# Tuesday, August 2, 2016

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

Cardiac Markers

## A-063

### Atherogenic index of plasma for the prediction of future CVD in prediabetes and diabetes population

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**Background:** Diabetes and prediabetes have become a worldwide public health problem. Diabetic patients develop various cardiovascular diseases (CVD) in different manner than predicted by traditional CVD risk factors. So, such risk stratification requires other factors to be taken into account but routine investigation of these biomarkers is difficult. Atherogenic index of plasma (AIP) which is calculated as logarithm of TG/HDLc is one of these non-traditional biomarkers. This study aims to evaluate the utility of AIP in diabetic and prediabetic population for prediction of future CVD.

**Methods:** Altogether we recruited 200 volunteers belonging 100 volunteers in each of diabetes and prediabetes group by purposive sampling method. We evaluated CVD risk by i) Biochemical CVD markers, ii) NCEP ATP III postulated risk factors, iii) Framingham Risk scores. Atherogenic index of plasma was calculated as logarithm of TG/HDLc ratio (Molar conc) and correlated with CVD risk. **Results:** Mean age of participants was 34±5 years, mean fasting blood glucose in prediabetes was 117±5 mg/dl and diabetes was 149±19 mg/dl. Similarly, mean AIP in prediabetes and diabetes was 0.39±0.11 and 0.45±0.14 respectively. AIP correlates with fasting total cholesterol (p=0.09), high TG (p=0.002), increased LDL (p=0.001), non-HDL cholesterol (p=0.005), decreased HDL (p<0.04), increased hsCRP (p<0.001), increased oxidized LDL (p=0.03), presence of multiple risk factors (p<0.001) and Framingham predicted 10 yr CVD risk (p=0.01). Compared to lowest quartile, highest quartile of AIP presented with 2.13 times higher CVD risk in prediabetes and 3.06 times higher CVD risk in diabetes group. **Conclusion:**

AIP is good indicator of future CVD in both diabetes and prediabetes.

## A-064

### Rule out myocardial infarction by admission limit of detection measurement using contemporary and high sensitivity troponin assays.

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Objective: To compare admission measurement of troponin for the rule out myocardial infarction (MI) when more sensitive troponin assays are used for the diagnosis using the universal definition of myocardial infarction.

Methods: The study was a sub study of the point of care arm of the RATPAC trial (Randomised Assessment of Treatment using Panel Assay of Cardiac markers), set in the emergency departments of six hospitals. Prospective admissions with chest pain and a non-diagnostic electrocardiogram were randomised to point of care assessment or conventional management. Blood samples were taken on admission and 90 minutes from admission for measurement of a panel of cardiac markers. An additional blood sample was taken at admission and 90 minutes from admission, separated and the serum stored frozen until subsequent analysis. All patients were followed up to 30 days for major adverse cardiac events (MACE). Samples were analysed for cardiac troponin I (cTnI) by

the Stratus CS (CS) (Siemens Healthcare Diagnostics), range 30 to 50,000 ng/L 10% CV 60ng/L 99$^{th}$ percentile 70 ng/L; the Beckman AccuTnI enhanced (B) (Access 2 , Beckman-Coulter) range 1 to 100,000 ng/L, 10% CV 30 ng/L, 99$^{th}$ percentile 40 ng/L, the Siemens Ultra (S) (ADVIA Centaur, Siemens Healthcare Diagnostics), range 6 to 50,000 ng/L, 10% CV 30 ng/L 99$^{th}$ percentile 50 ng/L. and cardiac troponin T (cTnT) by the Roche high sensitivity cardiac troponin T assay hs-cTnT (Elecsys 2010, Roche diagnostics), range 3 to 10,000ng/L, 10% CV 13ng/L, 99$^{th}$ percentile 14 ng/L.

The universal definition of myocardial infarction (MI) utilising laboratory measurements of cardiac troponin performed at the participating sites together with measurements performed in a core laboratory was used for diagnosis. The use of limit of detection of the assay was used to classify patients using the admission sample only. Values below the limit of detection were classified as no MI.

Results: Samples were available from 813 /1132 patients enrolled in the study, 487 male age 23.7-92.8 years median 53.8 years. Limit of detection measured on admission allowed accurate exclusion of MI in 98.5-99.2% of patients with a final diagnosis that excluded MI corresponding to 70.6-80.8% of all patients presenting. In those who ruled out based on a single admission measurement major adverse events occurred in 0.2-0.6% and comprised readmission with suspected acute coronary syndrome and 1 myocardial infarction on follow up.

Conclusion: In low risk chest pain patients, troponin below the limit of detection measured with a sensitive or contemporary sensitive assay identified a very low risk group who can be considered for immediate further investigation or discharge.

## A-065

### Evaluation of the Specificity and Concordance of a Cardiac Risk Prediction Array for the Detection of Multiple SNPs from One sample of Different Biological Matrices

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**Background:** Coronary Heart Disease (CHD) is the most common cause of death in the Western world. Research indicates a strong genetic predisposition to CHD. Genetic studies have identified CHD-associated single nucleotide polymorphisms (SNPs). Current risk prediction methods, *e.g.* QRISK / Framingham are suboptimal at predicting CHD. The development of a predictive risk tool, incorporating personalised genetic profiling for CHD is therefore warranted. The objective of this study was to determine specificity and examine concordance rates of genotypes generated with a Cardiac Risk Prediction Array, which enables simultaneous detection of multiple CHD-associated SNPs from one sample of different biological matrices.

**Methods:** Biochip Array Technology (BAT) allows the development of SNP genotyping arrays, utilising rapid multiplex allelespecific PCR and product hybridisation onto a biochip. Firstly, a multiplex PCR reaction is performed, where the products amplified correspond to the genotype of the patient sample. The PCR products are then hybridised onto the Cardiac Risk Prediction biochip array and imaged using the Evidence Investigator analyser (Randox Laboratories, Crumlin, UK) to identify which alleles are present. Patient samples can be genotyped within one day.

To determine specificity, participants from the second UK Northwick Park Heart Study ($n$=3,012) were genotyped for 19 CHD-associated SNPs by Sanger sequencing. A subset of participants ($n$=185) were genotyped using the Cardiac Risk Prediction Array for 19 CHD-associated SNPs. To assess concordance between different biological matrices, matched blood and saliva samples were collected from a cohort of participants ($n$=20) and the DNA extracted was genotyped using the Cardiac Risk Prediction Array. Sanger sequencing was employed for two blood-derived DNA samples to ensure validity of results.

**Results:** Overall, agreement between Sanger sequencing and the Cardiac Risk Prediction Array was 99.8% (3152/3158 genotypes). Agreement for individual SNPs ranged from a minimum of 98.7% to a maximum of 100%. Fourteen out of 19 SNPs achieved values of 100%. The concordance assessment showed 100% concordance (400/400 genotypes) when comparing genotypes generated from blood and saliva. Concordance data was confirmed by Sanger sequencing of two bloodderived DNA samples.

**Conclusion:** Results show high specificity of the Cardiac Risk Prediction Array when comparing with goldstandard methods *i.e.* Sanger sequencing. Furthermore, with this system genetic risk profiles both from DNA derived from blood and saliva can be accurately and robustly determined. This provides evidence of its potential use in direct-to-consumer rapid genotype testing for improved cardiac risk prediction.

## A-066

### Optimization and Validation of a Multiplex Cardiac Biomarker Assay

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**Background**: Multiplexed immunoassays enable individuals to obtain a wealth of biomolecular information more quickly and cheaply than traditional methods. In particular, the development of multiplexed assays is essential when patient samples are limited, a problem common when treating pediatric populations. The aim of our study was to develop and validate a multiplex cardiac biomarker assay consisting of NT-proBNP, cTnT, ST-2 and Galectin-3.

**Method**: We first measured these biomarkers in the plasma of 40 pediatric patients using FDA-approved ELISA-based assays. The data obtained from the standard FDA -approved assays was compared with results from our prototype multiplex assay. For our prototype, NT-proBNP and cTnT capture and detection antibody pairs were from Meso Scale Discovery (MSD), and ST-2 and Galectin-3 antibody pairs were from R&D Systems. Statistical analysis was performed using EP evaluator Version 9.0.

**Results**: The biomarker concentrations measured with the multiplex assay correlated well with the values measured in the respective singleplex assay. Correlation coefficients (r) were 0.87 for NT-proBNP, 0.99 for cTnT, 0.95 for ST-2, and 0.83 for Galectin-3 between multiplex and singleplex assays. For all the biomarkers, the recovery of the spiked samples was greater than 90% for all the concentrations tested. The measuring range was 0.75 - 20000 pg/ml for NT-proBNP, 10.4 -39900 pg/ml for cTnT, 0.69 - 8300 pg/ml for ST-2, and 6.25 - 4000 pg/ml for Galectin-3. Pooled patient samples was used to assess precision; within-run CV was <10.08% and total CV <16.63%. Due to the large dynamic range of our multiplex assay, a single dilution allowed us to measure all four biomarkers simultaneously. In contrast, the standard ELISA-based assay required multiple dilutions in order to accurately quantitate these biomarkers. Furthermore, the minimum sample volume needed to measure all four biomarkers using the traditional ELISA-based approach was 350 µl, whereas no more than 25 µl of plasma was needed to measure the same biomarkers using our multiplex assay.

