA1c results in the higher Hb disorder prevalence area.

Table 1. The results of Hemoglobin electrophoresis and HbA1c for five blood samples

Genotype	HbA(%)	HbF(%)	HbA2(%)	Others(%)	Bio-Rad VII	Bio-Rad VII - T 2.0	Sebia C2FP	Trinity Biotech Ultra²	Roche PPI
αα/αα;$\beta^{CD26}/\beta^{CD41-42}$	0	45	5.1	49.9	-	-	-	4.30%	5.10%
								23mmol/mol	32mmol/mol
αα/αα;$\beta^{IVS2-654}/\beta^{New-York}$	0	1.5	5	93.5	4.30%	4.50%	-	4.10%	4.20%
					23mmol/mol	26mmol/mol		21mmol/mol	22mmol/mol
αα/αα;$\beta^{CD41-42}/\beta^{New-York}$	0	1	5	94	4.50%	4.60%	-	4.30%	4.80%
					26mmol/mol	27mmol/mol		23mmol/mol	29mmol/mol
αα/αα;$\beta^{CD41-42}/\beta^{E-Bangkok}$	0	0.6	4.6	94.8	-	4.70%	-	4.70%	3.80%
						28mmol/mol		28mmol/mol	-
--SEA/--$^{4.2-Q-Thailand}\beta/\beta$	0	0	0	100	-	-	3.7%	5.3%	5.7%
							-	34mmol/mol	39mmol/mol

A-130

Clinical lab data of High-Risk HPV genotyping

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Persistent infection by high-risk human papillomavirus (HRHPV) contributed to cervical cancer. Accurate HRHPV genotyping helps identify the women with real risk of developing cervical cancer. The Centers for Disease Control and Prevention (CDC) also calls HPV genotyping to evaluate efficacy of HPV vaccine. We developed a multiplex PCR to amplify E6, E7 or L1 region for each of all 13 HRHPV types, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Validation of the genotyping compared with FDA approved Digene Hybrid Capture 2 (HC2) yielded an analytical specificity of 100%, and an analytical sensitivity of 98.3%. The type specific amplification has been confirmed by DNA sequencing.

The test has been applied in our reference lab for 3532 reflex or duel cases in conjunction with pap cytology. Total of 549 HRHPV positives were identified, in which single infection (one genotype) is 408 cases, 74%; double infection (two genotypes) is 115 cases, 21%; triple infection or more is 26 cases, 5%; HPV 16 and/or 18 infection is 49%. Surprisingly, only 25% of total HRHPV positive cases are HPV 16 and/or 18, counted either as single infection or double infection, which will be covered by the quadruple HPV vaccine. Almost half of 16, 18, the two most common types, co-infect with other types.

In the 581 reflex atypical pap cases, 189 were identified as HRHPV positives (32.5%). Among the HRHPV positive cases, 18 were cervical cancer or high-grade squamous intraepithelial lesion (HSIL). Single HRHPV infection has been found in all 10 cancer cases (carcinoma in situ), including HPV 16 in 7 cases; HPV 18 in one case; HPV 52 and HPV 35 in the other two cases, respectively. Among the 8 cases of HSIL and/or atypical glandular cells (AGC), five were single infection including three HPV 16, one HPV 59, and one HPV 33; the remaining cases are multiple infections, with HPV 16 and 59; HPV 33 and 35; HPV 16, 39, 52 respectively. Among the positive HRHPV cases, 89 pap cytology graded CIN1 and above were tested HRHPV positive, yielding a clinical sensitivity of 100%. Referenced with the pap cytology result as standard, our genotyping test yielded a clinical specificity of 86.6%.

Only persistent infection of HRHPV contributes to the progression to cervical high-grade lesions and cancer, while most women can remove the transient HPV infection by their own immune system. Accurate HPV genotyping can distinguish the two scenarios, to identify the women who are really at high risk as persistently infected with specific HRHPV('s). By our HRHPV genotyping, multiple infections can be detected simultaneously, which is not practical by Hybrid Capture 2 assay or other commonly available tests. Approximately 25% of HRHPV positive patients were infected by multiple types according to our data. It has been noted that patients with multiple infections are at risk for treatment failure, which further validates the clinical significance of accurate genotyping.

A-131

Comparative Study of Jaffe Kinetic And Enzymatic Creatinine In Indian Origin

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Introduction: Jaffe kinetic method is the most commonly used for measurement of serum creatinine. It is cost effective and easy to perform. However it is affected by endogenous metabolites or exogenous substances. Enzymatic method is a better alternative where creatinine and derived metabolites are converted by the combined use of creatinase, creatininase and sacrosine oxidase; the liberated hydrogen peroxide is used to form colorimetric indicator.

Aims and objectives: To compare serum creatinine concentration obtained by Jaffe kinetic with enzymatic method, to standardize the reference range in Indian population and to assess the interference of bilirubin in measurement of the same.

Materials and methods: This prospective study was carried out over perioed of six months in semi urban locality. 122 individuals with age 19 to 75 years were included. Out of these 25 had normal kidney function, 72 were known cases of chronic kidney disease and 25 had altered hepatic function (serum billirubin 2 to 9 mg%). 2 cc blood sample was collected in plain bulb and serum used to estimate creatinine by Jaffe Kinetic Autospan and Meril india kits were used. For Enzymatic method AGAPPE reagents were used. All the methods were standardised as per the pack insert and the quality control tests were run on daily basis and validated externally. Tests performed on semi automated analyzer.

Results: Following results were noticed for Enzymatic and Jaffe for the above said groups of normal, chronic kidney disease, hepatic dysfunction. In the normal group comparison, enzymatic creatinine with the mean Patient value of 0.79 mg % ± 0.21 and Jaffe with mean Patient value of 0.86mg% ± 0.25 were yield the p value > 0.09 CKD Group Enzymatic creatinine with the mean patient value of 5.68 mg% ± 2.78 and Jaffe with mean patient value of 6.25 ± 2.81 yield the P value > 2.06 altered hepatic function patients with the mean Bilirubin of 5.33 mg% ± 7.28 yield enzymatic results 0.8 mg% ± 0.22 and Jafe 0.44 mg% ± 0. 45 with P value > 0.9

Reference range female 0.7 to 0.9 mg % Male 0.8 to 1.1 mg % The hypotheses with the P values > 0.05 clearly prove better utility of enzymatic creatinine method.

Conclusion: Enzymatic creatinine shows better accuracy and precision and ease of use. Reagent & Calibration stability is > 20 days, smaller volume of sample is required.

A-132

A Retrospective Review of Paraneoplastic Panel Utilization at a Tertiary-Care Academic Hospital to Determine Predictors of Diagnostic Yield

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Background: Despite the recent advances in understanding of paraneoplastic neurologic syndromes (PNS), no clinical guidelines for diagnostic evaluation of PNS currently exist. Recommendations for the work-up of PNS include radiologic, CSF, and electrophysiologic studies in combination with onconeural antibody detection. However no consensus on the integration of these studies into an algorithmic approach has been made. Our goal for this study was to determine if the patient's prior history of malignancy or results of radiologic, CSF, and electrophysiologic studies could predict the detection of onconeural antibodies and guide utilization review.

Methods: The results of all patients that had either serum or CSF paraneoplastic panel testing sent to a reference lab between February 2015 and February 2016 were reviewed. Chart review was conducted for all patients that had detectable onconeural antibodies and a subset of patients that did not. The following information was collected during chart review: age, sex, and presenting symptoms at time of testing; performance and findings of MRI/CT studies of the CNS, chest, and abdomen, electrophysiological studies, and CSF analysis including electrophoretic evaluation conducted prior to testing; prior or current history of malignancy; and treatment modification following reporting of results.

Results: A total of 97 serum and 24 CSF studies were sent for paraneoplastic panel evaluation over the one year timeframe. Antibodies were detected in 18 of 97 (18.6%) serum samples and 0 of 24 CSF samples. Eleven of the CSF studies had paired serum samples. The ordering preference for and increased sensitivity of serum analysis mirrors findings in the primary literature. All 18 patients with detectable onconeural antibodies and a random subset (n=15) of patients with negative results underwent chart review. The rate of performance of MRI/CT CNS, MRI/CT chest and abdomen, FDG-PET, EEG, EMG, and CSF analysis for patients that underwent chart review was: 65.6, 15.6, 9.4, 9.4, 59.4, and 59.4%, respectively. The rate of onconeural antibody detection among tests ordered by neurologists was 16.5% (n=97), whereas the rate for all other providers combined was 8.0% (n=24). The sensitivity and specificity of abnormal findings in CSF analysis to predict detection of onconeural antibodies were both 54.5% with no significant correlation between abnormal CSF findings and positivity for onconeural antibodies found by chi-squared analysis with Yates correction (X^2 1.66, p=0.44). The sensitivity and specificity of a prior history of cancer to predict detection of onconeural antibodies performed even worse at 5.6 and 34.6%, respectively. Investigation of treatment outcomes in the 18 onconeural antibody positive patients revealed that 5 (28%) were lost to follow-up, 6 (33%) had no follow-up with appropriate imaging studies, and 7 (39%) were followed appropriately. As of this time no malignancy has been detected in the patients that have undergone appropriate follow-up.

Conclusion: Our results demonstrate that history of prior malignancy and abnormal CSF studies do not predict detection of onconeural antibodies in patients being evaluated for PNS. CSF evaluation for PNS provides no increase in sensitivity in our patient population. There is significant variability among providers on follow-up of patients with positive results.

A-133

A qRT-PCR Diagnostic System to Monitor Toca 511, a Cancer-Selective Gene Therapy for the Treatment of Patients with Recurrent High Grade Glioma

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OBJECTIVE: This study determines if Toca 511 can be effectively detected in multiple body fluids to support Toca 5, a Phase 2/3 clinical trial in recurrent high grade glioma (NCT01470794).

RELEVANCE: Toca 511 (vocimagene amiretrorepvec) is an investigational retroviral replicating vector that encodes the transgene cytosine deaminase. Toca 511 may be delivered by multiple routes and selectively infects and spreads in tumor cells. Subsequent oral administration of investigational extended-release 5-fluorocytosine (Toca FC) results in formation of the antineoplastic drug, 5-fluorouracil, within infected tumors. 5-FU kills cancer cells and myeloid derived suppressor cells resulting in durable and selective immune activity against the cancer in preclinical models. Monitoring levels of this virus in bodily fluids is intended to detect non-tumor-specific viremia if it were to occur.

METHODOLOGY: Multiple manufacturing lots of Toca 511 were spiked into Tocagen Buffer (TB), Base Pool (BP; Siemens modified formulation of the SeraConII human plasma product from SeraCare), saliva (SV), or urine (UR). Viral RNA was purified from each diluent utilizing the Siemens VERSANT kPCR Sample Prep instrument and measured using a Siemens optimized RT-qPCR assay targeting the Toca 511 polymerase gene (*pol*). Differences in mean RNA recovery from matrices were tested using ANOVA on the mean quantitation cycle (Cq) +/- standard deviation. Due to viscosity differences, SV samples were diluted 4-fold in TB before processing. Stability after freeze-thaws and incubation at room temperature were also evaluated.

VALIDATION: There was no statistically significant difference observed for mean Cq in TB and BP (27.614 Cq +/- 0.614 in TB; 27.554 +/- 0.992 Cq in BP). Viral samples in UR generated higher Cq values (28.3743 +/- 0.379 Cq; $P<0.05$). After adjusting the mean Cq of SV samples for this additional dilution factor, SV was found to be equivalent to TB and BP. The effect of 1-3 freeze thaws and incubations at room temperature up to 24 hours did not affect mean Cq in any matrix.

CONCLUSION(S): Toca 511 virus is stable and detectable when spiked into various matrices. Lower recovery was observed for the virus in urine. Dilution of SV was required for automated processing due to increased matrix viscosity. These results indicate that Toca 511 levels may be accurately and efficiently monitored in multiple sample types from clinical trial subjects being treated for recurrent high grade glioma.

A-134

Comparison of Presepsin and Neutrophil Gelatinase-Associated Lipocalin in Predicting Acute Kidney Injury in Cardiac Surgery Patients

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Background Acute kidney injury (AKI) is a common complication after cardiac surgery. Neutrophil gelatinase-associated lipocalin (NGAL) has been reported to be a promising marker for cardiac surgery-associated AKI (CSA-AKI). Also sepsis has consistently been shown to be a contributing factor for the development of AKI. Presepsin has proven as a sepsis marker with high diagnostic and prognostic validity in assessment of disease severity and association to kidney function in septic patients.

Objective The aim of the present study was to evaluate the diagnostic validity presepsin and NGAL to predict CSA-AKI in patients undergoing elective cardiac surgery in comparison with inflammatory, cardiac and renal markers (CRP, procalcitonin (PCT), NT-proBNP, Cystatin C, and creatinine).

Methods The marker concentrations were measured in pre- and postoperative plasma samples which were drawn in the late afternoon before and the early morning after surgery from 235 patients undergoing elective cardiac surgery. Outcome measures were occurence of acute kidney injury (AKI) during hospitalization. Presepsin was determined by using the PATHFAST Presepsin assay (LSI Medience corporation,Tokyo). NGAL was measured by using the NGAL Rapid ELISA Kit, BioPorto Diagnostics A/S, Gentofte, Denmark. CRP, PCT, NT-proBNP, Cystatin C, and creatinine were measured using routine clinical chemistry methods in the central laboratory.

Results AKI has been assessed according to AKIN classification: stages 1 (n=41), 2 (n=21), 3 (n=9). Patients who developed AKI (n=71, *30.2%*) had higher pre- and postoperative presepsin and NGAL levels than patients without AKI. Both markers revealed higher levels postoperatively than preoperatively. The postoperative values of NGAL and presepsin exceeded the preoperative values 1.8fold and 1.7fold in patients without and 3.0fold and 1.9fold in patients with AKI, respectively, but these factors showed less discriminatory power than the marker levels. Receiver operator curve (ROC) analysis of postoperative values for prediction of AKI occurrence revealed AUC values of 0.813 and 0.828 for NGAL and presepsin, compared to AUC values of 0.808, 0.785, 0.636 0.624 and 0.529 for NT-proBNP, cystatin C, creatnine, PCT and CRP, respectively. Examination of the predictive value of marker combinations by logistic regression demonstrated superiority for the combined postoperative presepsin and NGAL values compared to all other possible combinations. The increased AUC of 0.856 showed that the simultaneous assessment of NGAL and presepsin performed better than the markers alone.

Conclusion Presepsin and NGAL demonstrated comparable predictive power to identify patients who were at risk of developing CSA-AKI. Moreover, the combination of both markers was found to improve the diagnostic performance. The simultaneous assessment of NGAL and presepsin allows early diagnosis of AKI already at the first day after surgery and may enable individual risk stratification with appropriate individualized patient care.