**Conclusion**: The data obtained from the multiplex platform agreed well with the singleplex FDA-approved assays. Our data demonstrates that our 4-plex cardiac biomarker panel is suitable for simultaneous quantitation of cTnT, NT-proBNP, ST-2 and Galectin-3 in human plasma.

## A-067

### Impact of risperidone treatment of autistic disorders on metabolic risk factors in children and adolescents

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**Background:** Risperidone has been reported to affect cardiovascular and diabetic markers. Thus, we evaluated the influence of the dose and the duration of risperidone treatment on metabolic and diabetes risk markers in Thai children and adolescents with autistic spectrum disorders (ASDs).

**Methods:** In this cross-sectional analysis, cardiovascular and diabetic risk markers were measured in 172 ASD patients (88% male) treated with a risperidone-based regimen for ≥12 months. Based on FDA-approved dosing recommendations for pediatric patients, all patients were categorized into three groups: low dose, recommended dose, and high dose groups. Blood samples were analyzed for glucose and lipid metabolic markers, adiponectine, leptin, prolactin, cortisol, uric acid, creatinine, cystatin C and high sensitive C-reactive protein.

**Results:** The mean concentrations of fasting glucose, insulin, HOMA-IR, prolactin, and leptin levels significantly rose with risperidone dosage (all $P \leq 0.02$), but those of adiponectin, cortisol, uric acid, creatinine, and cystatin C did not. Dosage had minimal effect on triglycerides, total cholesterol, HDL-C, LDL-C, and lipoprotein subclasses (all $P > 0.10$). Insulin, HOMA-IR, triglyceride, and leptin levels rose with treatment duration (all $P < 0.03$). Conversely, adiponectin and HDL-C levels decreased with duration. Similar association patterns of dosage and duration of treatment with insulin, HOMA-IR and leptin were observed, all of which increased with dosage and duration.

**Conclusions:** Risperidone treatment disturbed glucose homeostasis and endocrine regulation (particularly leptin) in children and adolescents with ASDs, in a dose- and/or duration-dependent manner. It may directly impact glucose homeostasis by inhibiting the actions of leptin and insulin. The adverse metabolic changes associated with risperidone treatment suggest that risk for metabolic adverse effects, especially development of type 2 diabetes mellitus should be closely monitored, particularly in individuals receiving high doses and/or long-term treatment.

## A-068

### Comparison of Rapid Test Kits for Myocardial Markers

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Introduction

Myocardial marker levels increase during myocardial damage. Detection or measurement of such markers is useful for diagnosing acute coronary syndrome, including acute myocardial infarction. Two markers, human heart-type fatty acid-binding protein (H-FABP) and troponin T, are useful for the early detection of acute coronary syndrome. Rapid test kits are available for these markers. In this study, we compared 3 test kits and determined their characteristics.

Subjects and methods

1. Kits: We assessed a qualitative rapid H-FABP test kit Rapicheck® H-FABP (Rapicheck; DS Pharma Biomedical Co., Ltd.), a quantitative rapid H-FABP test kit Rapidchip® H-FABP (Rapidchip; Sekisui Medical Co., Ltd.), and a qualitative rapid test kit for troponin T (Trop T Sensitive® [Trop T]; Roche Diagnostics K.K.). All 3 kits are based on immunochromatography.

2. Subjects: We currently use the Rapicheck kit at our hospital, and the subjects were 36 patients (22 men and 14 women; mean age, 74.9 years) who underwent myocardial marker testing between July and September 2015.

3. Methods: Whole blood samples were collected into EDTA-2K blood collection tubes and were used for simultaneous measurement with the 3 kits immediately after collection. The Rapicheck and Trop T tests were assessed visually, while an H-FABP concentration ≥ 6.2 ng/mL was considered positive for the Rapidchip test.

Results

Of the 36 subjects, 30, 28, and 13 were positive by the Rapicheck, Rapidchip, and Trop T tests, respectively. Thirteen patients were positive by all 3 kits, and they had acute myocardial infarction, angina pectoris, heart failure, or similar diseases. The concordance rate between Rapicheck and Trop T was 52.8%, while it was 58.3% between Rapidchip and Trop T, being in the 50% to 60% range for these qualitative tests. When the results differed, patients were always positive by Rapicheck and Rapidchip, and were negative by Trop T. The concordance rate was a high 94.4% between Rapicheck and Rapidchip, both of which are qualitative H-FABP tests. The 2 patients in whom the results differed were positive by Rapicheck and negative by Rapidchip (assay values: 5.0-5.5 ng/mL). Among the 36 subjects, 4 had acute coronary syndrome. Three of them were positive by all 3 kits, while 1 was positive by Rapicheck and Rapidchip, but negative by Trop T. This patient had acute myocardial infarction, and blood was collected at 4.5 hours after the onset of chest discomfort.

Conclusions

H-FABP may indicate myocardial damage at an earlier stage than troponin T. However, some of the patients who were only positive for H-FABP showed elevation of this marker because of impaired excretion due to renal dysfunction or because of H-FABP from the skeletal muscle. The qualitative tests were associated with the problem of variation due to subjective judgment. In contrast, Rapidchip allows quantitative measurement of H-FABP and is free from subjectivity, making assessment of the results easy. Diagnostic sensitivity may be increased by using a combination of tests based on knowledge of the characteristics of each kit.

## A-069

### Standardization of Beckman Coulter Serum and Plasma Troponin-I assay in a Multi-Center Health System Using Multiple Testing Platforms

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**Background:** Cardiac troponin (cTn) are essential in the diagnostic evaluation for acute myocardial infarction. Our health system (WFBH, LMC, DMC) frequently assays cTnI concentration, however our hospitals use different cTnI testing platforms, sample types, and clinical cut-off values. This lack of standardization makes it difficult to monitor changes in cTnI when patients are transferred to the tertiary care hospital (WFBH). The aim of this study was to standardize cTnI testing in our care network by introducing the same cTnI method, comparing serum and plasma, and determining a clinical cut-off concentration for our patient population. This was achieved with pre- and post-study planning and evaluation between lab staff and clinicians. **Methods:** The WFBH Emergency Department collected 153 matched plasma (lithium heparin) and serum (SST) samples. Serum samples were assayed on the WFBH Siemens Centaur-XP immediately after collection and reported to physicians. Remaining samples were frozen until validation testing commenced. During validation testing, batches of samples (≈51/day) were assayed same-day on the Centaur and Beckman Coulter DxI 800 (AccuTnI+3) at WFBH and on the Access-2 at both DMC and LMC. Precision and linearity studies were conducted. Site-to-site variability was minimized by using the same lots of calibrators, QC materials, and reagents. Data were analyzed using EP Evaluator. **Results:** Linearity and precision agreed with the manufacturer's recommendations. The correlation studies are summarized below:

| Comparison | Passing-Bablok Regression | | | Sample No. |
|---|---|---|---|---|
| | Slope | Y-Intercept | $R^2$ Coefficient | |
| WFBH Centaur (Serum) vs WFBH DxI (Serum) | 0.609 | -0.0006 | 0.9849 | 134 |
| WFBH DxI (Serum) vs WFBH DxI (Plasma) | 1.000 | 0.0000 | 0.9996 | 153 |
| DMC Access-2 (Serum) vs DMC Access-2 (Plasma) | 1.038 | 0.0000 | 0.9996 | 119 |
| LMC Access-2 (Serum) vs LMC Access-2 (Plasma) | 1.033 | 0.0010 | 0.9998 | 130 |
| WFBH DxI (Plasma) vs DMC Access-2 (Plasma) | 1.000 | -0.0020 | 0.9995 | 131 |
| WFBH DxI (Plasma) vs LMC Access-2 (Plasma) | 1.109 | 0.0000 | 0.9984 | 131 |
| DMC Access-2 (Plasma) vs LMC Access-2 (Plasma) | 1.065 | 0.0020 | 0.9982 | 131 |
| WFBH DxI (Serum) vs DMC Access-2 (Serum) | 0.867 | -0.0009 | 0.9997 | 126 |
| WFBH DxI (Serum) vs LMC Access-2 (Serum) | 1.033 | -0.0002 | 0.9994 | 126 |
| DMC Access-2 (Serum) vs LMC Access-2 (Serum) | 1.083 | 0.0000 | 0.9997 | 126 |

Our observed correlation slope (m=0.609) was consistent with inherent differences between the immunoassays and supported by published CAP survey result comparisons. Qualitative method comparison between the Centaur and DxI showed a sensitivity of 93.5% and specificity of 91.0% at the manufacturer's suggested clinical cut-offs. The clinical cut-off value ≥0.025 ng/mL was appropriate for our patient population. **Conclusions:** Following this study, we will be able to standardize cTnI testing in our health system with the adoption of Beckman Coulter AccuTnI+3 reagents and instruments.

## A-070

### Correlation between conventional troponin and high sensitivity cardiac troponin assays in patients with chest pain

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**Background:** There are many methodologies available in the market, which use different targets and formats to assembly an assay, and depending on the type of assay and type of antigen used, different results and interpretations could be made. Due to the higher sensitivity and diagnostic accuracy for the detection of Acute Coronary Syndrome (ACS) at presentation, the time interval to the second cardiac troponin assessment can be shortened with the use of high-sensitivity assays

**Methods:** 40 consecutive samples were collected from patients of different ages admitted to the Emergency Department (ED) with chest pain and suspected ACS. The determination of Troponin I in serum samples were initially analyzed with a fluorescent immunoassay (ELFA) using the *Vidas* platform from *bio-Mérieux* ® (CT), wich was compared with a second method of high-sensitivity LOCI Cardiac Troponin I using a homogeneous sandwich chemiluminescent immunoassay in the *Dimension EXL 200* (*Siemens*) ® (HS)

**Results:** Excellent correlation coefficients were obtained, Pearson with r of 0.981 (expected &lt 0.95) and a concordance observed of 0.93 (expected $lt 0.90) and Kappa index of 0.85 (expected $lt 0.7).

**Conclusion:** There was a large correlation between the two Troponin I assays, in this particular situation both tests fulfilled their role for screening purposes using "Rule in" and "Rule out" algorithms. Further studies will answer whether there are advantages in replacing the CT methodology by HS, according to the clinical probability of ACS and delta obtained in serial samples

## A-071

### Identification of Predictive Proteomic and Metabolomic Biomarkers of Doxorubicin-induced Cardiotoxicity

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Doxorubicin (DOX)-based chemotherapy is commonly used for the treatment of a wide range of cancers. However, clinical use of DOX can cause cumulative dose-dependent and irreversible cardiotoxicity. Blood biomarkers such as cardiac troponin T (cTnT) and I (cTnI) have been suggested, but their ability to predict cardiotoxicity is limited. Therefore, development of new predictive biomarkers to identify potential cardiotoxicity prior to the occurrence of overt cardiac tissue damage and dysfunction would be extremely valuable for the prevention of permanent damage and/or identification of patients at risk for cardiac damage. The objective of this study was to identify potential biomarkers for presymptomatic detection of DOX-associated cardiotoxicity at the early doses of chemotherapy. Women treated for breast cancer with DOX-based chemotherapy at the University of Arkansas for Medical Sciences were enrolled in Institutional Review Board (IRB)-approved protocols of UAMS and FDA, and gave informed consent. All patients were treated following a predefined protocol which included a combination of DOX (Adriamycin, 60 mg/m$^2$) with cyclophosphamide (600 mg/m$^2$). Blood samples were collected prior to chemotherapy and after the first and/or second cycles of chemotherapy, and plasma was isolated. Cardiac function of all subjects was assessed by a multigated acquisition (MUGA) scan before the start of DOX treatment and at its completion of four cycles of chemotherapy. A decline of left ventricular ejection fraction (LVEF) by >10% or below 50% at completion of chemotherapy was considered abnormal or left ventricular dysfunction (LVD). Plasma samples of 27 patients, including 17 who maintained normal LVEF, 5 with LVEF decline by 5-10%, and 5 with LVEF decline by >10% at the completion of DOX treatment were analyzed using multiplex immunoassays for 82 proteins from 3 human cardiovascular disease biomarker panels (Millipore), one 40-plex human chemokine panel (Bio-Rad), one 9-plex human matrix metalloproteinases (MMPs) panel (Bio-Rad), and troponin T (Roche). This was to identify and verify potential utility of these proteins as predictive biomarkers of cardiotoxicity. It was found that higher abundance of CCL27, CXCL16, and GDF-15, and lower abundance of CXCL6, fibrinogen, and sICAM-1 ($p$<0.05) at the baseline level were associated with LVD (LVEF decline by >10%). After the second cycle of DOX treatment, increased abundance of CCL13, CXCL1, CXCL2, and MIF ($p$<0.05) was associated with LVD. In addition, a 1.6-fold increase of p-selectin after the second cycle of DOX treatment compared to patients' own plasma baseline levels ($p$<0.05) was observed in the group of patients with LVD. LC/MS-based metabolomic platform was also used to discover metabolic biomarkers in plasma. Metabolomics analysis revealed that plasma pyroglutamate and lysophosphatidylcholine (16:0) increased in the group of patients with LVD ($p$<0.05) while plasma docosahexaenoic acid and taurocholic acid decreased in both groups of patients with LVEF decline by 5-10% and by >10% ($p$<0.05) after the second cycle of chemotherapy. The differential plasma abundance of these proteins and metabolites at the baseline level or after the second cycle of DOX treatment could be potential predictive biomarkers of cardiotoxicity.

## A-072

### Relation of Interleukin-6 Level with Coronary Artery Disease Severity in Patients Undergoing Coronary Angiography

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**Background:** Atherosclerosis plays an important role in the pathogenesis of CAD. There is a closer relationship between atherosclerosis and inflammatory cytokines. Commonly used scoring systems to risk stratification in clinical practice are GENSINI and SYNTAX score for determining disease severity and method of revascularization. The aim of this study was to investigate the relationship between interleukin (IL)-6 levels and both GENSINI and SYNTAX scores in patients with CAD.

**Methods:** 118 patients who underwent to coronary angiography were enrolled into the study. GENSINI and SYNTAX were calculated for determining disease severity. IL-6 level was measured with immunometric assay method.

**Results:** There were no significant differences between the groups with respect to mean age, blood pressure, heart rate and use of alcohol. Gender and smoking status were significantly different between groups. GENSINI and SYNTAX scores of patients were significantly higher in CAD group than controls. IL-6 level was significantly higher in CAD group than controls. In correlation analysis, IL-6 level significantly correlated with GENSINI and SYNTAX scores and was an independent predictor of abnormal coronary angiography. Optimal cut-off level of IL-6 was 7.81 pg/mL to assess the ability of IL-6 to differentiate presence of CAD (Area under curve=0.78, sensitivity=78.3%, specificity=70.7%).

**Conclusion:** Patients with CAD have higher IL-6 level compared to control group. IL-6 level correlated with both GENSINI and SYNTAX scores. Moreover, IL-6 was an independent predictor of abnormal coronary angiography. An IL-6 value of 7.81 pg/mL

or higher predicted presence of CAD with a sensitivity of 78.3% and specificity of 70.7%.

## A-073

### Potential Risk Predictors for Cardiovascular Diseases in Patients with Obstructive Sleep Apnea Syndrome

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Background: An association exists between Obstructive Sleep Apnea Syndrome (OSAS) and increased incidence of cardiovascular diseases (CVDs). The high systemic oxidative stress in OSAS is considered as a major pathogenic mechanism leading to CVDs. There is limited and conflicting information in the literature investigating risk predictors for CVDs in OSAS Patients. Our aim was to investigate and define potential risk predictors for CVDs in newly diagnosed OSAS patients without manifest CVDs.

Methods: A total of 60 OSAS patients (13 moderate and 47 severe) diagnosed with polysomnography and 26 healthy volunteers were enrolled into the study. Patients were diagnosed having OSAS according to the apnea-hypopnea index (AHI) above 5. Patients were divided into subgroups according to their AHI: mild OSAS (AHI between 5 and 15); moderate OSAS (AHI between 15 and 30); and severe OSAS (AHI ≥30). There was no patient with mild OSA in the whole group. Blood samples were collected after overnight fasting, and plasma ischaemia-modified albumin (IMA), advanced oxidation protein products (AOPP), total oxidative status (TOS), total antioxidative capacity (TAC), copeptin level, myeloperoxidase (MPO) activity and soluble tumor necrosis factor receptor-1 (sTNF-R1) were measured in the patients and healthy controls. Statistical analysis was performed using SPSS Statistics Base 17.0 (SPSS Inc. Chicago, IL, USA). Receiver operator characteristic (ROC) curves were used to define sensitivity and specificity of the measured parameters for the diagnosis of OSA.

Results: Copeptin levels were significantly lower in both moderate and severe OSAS groups compared to the control group (0.42±0.18 and 0.49±0.26 ng/ml versus 0.64± 0.28; p=0.005, p=0.006, respectively). In contrast, we found no significant difference in copeptin levels between the OSAS subgroups (p=0.409). Plasma MPO activity and sTNF-R1 levels were significantly higher (43.2±21.65 vs. 30.44±8.05, p=0.0046; 2.379±1.2 vs. 1.086±0.86, p < 0.0001, respectively) in the patients compared to the control group. Plasma TOS (P<0.001) and AOPP levels (P =0.024) were significantly higher, in contrast to the TAC which was significantly lower (P=0.012) in the OSAS patients compared to the controls. There was a very strong negative correlation (r=-0.987, P<0.001) between TAC and AOPP levels. AOPP levels correlated significantly with TOS levels (P<0.001, r = 0.45). Plasma ischaemia-modified albumin levels were not statistically different between the patients and controls (P=0.74).