A-135

Plasma neuron derived exosomal protein biomarkers in the diagnosis of Alzheimer's Disease

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Background: Alzheimer's disease results in brain neuronal plaques composed of amyloid beta peptide (Aβ42) and neurofibrillary tangles composed of phosphorylated tau proteins (P-T181-tau and P-S396-tau). P-T181-tau and P-S396-tau are present at higher than normal concentrations and Aβ42 at lower than normal concentrations in the cerebrospinal fluid of AD patients. These proteins are not high in plasma samples of AD patients in part due to poor blood brain barrier transport and protease activities. Exosomes are shed by brain neurons, freely cross the blood brain barrier and protect and carry proteins from their cellular origin into plasma. We validated ELISA assays for Aβ42, P-T181-tau and P-S396-tau, and used them to quantify these proteins in neuron-derived exosomal extracts from normal and AD plasma samples.

Methods: Plasma samples were obtained from patients with mild cognitive impairment (MCI) and dementia due to Alzheimer's disease (AD), as well as matched normal controls. Exosomes were precipitated from the plasma samples using the ExoQuick preparation. Following centrifugation, they were suspended in a buffer containing protease inhibitors and phosphatase inhibitors. Antibody affinity purification with a solid phase mouse anti-human CD 171 antibody was used to enrich the content of neuron-specific exosomes. ELISA were validated for the biomarkers Aβ42, P-T181-tau and P-S396-tau including accuracy, precision, sensitivity, and specificity.

Results: ELISA assays for Aβ42, P-T181-tau and P-S396-tau were reproducible and the Inter-assay CVs were less than 15%. The sensitivity of the biomarker ELISAs varied from 2 - 10 pg/ml. Neuron-specific exosomes were prepared from the plasma of normal controls, MCI and AD patients. The reproducibility of the exosome preparations and biomarker levels were monitored in each ELISA. All biomarkers were elevated in MCI patients and AD patients compared to normal.

Conclusion: We have validated a reproducible procedure to isolate specific neuron-derived exosomes for quantification of specific protein biomarkers in plasma samples. The concentration of the biomarkers are high in patients with early dementia and Alzheimer's Disease. This procedure may be useful in the early diagnosis of Alzheimer's disease.

A-136

Noninvasive assessment of liver fibrosis staging using biomarkers in HCV carrier patients

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Background: Liver fibrosis involves excessive accumulation of extracellular matrix proteins (e.g., collagen) on liver cells, resulting in scar tissues. It occurs in most chronic liver disease, such as metabolic liver diseases and those associated with hepatitis B or C infection and alcohol consumption. Advanced liver fibrosis leads to cirrhosis, liver cancer, liver failure and portal hypertension. Currently liver biopsy is an optimal approach for detecting liver fibrosis and determining its severity. However, patient acceptance of this kind of examination is low, only less than 5% hepatitis patients are willing to perform biopsy, and thus many patients missed the appropriate treatment point. BioFibroScore® (General Biologicals Corporation, Taiwan) have been developed to predict liver fibrosis stages based on objective data from assays from three novel serum biomarker (urokinase-type plasminogen, matrix metal-loproteinase 9 and B-2-microglobulin) and the suitable algorithm. The present study aimed to analyze the accuracy of BioFibroScore® predicting liver fibrosis stages in HCV carrier patients.

Methods and results: A total of 100 frozen serum samples and their liver biopsy were provided by National Taiwan University Hospital, collected from HCV carriers. Numbers of these samples in each liver fibrosis stages were shown below: 12 samples of F0, 13 samples of F1, 25 samples of F2, 25 samples of F3 and 25 samples of F4. After analyzed by BioFibroScore® kits, we used two statistical strategies, logistic regression and SVM+KNN (suport vector machine + k-nearest neighbors algorithm), to construct prediction models that predict liver fibrosis stages of these 100 HCV carriers. The predicted results were compared with biopsy stages result. The accuracies with one stage tolerance of logistic regression model and SVM+KNN model were respectively 87% and 86%. The percentage of predicted liver fibrosis stages by logistic regression model lower and higher than biopsy stages were respectively 26% and 25%. The percentage of predicted liver fibrosis stages by SVM+KNN model lower and higher than biopsy stages were respectively 9% and 28%.

Conclusion: We obtained great accuracies by using these 100 HCV carriers samples to construct prediction models that could determine liver fibrosis stage. The potential clinical applications of BioFibrScore® are:

1. Reference for treatment decision
2. Monitor disease progression and prognosis
3. Outcome measurement for clinical trials.

A-138

CircRNAs in metabolic disease during pregnancy

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Background: Metabolic disease during pregnancy (MDP) includes mainly hypertensive disease of pregnancy (HDP) and gestational diabetes mellitus (GDM). Recently, it reported circular RNAs (circRNAs) may play important roles in the regulation of gene expression by acting as competing endogenous RNAs in diseases. So it may be a more effective early screening of circulating markers of disease. The lack of effective prediction scheme of MDP is the barrier of complications in prevention and management obstetrical severe cases. Here, we aim to investigate whether circRNA expression profiling can predict the metabolic disease during pregnancy before clinical diagnose.

Methods: A nested case-control study was performed at Guang Zhou Maternal-children Medical Center in 2015. Eighteen blood corpuscles were collected from groups (HDP, GDM and age and sample time matched control) before 24 weeks gestation (before clinical diagnose). The expression profile of circRNAs in blood corpuscles was performed using a human circRNA microarray which designed to simultaneously detect 5396 circRNA(Fig. 1).s. The express data of circRNAs was analyzed by volcano plot and heat map. The enrichment analysis and pathway annotation of these circRNAs' target genes were respectively done by DAVID.

Results: Volcano plot analysis shows the differentially expressed circRNAs among three groups using the parameters (P<0.01, fold change >50). We found that 293 circRNAs were differentially expressed among the three groups, in which 4 circRNAs were down-regulated and 15 circRNAs were up-regulated in the HDP group; 59 circRNAs were down-regulated and 215 circRNAs were up-regulated in the GDM group (Fig. 2). GO analysis of the circRNAs in the MDP group showed significant enrichment of biological processes, such as cell process, biological regulation (cell death, regulation of apoptosis, immune response, positive regulation of developmental process, positive regulation of cell differentiation, and regulation of angiogenesis). ; signaling (cell surface receptor linked signal transduction). The result of hierarchical clustering shows a distinguishable circRNA expression profiling among HDP, GDM and control group (Fig. 3).

Conclusion: CircRNAs may participate in the pathogenesis of MDP by involving in molecular function of ion binding, protein binding; glucuronosyltransfer et for HDP and of binding; cytoskeletal protein binding; Small GTPase binding; Ras GTPase binding,heterocyclic compound binding;organic cyclic compound binding et for GDM. HDP and GDM are distinguishable from gravidas by circRNA expression profiling of blood corpuscles before clinical diagnose. However, the results are preliminary and need to be validated in larger studies and other population. Here, hierarchical clustering was performed based on "All Targets Value - CircRNAs". The experiment consists of 18 different samples. The result of hierarchical clustering shows a distinguishable circRNA expression profiling among samples.