Conclusion: Copeptin levels were lower in the OSAS patients which might be due to low secretion of antidiuretic hormone. In contrast to our finding in the OSAS patients, copeptin levels are higher in CVDs. Thus, we conclude that measurement of copeptin is not proper to determine the risk of CVDs in OSAS patients. We found that a high systemic oxidative stress in OSAS as indicated by increased TOS and decreased TAC levels is reflected by increased AOPP without causing an increase in IMA. Elevated plasma MPO activity and sTNF-R1 levels in the OSAS patients indicate increased systemic inflammation which might contribute to the higher incidence of CVDs. Therefore, we recommend measurement of plasma MPO activity, sTNF-R1, TOS, TAC and AOPP levels in the OSAS patients as potential risk predictors for CVDs.

## A-074

### Alteration and association of galectin-3 levels in STEMI patients undergoing percutaneous coronary intervention

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**Background:** Galectin-3, secreted by activated macrophages, is both pro-inflammatory and pro-fibrotic but not cardiac specific. Most studies have focused on its prognostic utility in chronic heart failure, however few studies have investigated galectin-3 in acute coronary syndrome. Therefore, we initiated a study examining levels in adults presenting with acute ST-elevation myocardial infarction and undergoing percutaneous intervention.

**Methods:** Galectin-3 was measured using the Abbott chemiluminescent microparticle immunoassay on the ARCHITECT i2000 analyzer. Assay validation included precision studies and comparison with another laboratory using the Abbott assay (n=20) and one using an ELISA (n=20, BG Medicine Inc.). Results were analyzed with EP Evaluator (Data Innovations). Sample stability was studied using serum separator tubes: two patients with galectin-3 below and above the suggested cut-off (>17.8 ng/mL) were tested at 24 hour increments for 96 hours. Patient enrollment began in January 2016 and excluded those who might have preceding causes of elevated galectin-3 secondary to malignancy or severe chronic heart, renal, and liver disease. Study patients had galectin-3 measured at admission (baseline) and with up to two subsequent measurements prior to discharge.

**Results:** The Abbott method comparison results were: slope = 1.009 (0.956 to 1.061); intercept = 0.17 (-1.38 to 1.72); bias = 0.40 ng/mL (1.46%); R = 0.9944. Compared to the ELISA, results were: slope = 1.577 (1.279 to 1.875); intercept = -7.69 (-13.34 to -2.03); bias = 2.66 ng/mL (13.82%); R = 0.9253. Serum separator gel tube stability data over 96 hours produced a mean±SD of 9.26±0.65 ng/mL (CV 7.0%) at the lower concentration and 34.03±2.61 (CV 7.7%) at the higher concentration. Of the 13 study patients to date who have met inclusion criteria, baseline galectin-3 ranged from 13.7 to 43.6 ng/mL while baseline troponin I ranged from 0.03 to 19.9 ng/mL. Post-intervention CK-MB ranged from 1.8 to 300 ng/mL. No clear correlation was observed among these three cardiac biomarkers. Neither was there any concrete correlation found between galectin-3 and the number of ECG leads demonstrating ST-elevation nor with the number of coronary vessels exhibiting >50% stenosis. However, there was an emerging trend showing a sudden decrease in galectin-3 after percutaneous intervention with a mean -28.1% change from baseline in 7 of 12 patients within 27 hours and -24.7% in 7 of 9 patients within 63 hours. **Conclusion:** Our study demonstrates comparable inter-laboratory results when using the same Abbott method whereas significant proportional and fixed bias exists when compared to the ELISA method. The latter finding implies that any proposed cut-off value would depend on the particular assay used and highlights the concern that true industry wide harmonization is critical for reliable follow-up testing. Regarding our study patients, although enrollment and data acquisition is still in its early stages, we have observed a significant fall in galectin-3 immediately following cardiac catheterization in the majority of patients. This suggests that galectin-3 may yet have potential utility as an acute biomarker, perhaps associated with short-term myocyte recovery due to reperfusion, in addition to being a long-term indicator of cardiac fibrosis in chronic heart failure.

## A-075

### Development of a High Sensitivity Cardiac Troponin I Assay on the Siemens ADVIA Centaur® Immunoassay Systems*

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**Background:** The recent 2015 ESC guidelines for the management of NSTEMI patients have proposed algorithms for faster rule-in or rule-out of patients admitted in the acute care setting. Distinguishing acute from chronic c-Tn elevations using high sensitivity assays requires serial measurements to detect significant changes. High sensitivity cTn assays will allow more accurate and precise determination of serial changes or "delta's" which may afford acceptable rule-in and rule-out claims within 1 to 3 hours. The analytical performance of Siemens high sensitivity cTnI assay being developed for use on the Siemens ADVIA Centaur family of automated random access immunoassay analyzers is presented.

**Methods:** The Centaur TNIH assay employs streptavidin coated magnetic latex particles preformed with two biotinylated monoclonal anti-cardiac troponin I antibodies as the solid phase reagent. The detection reagent employs a recombinant sheep $F_{ab}$ antibody covalently linked to an acridinium ester-BSA carrier. The TSPAE acridinium ester is a new generation of high yield acridinium esters developed by Siemens for enhanced chemiluminescent detection. Simultaneous addition of solid phase reagent and detection reagent to the sample forms a classic sandwich immune complex which is subsequently washed. Chemiluminescence is initiated and measured. Relative light units are directly proportional to the cTnI concentration.

**Results:** The assay range is from the Limit of Detection, LoD, to 25,000 pg/mL. The LoD, is determined with 2 reagent lots on 2 Centaur XP's collecting n = 60 replicate measurements for each of 10 serum samples from a normal specimen collection. The LoD was 1.18 pg/mL and 1.24 pg/mL for two reagent lots. The Limit of Blank, LoB, was determined non-parametrically by rank order calculating the 95% percentile as described by CLSI Guideline EP17. The LoB is 0.32 pg/mL and 0.51 pg/mL for the two reagent lots. The LoQ is 2.57 pg/mL defined as the cTnI concentration at 20% Total CV. The dose at 10% Total CV is 3.97 pg/mL. The 99th percentile estimate using a healthy population of n = 194 had a non-parametric value of 37.51 pg/mL in agreement with the 99th of 40 pg/mL claimed for the ADVIA Centaur TnI-Ultra method. The % normals above the LoD exceeded 90%. The Total CV at the 99th is 4.2% determined on 3 Centaur XP's using 3 reagent lots collecting 324 replicate measurements over a period of 5 days.

**Conclusion:** The high sensitivity cTnI in development by Siemens for the ADVIA Centaur Immunoassay Systems has a 10% Total CV at a cTnI concentration 10-fold lower than the 99th percentile, while the 100 uL sample volume is unchanged compared to the previous contemporary sensitive assay generation. This new assay represents an 8- to 10- fold sensitivity increase over current contemporary cTnI methods and will afford a new analytical window at low cardiac troponin I levels allowing for safe evaluation of clinical delta changes. *Under development. Not available for sale. Product availability will vary by country. ADVIA Centaur and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc. or its affiliates.

## A-076

### Cardiac Troponin I Gender-Specific Reference Intervals and 99th Percentile Cutoffs of the Point-of-Care Assays PATHFAST™ cTnI and cTnI-II

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**Background**

cTn assays which have been classified as high-sensitivity assays demonstrated higher 99th percentile values in males vs. females. 1.2 – 2.4-fold higher 99th percentiles in males than in females have been reported for hs cTnI assays recently. Thus, the ability to determine sex-specific cutoffs has become an additional criterion for high-sensitivity cTn assays.

**Objective**

We thought to determine gender specific reference intervals including 99th percentile upper reference limits (URL) (sex-specific cutoffs) of cTnI for the POC system PATHFAST™ (LSI Medience Corporation, Tokyo).

**Methods**

We determined 99th percentiles for the assays PATHFAST™ cTnI (A) and PATHFAST™ cTnI-II (B) which are identical methods but standardized by using different concentrations of the NIST standard SRM 2921.

Lithium heparin plasma samples were obtained from 474 presumable healthy individuals (236 women and 238 men, mean age 51 ± 14 years, range 18 – 86 years) in whom chronic diseases or cardiac disorders were excluded by comprehensive evaluation including assessment of blood pressure, ECG registrations, oral glucose tolerance tests, TSH-, creatinine-, HbA1c-, NT-proBNP-concentration and cardiac magnetic resonance imaging without pathological findings.

**Results**

1. Higher cTnI values were found in males than in females: (A) mean (highest value): 5.58 (24.05) ng/L and 1.77 (14.49) ng/L; (B) mean (highest value): 6.11 (24.05) ng/L and 4.60 (42.84) ng/L, respectively.