A-139

Combination of Urinary Liver-Type Fatty-Acid Binding Protein and Albumin Improves Prediction of Acute Kidney Injury in Patients Hospitalized to CCUs

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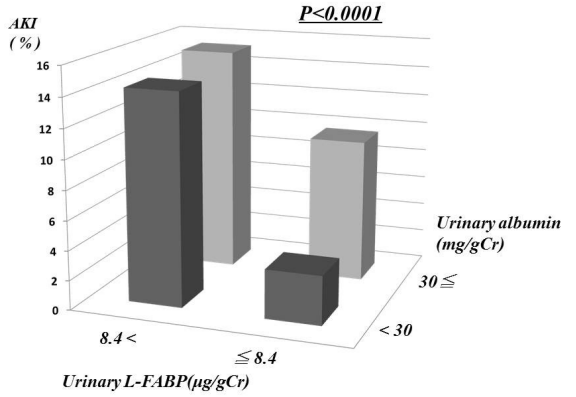
Background: Acute kidney injury (AKI) detected after admission to CCU is associated with very poor outcomes. Urinary albumin concentration reflects glomerular damage, and urinary concentration of liver-type fatty acid-binding protein (L-FABP) reflects tubulointerstitial damage.

Methods: We prospectively investigated the predictive value of combined urinary L-FABP and albumin on admission for AKI in 1,119 patients (mean age, 71 years) hospitalized to CCUs.Among these patients, 60% had heart failure; and 39.3%, acute coronary syndrome (ACS). AKI was defined as an increase of >50% in creatinine from baseline or an absolute increase of ≥0.3 mg/dL within 48 h after admission.

Results: AKI was detected in 177 (16%) patients. Multivariate logistic analysis identified urinary L-FABP (p=0.03) and albumin (p=0.002) as independent predictors of AK. Combined urinary L-FABP and albumin was associated with AKI incident rates (**Figure**).

Conclusion: The combination of urinary L-FABP and albumin could improve the prediction of AKI in patients hospitalized to CCUs.



A-140

The Discordance Between Serum and Vitreous Vascular Endothelial Growth Factor Levels in Proliferative Diabetic Retinopathy

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Background: In proliferative diabetic retinopathy (PDR), neovascularization occurs via hypoxia-induced vascular endothelial growth factor (VEGF), which is thought to be the major angiogenic agent. Vitreous VEGF level in eyes with PDR has been found higher than that in eyes with non-proliferative diabetic retinopathy (NPDR). There are conflicting results, however, regarding serum VEGF level in patients having PDR. Some studies indicate higher serum VEGF concentrations while others report that serum VEGF levels are not important. The objective of the present study was to find correlations between the vitreous and serum levels of VEGF in patients with type 2 diabetes mellitus that developed PDR.

Methods: Totally 88 subjects (63 diabetic and 25 non-diabetic) were enrolled. Diabetic patients were subdivided into 3 following categories: no diabetic retinopathy (noDRP, n=20), non-proliferative diabetic retinopathy (NPDR, n=20) and proliferative diabetic retinopathy (PDR n=23). Pars plana vitrectomy was performed with PDR while 15 out of 25 non-diabetics underwent vitrectomy for various reasons. Vitreous and serum samples were obtained during the vitrectomy procedures. Only serum samples were collected from the remaining participants. Serum and vitreous VEGF levels were analyzed using enzyme-linked immunosorbent assay (ELISA) by using Human VEGF-A Platinum ELISA kit from (BMS277/2, EBioscience). Total vitreous proteins and total serum proteins were also measured. The results were statistically compared. Statistical significance was accepted as P<0.05.

Results: There was significant difference for serum VEGF levels (P<0.001) and VEGF/total protein ratio (P<0.001) among 3 diabetic groups and non-diabetic control group. Higher vitreous VEGF levels were observed in eyes with PDR compared to non-diabetics (524.19±492.90 pg/mL [mean±standard deviation] *vs.* 97.97±108.72 pg/mL, P<0.001). The difference was still remained after correcting it for total vitreous protein levels (VEGF/total protein ratio) (P=0.001). Contrarily, serum VEGF levels did not differ between PDR and non-diabetic control group (493.15±372.02 pg/mL *vs.* 336.88±321.35 pg/mL, P=0.122). In non-diabetics, VEGF level in vitreous was significantly lower than that in their serum (P=0.001) while serum and vitreous VEGF levels were similar in subjects with PDR (P>0.05). However, there was no correlation between serum and vitreous VEGF levels of PDR and non-diabetic control group (P>0.05 and P>0.05, respectively).

Conclusion: Vitreous VEGF levels did not correlate with serum VEGF levels neither in patients with PDR nor in non-diabetics. It seems that the disruption of blood-retinal barrier was not the only part of proliferative retinopathy process. Elevated vitreous VEGF levels were mostly resulted of endogenous VEGF released from hypoxic retina. The increased VEGF level in serum may not be used as a significant marker for PDR development.

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A-141

Does heat shock protein 70 play a role on pathogenesis of proliferative diabetic retinopathy?

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Background: Diabetic retinopathy is the most common cause of blindness in productive age group of developed societies. Proliferative diabetic retinopathy, the severe stage of the pathology, manifests itself with retinal neovascularization and vitreoretinal proliferation. The exact mechanism of proliferative diabetic retinopathy has not yet been fully identified. An impaired antioxidant defense system probably contributes to the condition. The objective of the study was to find possible correlations between vitreous humor and serum levels of heat shock protein 70 (Hsp70) in eyes with proliferative diabetic retinopathy and compare them with non-diabetic subjects.

Methods: Overall 63 diabetic patients and 25 non-diabetic control subjects were enrolled in the study. Diabetic patients were divided into following 3 sub-groups according to their stage of retinopathy: no retinopathy (n=20), non-proliferative diabetic retinopathy (n=20) and proliferative diabetic retinopathy (n=23). Twenty-two eyes of 20 patients with proliferative diabetic retinopathy complicated with tractional retinal detachment and intravitreal hemorrhage as well as 15 eyes of 15 non-diabetic control group patients having macular hole, epiretinal membrane, rhegmatogenous retinal detachment underwent pars plana vitrectomy procedure. Vitreous humor and serum samples were collected from 37 patients at the time of vitrectomy. Serum samples were obtained from the rest of the subjects. The levels of Hsp70 in vitreous and serum were analyzed using enzyme-linked immunosorbent assay (ELISA). For this detection, Hsp70 high sensitivity ELISA kit was used (ADI-EKS-715, Enzo Life Sciences). Total vitreous proteins and total serum proteins were also measured. Kruskal-Wallis, Mann-Whitney U and Spearman correlation tests were performed. Statistical significance was set to $P<0.05$.

Results: There was a significant difference among 3 diabetic sub-groups and control group for serum Hsp70 levels ($P=0.024$) and for Hsp70/total protein ratio ($P=0.013$). Eyes that underwent vitrectomy for proliferative diabetic retinopathy had a significantly higher serum Hsp70 level (mean±standard deviation) compared to those of non-diabetic control group (201.06±80.44 pg/mL *vs* 85.43±27.47 pg/mL, $P=0.003$). Similar tendency was not observed between eyes with proliferative diabetic retinopathy and non-diabetic controls for vitreous Hsp70 level (149.79±56.60 pg/mL *vs* 184.41±154.40 pg/mL, $P=0.546$), and neither after adjusting it for total vitreous protein levels (Hsp70/total protein ratio) ($P=0.353$). In eyes with proliferative diabetic retinopathy, Hsp70 levels in serum and vitreous humor was similar ($P>0.05$). No correlation was found between serum and vitreous Hsp70 levels in non-diabetic control subjects ($P>0.05$).

Conclusion: The increased Hsp70 level in serum may be used as a significant marker for proliferative diabetic retinopathy development and may contribute to the elucidation of the pathogenesis of proliferative diabetic retinopathy.

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A-145

Multicenter characterization of a fully automated electrochemiluminescence immunoassay for the quantitation of serum periostin, a promising biomarker for guiding treatment in Type 2 asthma

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Objective: Periostin is a biomarker that may be effective in guiding treatment for Type 2 asthma with the drug lebrikizumab. This study aimed to characterize the Elecsys® Periostin immunoassay on the cobas e 601 analyzer under field conditions in 3 routine (field) laboratories. **Relevance:** Asthma, a chronic inflammatory airway disease with an increasing worldwide incidence, is a heterogeneous disorder that has at least two distinct molecular phenotypes defined by degree of Type 2 inflammation. The latter is caused by various cytokines, including the multifunctional and pleiotropic mediator IL-13. The physiological importance of IL-13 has made it a treatment target for Type 2 asthma using the monoclonal antibody lebrikizumab, and Phase II studies have indicated that lebrikizumab improves outcomes in patients identified with moderate-to-severe uncontrolled Type 2 asthma. Identifying Type 2 patients who will likely benefit from lebrikizumab treatment is important. Periostin is a surrogate biomarker of IL-13 activity in the airway that helps pinpoint asthma patients who are most likely to benefit from lebrikizumab therapy; periostin has potential for guiding therapy and improving both clinical and economic value of lebrikizumab treatment. However, for periostin to be used for guiding lebrikizumab therapy, an assay with potential for wide availability must be properly validated. **Methods:** Repeatability, intermediate precision, reproducibility and lot-to-lot variability were assessed according to CLSI-EP5-A3 guidelines, using sample panels consisting of 11 human serum samples and 3 concentration levels of quality control materials (14 samples in all) at 3 field laboratories. Materials were shipped to the 3 sites; each site used the cobas e 601 system (Roche Diagnostics) and a combination of 2 out of 3 available assay lots for measurements. The 11 individual sample pools and 3 control levels were run in randomized order. Study design included 1 run per day for each lot, with 5 replicates of each sample over 5 separate days. The total reproducibility and variance components were calculated for each of the samples and expressed as %CV using variance components analysis that included site, lot, day, run and within-run precision. **Validation:** For all 14 samples analyzed, reproducibility ranged between 1.7% and 3.1%. Intermediate precision was 1.2-1.7% CV. Repeatability ranged between 0.9% and 1.5% CV. **Results and Conclusion:** The Elecsys® Periostin immunoassay on the cobas e 601 system demonstrated the specified level of reproducibility in the field, with %CV values of <3.1% that are required for effective use in guiding lebrikizumab treatment. Lot-to-lot variability was satisfactory at 0.6–2.5% CV. The Elecsys® Periostin immunoassay has been used in the lebrikizumab Phase III LAVOLTA trials (NCT01867125/NCT01868061) to classify patients as periostin low (<50ng/ml) or periostin high (≥50ng/ml) based on pretreatment levels. This cut-off was previously established in the lebrikizumab Phase II study MILLY (NCT00930163) and confirmed in the Phase IIb studies LUTE (NCT01545440) and VERSE (NCT01545453). Our findings indicate that the Elecsys® Periostin immunoassay has the appropriate performance characteristics for use in identifying patients who may benefit from lebrikizumab treatment. **Disclaimer:** This product is not cleared or approved for use in the USA or elsewhere.

A-146

Evaluation of Astute Medical NEPHROCHECK © Test System as a risk assessment device for moderate to severe acute kidney injury (AKI)

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Background: Acute kidney injury (AKI) is defined as abrupt decrease in kidney function as measured by an increase in serum creatinine or decrease in urine output. However in the non-steady state setting of AKI, serum creatinine does not accurately reflect the glomerular filtration rate (GFR), and may lead to a delay in the diagnosis of AKI. Additionally, serum creatinine often does not increase until kidney function has declined by at least 50%. AKI is independently associate with increased morbidity and mortality in critically ill intensive care unit (ICU) patients. A sandwich immunoassay has been developed to measure urinary concentration of insulin-like growth factor binding protein 7 (IGFBP-7) and tissue inhibitor of metalloproteinase 2 (TIMP-2). These markers have been shown to be elevated upon renal tubular injury and prognosticate the future development of AKI. The objective of this study was to evaluate the performance of this new assay for risk assessment of human AKI.

Methods: The study examined urine samples of 43 patients who are 21 years of age or older without gender specification. The inclusion criteria for this study are at least one of the following two acute conditions within 24 hours prior to enrollment: 1.) Respiratory sepsis-related organ failure assessment (SOFA) score of ≥ 2 (PaO_2/FiO_2 <300). 2.) Cardiovascular SOFA score of ≥ 1 (MAP < 70 mm Hg and/or any vasopressor required). Patient had not been diagnosed with AKI or had stage 1 AKI (50% or 0.3 mg/dL increase in serum creatinine from baseline) based on the Kidney Disease Improving Global Outcomes (KDIGO). During evaluation, NEPHROCHECK® Calibration Verification Kit was used as linearity material and Liquid Control Kit for precision study. Both kits contain TIMP-2 and IGFBP7 markers used to derive the AKIRISK™ Score.

Results: The Astute Medical NEPHROCHECK® Test System demonstrated good linearity for the calculated measurement of IGFBP-7 and TIMP-2 concentration converted to a risk score range of 0.06-6.21 $(ng/mL)^2/1000$ with an $R^2>0.999$. The total precision displayed CVs at <15% for all QCs. Interference study showed assay was not affected by hemolysis index up to 200 mg/dL and bilirubin index up to 16 mg/dL. Comparison between two NEPHROCHECK® devices showed the equation $y= 1.03 x + 0.005$ ($R^2> 0.99$, $n= 43$), with an acceptable overall bias of 0.05.

Conclusion: The data demonstrates good analytical performance of precision and correlation and is acceptable for clinical implementation.

A-147

BIOCHEMICAL EVALUATION OF SEMINAL PLASMA IN AZOOSPERMIC SAMPLES

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Background: Infertility is considered one of the main public health issues, as it affects about 15% of the couples of reproductiveage. The male factor is involved in 40% - 50% of infertility cases and azoospermic men constitute approximately 10 to 15% of all infertile men . The aim of our study is to determine the relationship between biochemical parameters in seminal plasma and the complete absence of sperm in the ejaculate.

Methods: Prospective study, patients older than 18 years who asked for a spermiogram were included, those who did not follow the pre-analytical guidelines recommended by the WHO and lack of Informed Consent were excluded. Patients were classified into two categories: one group with presence of sperm in the ejaculate and the other group with azoospermia. Several biochemical parameters (glucose, creatinine, urea, AST, ALT, CK, lactate dehydrogenase, CRP, total protein, alkaline phosphatase, Na, K, chloride, calcium, cholesterol) and hormones (TSH, testosterone, FSH, LH, prolactin, cortisol) were determined in the seminal plasma and serum on the COBAS MODULAR 711 (Roche Diagnostic). **Results:** Forty-six men with a mean age of 36.65 (range between 23 and 54) years were studied. Six cases had azoospermia. Azoospermia patients had significantly lower levels of urea 54 (42-55) mg/dl vs. 62 (58 to 74.5) mg/dl; p=0.026, ALT 32 (8-39) IU/L vs. 47 (38-58) IU/L; p=0.013 and AST 172 (131-192) IU/L vs. 285 (231-357) IU/L; p=0.01. TSH was significantly higher in the azoospermic samples 0.14 (0.12-0.16) mU/L vs. 0.08 (0.052-0.1) mU/L; p=0.008. The serum parameters did not correlate with the seminal plasma ones. The following table shows the areas under the ROC curves (AUC) and the optimal cutoff points with corresponding sensitivity and specificity.**Conclusion:** Decreased urea, ALT and AST levels and high TSH value in seminal plasma may predict the presence of azoospermia.