2. Only slightly higher cTnI values were found in subjects aged >65 years compared to subjects aged <65 years. The highest values in males aged >65 years were 12.08 ng/L (A) and 27.40 ng/L (B) and didn`t exceed the manufacturer recommended 99th percentile cutoffs (A: 20 ng/L; B: 29 ng/L). Nevertheless, a slightly increasing tendency of cTnI concentration with age could be observed.

3. According to CLSI C28-A3 the following 99th percentiles were obtained: A overall 15.46 ng/L, males 16.9 ng/L, females 11.5 ng/L; B: overall 27.47 ng/L, males 31.3 ng/L, females 24.9 ng/L.

4. cTnI values were detected above the LoD (A: 1.0 ng/L; B: 3.0 ng/L) in 362 and 355 samples, respectively, demonstrating that 76.3 % and 74.9% of normal subjects

revealed detectable values by using PATHFAST™ cTnI and PATHFAST™ cTnI-II, respectively.

**Conclusion**

PATHFAST cTnI is the first POC assay demonstrating fulfillment of the analytical criteria for high-sensitivity cTn assays: Imprecision (CV) at the 99th percentile value < 10%, detectable values above the limit of detection in 76.3% respectively 74.9% of healthy individuals.

Additionally, we could establish overall and gender specific reference intervals demonstrating significantly higher 99th percentile cutoffs in males than in females. Remarkably, the cutoffs in males differed not significantly from the overall cutoff and might be regarded as negligible in view of the biological variability. In contrast, the cutoffs in females were significantly lower than the overall cutoffs and should be taken into consideration for the diagnostic interpretation of PATHFAST cTnI/cTnI-II values to avoid underdiagnosing of acute and chronic cardiovascular diseases in women.

## A-077

### Plasma kynurenine-tryptophan ratio reflects metabolic inflammation, kidney function and homoarginine levels in a cardio-vascular high-risk cohort

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**Background:** Kynurenine (KYN) is a metabolite of tryptophan (TRP) produced by the enzyme indoleamine 2,3-dioxygenase. Apart from exerting effects on neuro-regulation and cancer immunology, KYN and its metabolites impact on vascular inflammation, endothelial integrity, and oxidative stress. KYN or the KYN-TRP ratio have recently been suggested to predict acute and chronic prognosis in cardio-vascular disease.

**Methods:** This study aimed at investigating plasma KYN and KYN/TRP and their dependence on various clinical and biochemical factors in 229 patients carrying automatic implantable cardioverters/defibrillators (AICD), with ~75% of them suffering from systolic heart failure (CHF), ~69% from coronary artery disease (CAD), and 25% from type-2 diabetes (T2D).

**Results:** Univariate linear regression analysis showed that KYN/TRP was associated with plasma high-sensitivity C-reactive protein (hsCRP), glomerular filtration rate (GFR), homoarginine, zonulin, carbonylated proteins, ADMA, and left ventricular ejection fraction, but not with other clinical routine and biochemical parameters (body mass index, sex, age, CAD severity, NT-proBNP, oxidized LDL, calprotectin, myeloperoxidase, nitrotyrosine). The subsequent multiple linear regression analysis (Generalized Linear Model) showed highly significant associations with hsCRP (positive) and GFR (negative) ($p < 0.001$ each) and a moderate negative association with homoarginine ($p = 0.030$).

**Conclusion:** These findings may support reports on the involvement of KYN in the phenomenon of metabolic inflammation; the negative correlations of KYN/TRP with GFR and homoarginine possibly reflect both impaired excretion and vascular dysfunction in patients with CHF and CAD.

## A-078

### Plasma homoarginine associates with circulating zonulin and tryptophan in a cardio-vascular high-risk cohort

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**Background:** Homoarginine (hArg) is an endogenous, non-proteinogenic amino acid which differs from arginine by an additional methyl group. It is mainly produced in the kidneys where the enzyme L-arginine:glycine amidinotransferase (AGAT) converts it from lysine. Recently, several epidemiological studies have identified low hArg levels as an independent risk marker for cardiovascular, cerebrovascular and renal diseases as well as for mortality. Whether hArg is a causal pathophysiological factor is a matter of ongoing debate; hArg is known to impact on NO synthesis as well as on energy metabolism.

**Methods:** This study aimed at investigating plasma hArg and its dependence on various clinical and biochemical factors in 229 patients carrying automatic implantable cardioverters/defibrillators (AICD), with ~75% of them suffering from systolic heart failure (CHF), ~69% from coronary artery disease (CAD), and 25% from type-2 diabetes (T2D).

**Results:** Univariate linear regression analysis showed that hArg was associated with plasma zonulin, tryptophan, glomerular filtration rate (GFR), and body mass index (BMI), but not with other clinical routine and biochemical parameters (sex, age, left ventricular ejection fraction, CAD severity, NT-proBNP, oxidized LDL, calprotectin, myeloperoxidase, nitrotyrosine, carbonylated proteins, ADMA, hsCRP, kynurenine). The subsequent multiple linear regression analysis (Generalized Linear Model) showed highly significant positive associations with zonulin ($p < 0.001$) and tryptophan ($p = 0.004$ each) and a moderate positive association with BMI ($p = 0.025$).

**Conclusion:** The hitherto unknown association of hArg and zonulin, an important tight-junction regulator, deserves further investigation and may pose a novel pathophysiological link. Regarding hArg and tryptophan, both are inhibitors of different isoenzymes of alkaline phosphatase and may be linked via the metabolism of biogenic amines.

## A-079

### Measurable and Undetectable Cardiac Troponin Concentrations in Men and Women Using High-Sensitivity Assays With Sex-Specific 99th Percentiles.

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**Background**: Using high-sensitivity cardiac troponin (hs-cTn) assays, sex-specific 99$^{th}$ percentiles are recommended. Our objective was to determine whether differences exist between men and women in both the proportion of measurable (≥LoD to sex-specific 99$^{th}$ percentiles) and undetectable (<LoD) cTn concentrations in healthy individuals.

**Methods**: Lithium heparin plasma samples were obtained from apparently healthy volunteers and cTn was measured across five hs-assays: 1) Abbott ARCHITECT hs-cTnI, 2) Beckman Access 2 hs-cTnI, 3) Roche Cobas e601 hs-cTnT, 4) Siemens Dimension Vista hs-cTnI, and 5) Singulex Erenna hs-cTnI. LoD and sex-specific 99$^{th}$ percentiles according to published peer-reviewed literature are shown in Table.

**Results**: The proportion of measurable (≥LoD to sex-specific 99$^{th}$ percentiles) and undetectable (<LoD) cTn concentrations was examined across hs-assays. Men had a higher proportion of measurable cTn concentrations than women in 4 out of 5 hs-assays. All hs-cTnI assays provided measurable concentrations above 50% for men and women, while hs-cTnT did not. The proportion of undetectable cTn values varied significantly across assays with women having a higher proportion of undetectable values in contrast to men in 4 out of 5 assays. Using the hs-cTnT assay, marked differences were observed between men and women in both the proportion of measurable (F 7% vs. M 43%) and undetectable (F 93% vs. M 57%) values.

**Conclusion**: Substantial variation exists between the proportion of measurable and undetectable cTn values using hs-assays among men and women. These findings have significant clinical implications, as the ability to provide measurable and undetectable hs-cTn concentrations is key when considering the use of hs-assays for both primary prevention and ruling-out acute myocardial infarction.

| Manufacturer, assay | N | LoD, ng/L | 99$^{th}$ percentile, ng/L | Excluded values above the 99$^{th}$ percentile, n | Measurable values ≥LoD – 99$^{th}$ percentile | Proportion of undetectable values (<LOD) |
|---|---|---|---|---|---|---|
| Abbott ARCHITECT hs-cTnI | F: 252 M: 272 | 1.9 | F: 16 M: 34 | F: 2 M: 2 | F: 67% (168/250) M: 80% (215/270) | F: 33% (82/250) M: 20% (55/270) |
| Beckman Access 2 hs-cTnI | F: 252 M: 272 | 2.5 | F: 9 M: 11 | F: 12 M: 16 | F: 73% (175/240) M: 87% (222/256) | F: 27% (65/240) M: 13% (34/256) |
| Roche Cobas e601 hs-cTnT | F: 252 M: 272 | 5 | F: 14 M: 22 | F: 1 M: 2 | F: 7% (17/251) M: 43% (115/270) | F: 93% (234/251) M: 57% (155/270) |
| Siemens Dimension Vista hs-cTnI | F: 239 M: 264 | 0.5 | F: 33 M: 55 | F: 5 M: 3 | F: 82% (191/234) M: 90% (234/261) | F: 18% (43/234) M: 10% (27/261) |
| Singulex Erenna hs-cTnI | F: 252 M: 272 | 0.1 | F: 15 M: 27 | F: 8 M: 5 | F: 100% (244/244) M: 100% (267/267) | F: 0% (0/0) M: 0% (0/0) |

## A-080

### Improved Diagnostic Accuracy for Type 1 and Type 2 Acute Myocardial Infarction of High Sensitivity Cardiac Troponin I Compared to Contemporary Cardiac Troponin I at Presentation to an Emergency Department

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**Background**: The diagnostic accuracy of acute myocardial infarction (AMI) defined by the Third Universal Definition of Myocardial Infarction is predicated on the clinical sensitivity and specificity of cardiac troponin (cTn) measurements defined by increases above the 99$^{th}$ percentile. Our objective was to determine the clinical sensitivity and clinical specificity for AMI of the initial presentation measurement of the Abbott Architect high sensitivity (hs) - cTnI assay compared to the Abbott contemporary cTnI assay for both type 1 MI and type 2 MI in men and women.

**Methods**: Plasma (EDTA) from 1927 patients (UTROPIA, NCT02060760) presenting to an inner-city emergency department with symptoms suggestive of ischemia had both a contemporary cTnI and a research hs-cTnI measured (Abbott ARCHITECT). 99$^{th}$ percentile upper reference limits (URL) were: hs-cTnI gender defined, 16 ng/L for females and 34 ng/L for males; the contemporary assay had a single URL at 0.030 µg/L. All patients were adjudicated for type 1 and type 2 AMI predicated independently on both the contemporary and the research hs-cTnI assays.

**Results**: Overall there were 208 MIs (10.8%), 84 type 1 (4.4%) and 124 type 2 (6.4%), per adjudication based on hs-cTnI assay. For the hs-cTnI assay, clinical sensitivities for both males and females at presentation were both 95%, and significantly greater ($p< 0.01$) than for the contemporary cTnI assay: 80% for males, 65% for females. Clinical sensitivities for men were 95% for both type 1 and type 2 MI. For women, clinical sensitivity was higher for type 1 MI (100%) than for type 2 MI (92%). Clinical specificity for any MI for hs-cTnI was 82.8% compared to 81.7% for the contemporary cTnI (NS).

For the hs-cTnI assay, clinical specificities were lower in women for either MI type (79%) compared to men (85%) ($p< 0.01$).

**Conclusion**: A single hs-cTnI measurement at presentation in patients presenting with symptoms suggestive of ischemia demonstrated the following diagnostic accuracy: a) higher clinical sensitivity for detection of type 1 and type 2 MI in both men and women compared to a contemporary cTnI assay, b) higher clinical sensitivity in women for detection of type 1 MI compared to type 2 MI, and c) lower clinical specificities in women for both type 1 MI and type 2 type MI compared to men.

## A-081

### Preliminary performance assessment of a new integrated POC biosensor for quantitative detection of BNP

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**Background:** Based on proprietary Magnotech biosensor technology, Philips is developing the Minicare system which consists of a handheld analyzer, the Philips Minicare I-20, and a disposable self-contained cartridge. The system is able to show test results on the reader display within minutes, at the point-of-care (POC). The Philips Minicare BNP* assay under development is for the quantitative measurement of B-type natriuretic peptide (BNP) in EDTA blood or plasma specimens using the Minicare I-20 instrument. It is targeted to be used in the emergency department as an aid in the diagnosis of heart failure. The Minicare BNP* assay is a one-step sandwich immunoassay. An anti-BNP antibody printed onto the base cartridge captures the analyte while a second anti-BNP antibody coupled to magnetic nanoparticles recognizes complexes of capture antibody and BNP thereby allowing detection by f-TIR detection optics.

**Objective:** To assess the preliminary performance of the Philips Minicare BNP* prototype against commercially available BNP assays.

**Methods:** All samples were collected at Diagnostiek Voor U, a diagnostic services lab which receives samples from hospitals and GP offices in the Netherlands. 121 fresh EDTA whole blood samples from patients who had a BNP test request were tested on the Minicare BNP* and on a commercially available POC BNP test (Alere Triage, Alere, Galway, Ireland). Next, collection tubes were centrifuged and EDTA plasma samples were run on a core lab BNP assay (BNP Centaur XP, Siemens, Marburg, Germany). Measurements on all three analyzers were performed within 8 hours after blood collection.

**Results:** The median BNP concentration measured on the Minicare BNP* was 62.8 ng/l with interquartile ranges (IQR) of 32.3 and 219.8 ng/L. Passing-Bablok regression demonstrated a Pearson correlation coefficient for the Minicare BNP* vs Alere Triage of R=0.979. Similarly, when the Minicare BNP* was compared to the core lab assay (Centaur XP), the correlation coefficient was R= 0.947.

**Conclusion:** The Minicare BNP* correlated very well with both, POC and core lab BNP assays, indicating that the future Minicare BNP* POCT will be able to deliver on-the-spot results on whole blood samples, comparable to core lab assays without the need for sample pretreatment or centrifugation.

*Minicare BNP is under development. Not available for sale.

## A-082

### Lipid dual index: Measure HDL quality not quantity in myocardial infarction patients from India.

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Background: Myocardial infarction (MI) is a leading cause of mortality and morbidity worldwide. Extensive research is needed to identify a risk factor which can predict the risk of myocardial infarction. Total cholesterol, lipoproteins, homocysteines etc are known risk factors, Atherosclerotic plaques progression has been known to be correlated to elevated circulating homocysteine (Hcy) due to increased thrombogenicity, oxidative stress and endothelial dysfunction. High density lipoproteins has protective effect against MI and this protective effect of HDL is due to its associated enzyme paraoxonase 1 (PON 1) which has homocysteine thiolactonase activity (PON1 HCYTase activity). Objective: To determine the role of ratio of low density lipoproteins and PON1 homocysteine thiolactonase (LDL/PON1 HCYTase) activity in prediction of MI compare to other conventional lipid ratio. Material and methods: Study group consists of 40 age and sex matched MI cases and 40 healthy controls (18-65 years). Serum Total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDL) estimations were done by enzymatic methods and low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were calculated by Friedewald equation. PON1 HCYTase activity (micromoles/liter) was measured spectrophotometrically. Odd's ratio and Nagelkerke's $R^2$ were calculated after applying binary logistic regression for TC/HDL, LDL/HDL, and LDL/PON1 HCYTase activity. Area under curve (AUC) was also calculated for these ratios. Result: All data were Gaussian distributed checked by shapiro-wilk test. Means were compared by unpaired student t test. The mean ± standard deviation of TC/HDL ratio was 7.32 ± 4.03 and 3.98 ± 2.43 in MI cases and controls respectively. The LDL/HDL ratio was 5.16 ± 3.44 and 2.31 ± 2.21 in MI cases and controls respectively and LDL/PON1 HCYTase activity was 6.013 ± 2.83 and 2.65 ± 1.56 in MI cases and controls respectively ($p<0.05$). AUC for TC/HDL, LDL/HDL and LDL/ PON1 HCYTase activity were 0.802, 0.808 and 0.864 respectively and Nagelkerke's $R^2$ were 0.306, 0.302 and 0.497 respectively. Odd's ratio for TC/HDL, LDL/HDL and LDL/ PON1 HCYTase activity were 1.518 (Confidence interval (CI): 1.185-1.946), 1.620 (CI: 1.512-3.0230 and 2.138 (CI: 1.221-2.150) respectively. Conclusion: These findings suggest the newly proposed lipid dual index, LDL/PON1 HCYTase activity is a better discriminator of MI risk than other conventional lipid ratio. So LDL/ PON1 HCYTase activity may be suggested as a better predictor for the risk assessment of MI than other lipid ratio.

## A-083

### Comparison of three troponin assays for rates of analytic false positive and negative results and percent of samples from emergency department patients exceeding 99th percentile values

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**Background**

Analytic false positive troponin results have been described for most methods and adversely impact interpretation of troponin results and management of patients. Use of more sensitive troponin reagents in the Emergency Department (ED) may result in more troponin values exceeding the 99th percentile value, complicating rapid evaluation of patients for acute coronary syndrome. In this study we compared the analytic false positive and false negative rates of the Roche 4th generation Troponin T STAT (cTnT), Abbott Architect Troponin I (cTnI), and Abbott Architect high-sensitive Troponin I (hs-TnI) assays. We also compared the number (percent) of samples submitted from ED patients that were above the 99th percentile cut-off value for each assay.

**Methods**

Rapid clot serum tubes (RST) collected from adult ED patients received for clinical cTnT testing on the Roche cobas e411 immunoassay analyzer (e411) were used for the study. Samples were centrifuged for 5 minutes at 4000 x g prior to cTnT analysis. Within 15 minutes of testing on the e411, RST samples (N=3023) were placed on an Abbott Architect 2000i for analysis of cTnI and hs-TnI, then refrigerated at 2-8°C within 2 hours of Architect analysis. Within 24 hours of initial analysis, samples were warmed to room temperature, aliquoted and re-centrifuged at 1500 x g for 15 minutes, and re-analyzed in duplicate on all three methods.