	AUC (CI 95 %)	Cutoff	Sensitivity (CI 95 %)	Specificity (CI 95 %)
Urea	0.892 (0,765-0,964)	55 mg/ml	100% (100-100)	85% (70.2-94.3)
ALT	0.860 (0.722-0.945)	39 UI/L	100% (100-100)	73.7 % (56.9-86.6)
AST	0.925 (0.808-0.981)	192 UI/L	100% (100-100)	85 % (70.2-94.3)
TSH	0.948 (0.823-0.992)	0.1 mU/L	100% (100-100)	81.3 % (63.6-92.7)

A-148

S100B protein as serum marker of brain damage after general anesthesia

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Background. S100B protein is a serum marker of cerebral damage. The objective was to evaluate the brain damage caused for general anesthesia, by determining the concentration of serum S100B protein before and after of general anesthesia. **Methods.** Patients with chronic adenotonsillar hypertrophy and indications for tonsillectomy were included. Venous blood sample was taken from patients before general anesthesia (basal sample). The patients were anesthetized using the following intravenous anesthetic drugs: midazolam, fentanyl and propofol; and sevoflurane inhaled. Second venous blood sample (sample postoperative) was taken from patients after the surgery, in the operating room. The concentration of serum S100B protein was determined in the basal sample (S100Bb) and postoperative sample (S100Bp) by immunoassay electro-chemiluminescence in MODULAR E-170 (Roche Diagnostics®). **Results.** 76 patients were included, 46 males and 30 females, with age between 3 to 14 years (median = 5 years). Descriptive statistics are showed in following table (CI: confidence interval; IR: interquartile range):

	Lowest	Highest	Median (95% CI)	IR
S100Bb (ng/L)	41,0	205,0	94,5 (89,0-103,0)	41
S100Bp (ng/L)	57,0	1052,0	164,0 (145,0-189,0)	149
S100Bp-b (ng/L)	7,0	955,0	58,0 (42,0-75,0)	136

In all patients, serum S100B protein levels have increased after general anesthesia. The values of S100Bp (median = 164.0 ng/L) were significantly higher than values of S100Bb (median = 94.5 ng/L). The median of difference between S100Bp and S100Bb was 58.0 ng/L. There were statistically significant differences between S100Bb and S100Bp using the Wilcoxon test (p<0.0001). **Conclusions.** The concentration of serum S100B protein has increased significantly after general anesthesia. This indicates that general anesthesia may cause brain damage.

A-151

Stabilization of glucose concentration in the new VACUETTE® FC Mix blood collection tube for diagnosis of gestational diabetes

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Background: Reliable detection of Gestational Diabetes Mellitus (GDM) is required to prevent maternal and fetal complications. With transport times of up to 48h, the aim of the study was to demonstrate long term stability of initial glucose concentration in specimens centrifuged directly after collection and compared to whole blood specimens stored at room temperatures. Recent guidelines from the German Diabetes Association recommend use of tubes with an additive composed of citrate, EDTA and sodium fluoride, as in VACUETTE® FC Mix tubes, to effectively stabilize glucose levels for the diagnosis of GDM due to the incomplete inhibition of glycolysis by sodium fluoride alone.

Methods: The current study was conducted at ISALA Hospital (Zwolle, Netherlands) using VENOSAFE™ FC Mixture® versus VACUETTE FC Mix blood collection tubes. Altogether, 43 pregnant donors who were healthy (n=19) or diagnosed with gestational diabetes by 75g-Oral Glucose Tolerance Test (n=24) were recruited. Informed consent was given by all donors and the study was approved by EC Netherlands. Venous blood was drawn from each donor into four tubes (two tubes each tube type). One tube of each type was centrifuged directly after blood collection according to manufacturer recommendations and the second one after whole blood storage for 48h at room temperature. Following collection, plasma was measured immediately after centrifugation to obtain initial values (fasting) and after 48h for evaluation of glucose stability using the Hexokinase method on a COBAS 8000 (Roche Diagnostics, Mannheim, repeatability VC 1%, total precision VC 1.7%). Statistical evaluation was done by STATISTICA 12.

Results: Evaluation of all clinical results for glucose concentration and any deviations was done on the basis of maximal allowed deviation for a single value (for glucose 11%) according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of both tubes for performance testing did not reveal any clinically nor statistically significant deviations (p<0.05). The values of both tubes resulted in an initial highest deviation of 5.5%, and 6.4% after 48h (both healthy). Comparable highest deviations for initial values in relation to 48h values were obtained for VENOSAFE and VACUETTE tubes with 5.4% (healthy) and 6.6% (GDM), respectively. The storage of whole blood specimens for 48h showed no significant deviation (10.5%, healthy).

Conclusion: Based on these results, the VACUETTE FC Mix blood collection tube is suitable for reliable determination of blood glucose, one of the most frequently measured laboratory analytes and of primary importance in diagnosis, monitoring and therapy of GDM. The stability of glucose concentration in whole blood specimens drawn in the VACUETTE FC Mix tube and stored up to a 48h at room temperature has been shown. Use of this tube will improve the stability of glucose with extended transport times up to 48h, which is more common with centralization of laboratory testing, negate the need for sample aliquotting and allow for good obstetric practice.

A-152

Use of Selected Clinical Laboratory Tests - Claims Data from a Sample of Commercially Insured and Medicare Supplemental Enrollees, 2009-2014

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Background: Identifying reimbursement changes for specific clinical laboratory tests may provide information to assess the effectiveness of test recommendations to screen for or treat diseases, inform practice guidelines, and provide needed information for health policy decisions. Laboratory reimbursements rates for Medicare Part B enrollees in 2000-2010 for the most commonly ordered clinical laboratory tests were previously evaluated (Shahangian S, et al. *Arch Pathol Lab Med*. 2014;138:189-203). However, that study did not include the younger, commercially insured U.S. population. The objective of this study was to provide information useful for more effective implementation of evidence-based recommendations by evaluating test ordering trends of specific clinical laboratory tests or test panels in 2009-2014. Methods: Claims data were collected using Truven Health Analytics' MarketScan databases for commercial claims and encounters and Medicare Supplemental in 2009-2014 (92 million). These populations constituted an average of ~30% of privately insured U.S. population, as well as ~5% of Medicare enrollees. The ratio of the most recent (2014) divided by the oldest (2009) reimbursed test utilization rate (rate ratio or RR) was used as a measure of trend. A two-sided Poisson regression, adjusted for potential overdispersion, was used to determine *P* for trend. Trends were considered significant at $P < 0.050$. Results: Of the 77 laboratory tests and 6 test panels examined, 59 (77%) showed very significant ($P < 0.0001$) trends; and in all but five of them utilization rate increased. Exceptions were tests for myoglobin (RR, 0.62), phenytoin (RR, 0.64), electrolytes (RR, 0.68), carbohydrate antigen (CA) 125 (RR, 0.83), and carbamazepine (RR, 0.86). There were also less significant downward trends for two tests with RR < 0.85: digoxin (RR, 0.76; $P = 0.002$) and cervical cytology (RR, 0.83; $P = 0.0003$). The greatest RR values were seen for testing of drugs with potential for abuse or overuse from ethanol (RR, 2.31) to nicotine (RR, 15.09), $P < 0.0001$. Seventeen other tests showed significantly increasing use over time (RR, ≥1.50; $P < 0.0001$): natriuretic peptide (RR, 1.50; $P = 0.0005$), human papilloma virus (HPV) DNA (RR, 1.52), D-dimer (RR, 1.53), IgM (RR, 1.59), herpes in women (RR, 1.65), folic acid (RR, 1.67), herpes in men (RR, 1.75), free PSA (RR, 1.76), glycohemoglobin (RR, 1.76), vitamin B-12 (RR, 1.84), testosterone in men (RR, 1.97), fibrinogen (RR, 1.99; $P = 0.001$), hepatitis C virus antibody (RR, 2.18), chlamydia in men (RR, 2.29), gonorrhea in men (RR, 2.35), vitamin D (RR, 2.49), and apolipoproteins (RR, 3.11). Conclusion: These results show changes in utilization practices for laboratory tests over a 6-year time frame, with some trends likely to have been positively impacted by evidence-based recommendations such as decreasing CA 125 screening for ovarian cancer, while others showing that recommendations have not been as effective such as increasing vitamin D testing. Given that some laboratory utilization trends do not follow evidence-based recommendations for certain laboratory tests, there is a need to understand the underlying factors so that measures can be adopted to promote better laboratory utilization practices.