We defined analytic false positive as:

• initial value >99th percentile for assay; with both replicates ≤99th percentile ***and*** both replicates differing by >10% of initial value (failure to repeat within 10% around cut-off value) ***or***

• any replicate >99th percentile for assay and differing by >50% from any other replicate (flier or outlier)

We defined analytic false negative as:

• initial value ≤99th percentile; with both replicates > 99th percentile and both replicates differing by >10% from initial value (failure to repeat within 10% around cut-off)

The 99th percentile values used were < 0.01 ng/mL (cTnT), ≤ 0.028 ng/mL (TnI), and ≤ 26 ng/L (hsTnI).

**Results**

For cTnT 19/3023 (0.06%) ED samples analyzed resulted in analytic false negatives, while 21/3023 (0.07%) were analytic false positives. For cTnI only 4/3023 (0.01%) analytic false negatives were observed, while 100/3023 (3.3%) of samples were analytic false positives upon repeat analysis. hsTnI demonstrated the best analytic performance with 2/3023 (<0.01%) analytic false negatives and 9/3023

(0.03%) analytic false positives. 1997/3023 (66%) of cTnT values among ED patients were <99th percentile; compared to 2217/3023 (73%) of cTnI values and 2143/3023 (71%) of hsTnI values. Using gender-specific hsTnI 99th percentile values of ≤ 15 ng/L (female) and ≤ 36 ng/L (males), 2129/3023 (70%) of ED samples submitted were ≤ 99th percentile.

**Conclusions**

Abbott hsTnI had fewer analytic false positive results than Abbott cTnI, and fewer false negative and positive results than Roche cTnT. The percent of ED samples analyzed that were >99th percentile did not differ significantly between assays. The results suggest that use of hsTnI will improve analytic performance in stat measurement of troponin without impacting ED workflow in the evaluation of patients for acute coronary syndrome.

## A-084

### Development of an NT-proBNP Assay* Using Acridinium Ester Technology on the ADVIA Centaur Immunoassay Systems

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**Background:** The measurement of NT-proBNP is a useful aid in the diagnosis and assessment of severity of congestive heart failure. Siemens Healthcare is currently developing an NT-proBNP assay for serum and plasma on the ADVIA Centaur® Immunoassay Systems.

**Methods:** The ADVIA Centaur NT-proBNP (PBNP) assay* is a fully automated two-site sandwich immunoassay using direct chemiluminescent technology. The development of a new acridinium ester (TSP-AE) by Siemens has enabled good precision throughout the assay range. NT-proBNP reagents include a biotinylated sheep monoclonal antibody in the Ancillary reagent, a second sheep antibody labeled with the newly developed acridinium ester in the Lite reagent, and streptavidin-coated paramagnetic latex particles in the solid phase. Samples are incubated with the Ancillary reagent and solid phase to form a PBNP/biotinylated antibody/solid phase complex. Lite reagent is added and allowed to incubate, resulting in the formation of an acridinium ester/PBNP/biotinylated antibody/solid phase complex. Separation occurs, and the signal is proportional to the concentration of NT-proBNP in the sample.

**Results:** The method requires 20 µL of serum or plasma. Time to first result is 18 minutes, with stable calibration for 28 days. 28-day open-well stability has been achieved. Linearity was demonstrated over the range of <LOQ to >35,000 pg/mL. With automated dilution, the measuring interval is extended to 350,000 pg/mL. Equivalent results were obtained among serum, lithium heparin plasma, and EDTA plasma. Reproducibility was assessed using the CLSI EP5-A2 protocol with serum samples ranging from 84 to 30,145 pg/mL. Repeatability CVs ranged from 1.4 to 3.5%. Within-lab CVs ranged from 2.0 to 4.3%. In accordance with the CLSI EP7-A2 protocol, no interference was observed with 75 ng/mL biotin. Split-sample correlation between this method and the Roche cobas e 411 proBNP II assay produced the following statistics by Passing-Bablok analysis: slope = 1.06, intercept = -5 pg/mL, r = 0.9940, and n = 178 over a concentration range of 31-30,542 pg/mL. Over the concentration range of 31-4819 pg/mL, these statistics are as follows: slope = 1.07, intercept = -11 pg/mL, r = 0.9965, and n = 142.

**Conclusion:** The ADVIA Centaur NT-proBNP assay demonstrates good precision and good correlation to the Roche proBNP II assay.*Under development. Not available for sale.

## A-085

### N-glycosylation of NT-IGFBP-4 does not influence its immunodetection by the neo-epitope specific sandwich immunoassay

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**Background:** N- and C-terminal proteolytic fragments of IGFBP-4 (NT-IGFBP-4, 14.6 kDa, and CT-IGFBP-4, 11.3 kDa) are strong predictors of major adverse cardiac events risk in patients presented with ischemia and stable patients with type 1 diabetes. The presence of these fragments in the circulation depends on the proteolytic activity of metalloprotease PAPP-A, which specifically cleaves IGFBP-4 between Met135 and Lys136. We have previously developed sandwich immunoassays based on monoclonal antibodies that are specific to proteolytic neo-epitopes on NT-IGFBP-4 and CT-IGFBP-4 and have less than 1% of cross-reactivity to the full-length IGFBP-4. It has been shown that a fraction of circulating IGFBP-4 contains glycosylated Asn104 that is located close to the PAPP-A-specific cleavage site. Our assay for NT-IGFBP-4 relies on an antibody that has a binding epitope which is in the vicinity of Asn104 and hence the NT-IGFBP-4 assay may be affected by the glycosylation of this amino acid residue. Therefore, the aims of this study were: a) To determine NT-IGFBP-4 glycosylation levels in individual EDTA-plasma samples and... b) To evaluate the influence of glycosylation on the immunodetection of NT-IGFBP-4.

**Methods:** IGFBP-4 and its proteolytic fragments were extracted from twelve individual EDTA plasma samples of ACS patients by immunoprecipitation and analyzed using enhanced chemiluminescence immunoblotting (ECL). Concanavalin A sepharose was used to verify the presence of glycosylated forms of IGFBP-4 and NT-IGFBP-4. The levels of IGFBP-4 and NT-IGFBP-4 were measured using sandwich HRP immunoassays. The precise masses of purified glycosylated and non-glycosylated NT-IGFBP-4 were confirmed by mass spectrometry. Increases in the concentrations of the proteolytic fragments of IGFBP-4 during the incubation with recombinant PAPP-A were used as measures of the proteolysis rates of glycosylated and non-glycosylated IGFBP-4.

**Results:** The investigation of individual EDTA plasma samples revealed that of the total circulating IGFBP-4, 47.2-61.7% was glycosylated. Meanwhile, of the total NT-IGFBP-4, only 9.8-23.5% was glycosylated. Mass spectrometric analysis of NT-IGFBP-4 extracted from pooled EDTA plasma revealed two peaks of 17260 and 14615 Da that corresponded to glycosylated and non-glycosylated forms respectively. The immunoreactivities of endogenous glycosylated and non-glycosylated NT-IGFBP-4 differed by less than 10% when measured using HRP or ECL based immunoassays. PAPP-A-dependent proteolysis of glycosylated IGFBP-4 was 3-4 times less efficient compared to proteolysis of non-glycosylated IGFBP-4.

**Conclusion:** For the first time, the presence of glycosylated NT-IGFBP-4 in human plasma was shown and the proportion of glycosylated and non-glycosylated NT-IGFBP-4 was measured. PAPP-A-dependent proteolysis of glycosylated IGFBP-4 is less efficient if compared with the proteolysis of non-glycosylated IGFBP-4, although it is not completely inhibited. The glycosylated NT-IGFBP-4 displays the same immunoreactivity as non-glycosylated NT-IGFBP-4 in the fragment-specific immunoassay. Thus, this sandwich immunoassay can be used for the reliable measurement of NT-IGFBP-4 in the blood of patients.