A-153

Stabilization of organisms in the VACUETTE® CCM urine collection tube for diagnosis of urinary tract infections

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Background: Reliable test results are of utmost importance for diagnosis, monitoring and therapy of patients with urinary tract infections (UTI), one of the most common healthcare associated infections. With regard to delays in delivery to the laboratory, an increase in microbial counts due to lack of preservative or inappropriate transport temperatures may lead to false results. The new VACUETTE® Urine CCM Tube contains a novel preservative that stabilizes urine samples at room temperatures (20-25°C) for up to 48 hours making it appropriate for collection, transport, storage and microbiology testing. Urine culture results showing microbial counts of $\geq 10^5$ CFU/ml are indicative of a UTI. Counts below usually indicate contamination of the urine sample.

Methods: A study was designed to evaluate the stability of organisms (bacteria and yeast), which occur frequently in urine, for a storage period of up to 48h at room temperature relative to initial counts in the specimen. Urine samples (n= 170, partly spiked) from clinically inconspicuous and conspicuous (nitrite and leucocyte positive with dipstick urinalysis) specimens were used. Midstream urine was collected from healthy subjects and clinical patients using a urine beaker (100ml) with integrated transfer unit. All specimens were transferred to the CCM tube and examined within 2 hours (initial), 24h and 48h after filling. On the basis of adjusting the McFarland, spiked-in urine samples were prepared with a target concentration of 10^3 CFU/ml (low) and 10^5 CFU/ml (high) from 120 healthy subjects. The organisms were selected based on CLSI, M40-A. Samples were assessed for stability of the following pathogenic organisms: *Escherichia coli (ATCC®25922), Enterococcus faecalis (ATCC®29212), Pseudomonas aeruginosa (ATCC®BAA-427), Staphylococcus saprophyticus (ATCC®15305), Proteus mirabilis (ATCC®7022),* and *Candida albicans (ATCC®24433)*. Agar test plates were innoculated with 10µl of urine and incubated for 24 to 48h at 37°C, followed by visual counting of colonies. Counts were recorded as CFU/µl. This innoculation method yields a detection limit of 0.1 CFU/µl, equivalent to 100 CFU/ml.

Results: Of all clinically inconspicuous urine specimens, 26 contained <1,000 CFU/mL at the initial time point, 15 showed a bacterial count between 1,000-10,000 CFU/mL, 6 between 10,001-100,000 CFU/mL, and 3 specimens >100,000 CFU/mL. Of all clinically conspicuous urine specimens, 18 contained <1,000 CFU/mL at the initial time point, another 8 between 1,000-10,000 CFU/mL, 2 between 10,001 - 100,000 CFU/mL, and 22 specimens >100,000 CFU/mL. None of the tubes showed a significant deviation in relation to the initial bacterial count. Additionally, none of the urine tubes spiked with facultative pathogenic organisms in either concentration ($1*10^3$ CFU/mL or $1*10^5$ CFU/mL) showed significant deviation.

Conclusion: These results demonstrate the suitability of the VACUETTE Urine CCM Tube for microbial testing. This tube stabilizes the tested organisms 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 improves preanalytic variables in urine culture.

A-155

Modified fasting glucose cutpoints reduce un-necessary tolerance testing in pregnant women from a large urban and rural population

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Background: Calgary Laboratory Services (CLS) is the sole provider of laboratory testing for Calgary and surrounding communities in Alberta, Canada (>1.4 million people). Glucose tolerance testing is performed by CLS at outpatient laboratories in hospitals and patient service centers (PSCs) in Calgary, rural hospitals and community health centers. At each site, the 75 gram glucose drink is administered only if a fasting glucose is <7.8 mmol/L unless otherwise requested. This is because a fasting glucose ≥ 7.0 mmol/L combined with clinical symptoms is compatible with a diagnosis of diabetes. This decision is made by rapid fasting venous glucose at all sites except PSCs, where a capillary glucose by glucometer is used to decide whether to proceed while a venous sample is sent for later testing at a central lab. Glucose tolerance testing (75 gram) is performed in pregnant women if their gestational diabetes (GDM) screening test is inconclusive (1-hour glucose post 50 gram load = 7.8-11.0 mmol/L). GDM is diagnosed by either a fasting glucose ≥ 5.3 mmol/L, a 1-hour glucose ≥ 10.6 mmo/L, or a 2-hour glucose ≥ 9.0 mmol/L. Reducing the fasting cutpoint value from 7.8 to 5.3 mmol/L would reduce un-necessary testing and potential harm. However because diagnosis cannot be made by glucometer alone, a glucometer cutpoint must be chosen with adequate sensitivity and specificity for diagnosis by the fasting venous result which is returned later. Our objectives were to determine how many pregnant women will be saved from glucose tolerance testing by (1) changing the fasting cutpoint from 7.8 to 5.3 in urban hospitals and rural sites, and (2) selecting a glucometer cutpoint with a false positive rate that would minimize the number of patients required to repeat the tests. **Methods:** Two years of glucose tolerance testing results (venous fasting glucose, 1-hour glucose, 2-hour glucose) for pregnant women were extracted along with accession number from the CLS lab information system. The number of GDM diagnoses made by diagnostic criteria were determined. Venous results were linked by accession number to a fasting glucometer result from a separate server. Receiver operating characteristic (ROC) curve analysis was used to select a glucometer cutpoint with a false positive (GDM) rate of 1 patient per month.

Results: Across CLS, 4400 GDM tolerance tests were performed annually resulting in 1314 GDM diagnoses, 409 (31%) of which were by fasting glucose alone. As 11% of tests were performed at urban hospitals and rural sites, approximately 45 patients would be saved annually from testing by changing the fasting cutpoint to 5.3 mmol/L. ROC analysis of PSC capillary (glucometer) glucose and GDM diagnosis by fasting venous glucose yielded a c-statistic of 0.97. A glucometer cutpoint of ≥ 5.8 mmol/L resulted in a sensitivity of 42% and specificity of 99.7%, which if implemented would save 153 patients annually from having to undergo tolerance tests, and result in 1 patient per month having to be re-tested.

Conclusion: Modifying fasting glucose cut-points for pregnant women will eliminate un-necessary tolerance testing in 198 patients annually, resulting in only 1 re-test per month.

A-157

A Comparison Between Hemolyzed Specimen Rejection Rates and Blood Collection Techniques In Emergency Department

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Background:

The employment of industry standard blood collection techniques greatly impacts the quality of patient care (QPC) delivered at medical facilities, as improper techniques can lead to hemolysis thus decreasing the accuracy of clinical results by producing various testing interferences[1]. Moreover, QPC is affected through subsequent re-draws leading to an increase in patient turnaround time. The objective of this study was to identify the primary *in vitro* factors resulting in the hemolysis of blood samples from the Emergency Department (ED) at Southlake Regional Health Centre (SRHC). This was accomplished through a two phase study in which initial retrospective analysis of rejected specimens' database was carried out to determine the rate of rejection due to hemolysis in the ED compared to other in-patient units, followed by observation of techniques deemed through literature to be factors that may result in hemolysis of blood samples[1].

Methodology:

Collection date and time, ordering unit, patient accession number and reason for rejection were extracted from the laboratory information system of Southlake Regional Health Centre (March to June 2015). The rate of rejection due to hemolysis was compared between the ED and other in-patient units followed by development of an observation checklist which included observations for: phlebotomy site, equipment used, needle gauge, length of tourniquet placement, number of punctures, filling of tubes, degree of mixing, and mechanical trauma. Observations of 114 randomly selected phlebotomy procedures were conducted in the ER from July 18 to August 25 followed by an analysis to determine correlation between the observed techniques and hemolysis of blood samples.

Results:

Of the 274 specimens rejected due to hemolysis from March to June 2015, 191 were rejected due to hemolysis in the ED (70%). Of the 114 randomly selected phlebotomy observations in the ED, 27 produced some degree of in-vitro hemolysis (24%). The greatest correlation was found between the use of 20 gauge IV catheters for blood collection and the presence of hemolysis in the blood sample. Use of IV catheters observed to produce 67% of hemolyzed samples (n=18), remaining 33% of blood samples were collected using straight or butterfly needles (n=9). All other factors were not found to correlate significantly with the presence of hemolysis in the blood samples.

Conclusion:

The results of the study support previous findings indicating that collection through IV catheters is the leading cause of hemolysis[2]. Other factors were determined to be insignificant contributors to the high rate of hemolysis in the ED at SRHC, providing insight to the possibility that the leading *in vitro* causes for hemolysis varies between hospitals as a result of differing blood collection techniques. Future research can be aimed at studying the components of the IV catheter and their potential effects on hemolysis, in order to improve QPC.

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A-159

Monocyte Chemoattractant Protein-1 Levels are Associated with Reduced Myocardial Reperfusion after Primary Percutaneous Coronary Intervention for ST-Segment Elevation Myocardial Infarction

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Background: : Inflammation is an important factor in atherosclerosis and the development of coronary heart diseases. The CC chemokine monocyte chemoattractant protein (MCP)-1 is involved in the formation, progression, and destabilization of atheromatous plaques. Emerging evidence suggests that MCP-1 plasma levels have prognostic value in the acute and chronic phase following acute coronary syndrome, providing information independent of standard clinical variables. The aim of this study was to investigate the time course and possible associations between MCP-1 levels and myocardial reperfusion after primary percutaneous coronary intervention (pPCI) for acute ST-elevated myocardial infarction (STEMI).

Methods: A total of 43 consecutive patients with the first anterior STEMI successfully treated by pPCI (< 20% of residual stenosis and TIMI flow 3) within 6 hours after the onset of the chest pain were included. Serum was sampled at baseline, 4, 12, 24 hours after pPCI, 2 days and 7 days after pPCI. Samples were stored at -80°C until analysed. Quantification of MCP-1 concentration was performed by Randox, Ltd. (Crumlin, UK), by using a biochip array analyzer (Evidence Investigator®). Coronary angiograms post-PCI were analysed for myocardial blush grade (MBG) as indicator of myocardial reperfusion injury and reflects the microvascular damage. Myocardial blush grade was categorized as follows: 0 (no myocardial blush, or contrast density), 1 (minimal myocardial blush), 2 (moderate myocardial blush but less than that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery) and 3 (normal myocardial blush comparable to that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery). The primary analysis was MBG (reduced vs normal, 0, 1, 2 vs 3). Coronary angiograms were analysed by a physician blinded to clinical data. Univariate and multivariate logistic regression analyses were used to explore the association between MCP-1 levels and myocardial blush grade.

Results: The average age was 56 ± 11 years and 80% were male. Seventy percent of the patients had hypertension, 27% had diabetes mellitus, 56% hyperlipidemia, 30% a positive family history of coronary heart disease, and 40% were current smokers. The median time from symptom onset was 2.5 h and did not differ significantly between groups (p>0.05). Clinical characteristics did not significantly differ between patients with reduced and patients with normal MBG. MCP-1 level at baseline was 202 (153-237) pg/mL in patients in MBG normal group and 324 (IQR 239-457) pg/mL in MBG reduced group. In both MBG groups, MCP-1 level decreased over time. This decrease was more pronounced in patients with optimal reperfusion compared to patients with reduced reperfusion. After multivariate adjustment, baseline MCP-1 was an independent predictor of the optimal MBG (OR 1.012, 95% CI 1.002-1.023, P=0.021).

Conclusion: Our study shows an association of MCP-1 with MBG after pPCI in STEMI patients.

A-160

The incidence of *Streptococcus pneumoniae* strains in patients with otitis media in hospital and ambulatory

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Introduction: Otitis media is common especially in children, representing an important socio-economic issue both through expanded use of financial and human resources. The development of mechanisms of bacterial resistance to antibiotic treatment is a problem in otitis therapeutic management.

Aim: To assess the incidence of pneumococcal strains involved in the etiology of otitis media and the development of prophylactic regimens in a population group of hospital and ambulatory.

Material and methods: We performed a prospective study of pathogens isolated from patients with acute otitis media, diagnosed on the basis of otoscopic findings of either middle ear effusion or purulent otorrhea with a duration of less than 24 h. Isolation on conventional culture media (blood and chocolate agar) and identification of germs were performed at the hospital laboratory. Identification of *Streptococcus pneumoniae* and extensive antimicrobial tests (by dilution antimicrobial susceptibility tests) were performed using the BioMerieux® VITEK2 automated microbiology system, with Vitek 2 GP and Vitek 2 AST P533 cards (EUCAST standards). Quality control strains used in the testing was *Streptococcus pneumoniae* ATCC 49619.

Results: We analysed 50 patients samples. Out of these 40%(20 patients) were *Streptococcus pneumoniae*, 20%(10 patients) nontypable *Haemophilus influenzae*, 3%(6 patients) *Moraxella catarrhalis*, 8%(4 patients) *Streptococcus pyogenes* positive. *Staphylococcus* spp. and *Enterobacteriaceae* strains were interpreted as a contaminant 19%(10 patients). Of 20 isolates of *S. pneumoniae* tested in our study,

35% were intermediately or fully resistant to penicillin. Finally, 47% of intermediately and fully penicillin-resistant isolates were resistant to multiple antimicrobial classes in our study. Only 1% of the isolates of *S. pneumoniae* were resistant to amoxicillin in our study.

Conclusions: Our findings may have clinical implications because the selection of antibiotic therapy for intermediately or fully penicillin-resistant *S. pneumoniae* infections. The need for parenteral agents in patients with acute otitis media is especially doubtful because of the low morbidity, high frequency of spontaneous recovery, and frequent resolution of clinical signs and symptoms, despite the persistence of bacteria in middle ear fluid.