## A-086

### Influence of troponin-specific autoantibodies on measurements of cardiac troponin I in binary and ternary complexes

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**Background:** Autoantibodies specific to cardiac troponins (TnAAbs) have been found in the blood of 6-7% of the population. It is believed that TnAAbs may negatively affect cardiac troponin I (cTnI) measurements in the blood of patients with acute myocardium infarction (AMI) by immunoassays that utilize monoclonal antibodies (mAbs) recognizing certain epitopes in the mid-part of the cTnI molecule. In the current study we investigated the epitope specificity of TnAAbs and their influence on the cTnI immunodetection in AMI samples. **Methods:** The presence of TnAAbs in plasma samples from healthy donors was detected according to Eriksson et al., 2004. 191 plasma samples were spiked with ternary cTnI-cTnT-TnC complex and cTnI recovery was measured using an immunoassay sensitive to the presence of TnAAbs. Twelve plasma samples showing low cTnI recovery were selected and studied for TnAAbs epitope specificity. cTnT recovery was measured in the same plasma samples. The mapping of sites on cTnT that are affected by TnAAbs was performed by using eleven anti-cTnT mAbs. The effect of TnAAbs on the measurements of cTnI in blood of AMI patients was analyzed after mixing TnAAbs-containing plasmas 1:1 with the plasma samples of AMI patients (n=35; cTnI concentrations from 2.5 to 35.1 µg/L). **Results:** Human cardiac troponins (cTnI, binary cTnI-TnC or ternary cTnI-cTnT-TnC complex) were spiked into twelve TnAAbs-containing plasma samples to the concentration 50 µg/L and the recoveries of cTnI were analyzed. The well-pronounced inhibitory effect of TnAAbs on cTnI measurements (mean recovery 10.3%) was observed only when cTnI was added in the form of a ternary complex. The inhibitory effect was significantly lower with spiked cTnI-TnC complex or free cTnI (mean recoveries 71.0% and 96.5%, respectively). Since cTnT appeared to be important for the manifestation of the negative interference of TnAAbs on cTnI measurements, the influence of TnAAbs on cTnT measurements was also studied. Only one epitope (223-242 aar) of cTnT was influenced by TnAAbs. The inhibitory effect of TnAAbs on cTnT detection (mean recovery 14%) was only found with spiked ternary complex, whereas it was much less pronounced with spiked free cTnT (mean recovery 73%). Since the inhibitory effects of TnAAbs on the detection of both cTnI and cTnT were observed only for the cTnI-cTnT-TnC complex, we suggest that TnAAbs are specific to structural epitopes that are formed by closely located cTnI and cTnT polypeptide chains. The negative effect of TnAAbs on the measurements of endogenous cTnI in AMI samples was significantly less pronounced compared to measurements of spiked cTnI in the form of ternary complex. The mean recovery was 61.4% vs. 10.3%, respectively. **Conclusion:** Unlike the common notion, our study shows that the anti-cTnI TnAAbs are not specific to cTnI per se but to the structural epitopes formed by cTnI and cTnT polypeptide chains. In our experiments, added TnAAbs have a rather limited impact on the immunodetection of cTnI in AMI samples probably because TnAAbs affect the measurements of cTnI and cTnT in ternary complex, whereas the predominant form of cTnI in the blood of patients is believed to be a cTnI-TnC binary complex.

## A-087

### Antibodies and recombinant standards for the Lp-PLA2 fluoroimmunoassay.

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Background. Lipoprotein-associated phospholipase A2 is an enzyme that plays an important role in vascular inflammatory processes involved in the development of atherosclerosis. The blood level of Lp-$PLA_2$ has been shown to correlate with the risk of adverse coronary events in apparently healthy people as well as in patients with stable coronary disease. The blood concentration of Lp-$PLA_2$ is currently measured by immunoassays. The aim of this study was to develop a reliable and sensitive Lp-PLA2 immunoassay. To facilitate this, we developed monoclonal antibodies that detect Lp-$PLA_2$ with high sensitivity and are capable of recognizing Lp-$PLA_2$ as a part of lipoprotein complexes, as well as a recombinant antigen with biochemical and immunochemical properties similar to the native protein presented in human blood.

Methods. Recombinant human Lp-$PLA_2$ was produced in two different expression systems: human cell line expi293f and insect cell line HighFive. The recombinant proteins were purified by several chromatographic steps. The recombinant Lp-PLA2 was used as the immunogen in antibody development. Six murine monoclonal antibodies (mAbs) specific to human Lp-$PLA_2$ that was obtained were tested as capture and detection (labeled with stable $Eu^{3+}$ chelate) antibodies in sandwich fluoroimmunoassays. Serum samples from patients with acute myocardial infarction (AMI) and healthy donors were used in the analyses. Serum dilution experiments were performed in serum specimens from healthy donors.

Results. Two mAb combinations, PL26- PL4 and PL42-PL46 (capture - detection) recognized both recombinant Lp-$PLA_2$ preparations in a similar manner. They demonstrated a good linearity range (2-600 ng/ml and 1-1000 ng/ml respectively) and required sensitivity (0.2 ng/ml for PL42-PL46).

Both assays also recognized native human Lp-$PLA_2$ in the blood of two groups of donors - healthy volunteers and AMI patients. The titration curves of serum samples and recombinant proteins were parallel, which suggests that native (from human blood) and recombinant forms of Lp-$PLA_2$ have similar immunoreactivity in these fluoroimmunoassays. When spiked into normal human serum both recombinant Lp-$PLA_2$ proteins formed complexes with serum lipoproteins and in gel-filtration studies were found in the same lipoprotein fractions as native Lp-$PLA_2$. In stability studies, both recombinant Lp-$PLA_2$ forms demonstrated consistent immunoreactivity during 6 months of incubation of the protein solution (1 mg/ml) at +4°C.

Conclusions. Monoclonal antibodies obtained in this study could be used for the development of a sandwich immunoassay for quantitative measurements of Lp-$PLA_2$. Both recombinant forms of human Lp-$PLA_2$ demonstrate immunoreactivity which is similar to that of native protein presented in human blood and could be used as standards or calibrators in an Lp-$PLA_2$ immunoassay.

## A-088

### Different susceptibility of BNP and proBNP to neprilysin cleavage suggests a limited effect of neprilysin inhibition by LCZ696 on the level of immunoreactive BNP

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**Background:** The new FDA approved HF drug LCZ696 (Entresto™, Novartis), which combines the neprilysin inhibitor and the angiotensin II receptor inhibitor, has stimulated the interest of the cardiology community in neprilysin. This ubiquitous protease is responsible for the degradation of various important vasoactive peptides, including natriuretic peptides (NPs). In light of this, the inhibition of neprilysin activity for the augmentation of the endogenous NPs concentrations has been considered to be a potential therapeutic strategy in HF. It was suggested that by increasing the circulating concentrations of B-type natriuretic peptide (BNP), LCZ696 could make BNP measurements ambiguous and misleading from a diagnostic perspective. However, the major form of plasma BNP-immunoreactivity in HF patients is represented by its uncleaved precursor proBNP, which differs from BNP due to the presence of the 76 amino acid N-terminal extension. Therefore, the inhibition of neprilysin in HF patients should affect the level of proBNP rather than BNP. To the best of our knowledge, neprilysin mediated degradation of proBNP has never been analyzed. Furthermore, susceptibility of different BNP epitopes to neprilysin-dependent proteolysis is unknown. Hereby, the aim of the present study was to compare the susceptibility of two different epitopes within the central region of BNP and proBNP to cleavage by neprilysin.

**Methods:** BNP 1-32 (synthetic) as well as non-glycosylated (expressed in E. coli) and glycosylated (expressed in mammalian cells) forms of proBNP 1-108 were incubated with human recombinant neprilysin for different time periods. The susceptibility of two different epitopes of BNP and proBNP that are recognized by antibodies in commercial BNP assays to neprilysin cleavage was analyzed using two sandwich immunoassays. In the first assay, the mAb KY-BNP-II (epitope 14-21) was used as a capture antibody and the mAb 50E1 (epitope 26-32) was used as a detection antibody. The second assay was the Single Epitope Sandwich BNP assay (SES-BNP™) that is specific to the epitope 11-17. Mass spectrometry was applied to determine the sites of BNP cleavage by neprilysin.

**Results:** Both forms of proBNP, glycosylated and non-glycosylated, were resistant to the degradation by neprilysin. As follows from both immunochemical and MS analysis, proBNP remained intact even after prolonged incubation with neprilysin. In the case of BNP, the epitope 14-21 that contains the known $Arg_{17}$-$Ile_{17}$ neprilysin cleavage site was much more susceptible to cleavage by neprilysin than the epitope 11-17 that was utilized in the SES-BNP assay.

**Conclusion:** Our findings demonstrate that the major BNP-immunoreactive form, proBNP, is not susceptible to neprilysin cleavage. On this basis, we speculate that modulation of neprilysin activity by specific inhibitors (e.g. LCZ696) may not greatly affect the circulating concentrations of immunoreactive BNP, which in HF is mostly represented by intact proBNP. The different stability of BNP epitopes highlights the importance of the choice of antibodies for reliable BNP immunodetection: BNP immunoassays that utilize antibodies with epitopes comprising the site $Arg_{17}$-$Ile_{18}$ are expected to be more sensitive to proteolysis by neprilysin than immunoassays that utilize antibodies with other specificity.

## A-089

### Younger age and women lower the upper reference limit for high-sensitivity troponin T in a large multi-ethnic population

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**Purpose**: We sought to ascertain the effect of age on the 99th percentile upper reference limit (99PURL) for high-sensitivity cardiac troponin-T (hs-cTnT) from a large multi-ethnic population as the current recommended cut-point of 14 ng/L is derived from younger subjects while patients evaluated for chest pain are older.

**Methods**: Previously we found the hs-cTnT all-subject, male, and female 99PURL to be 16.0, 18.0 and 11.0 ng/L respectively from 1659 (816 men) apparently healthy subjects (questionnaire) aged 30-65 (mean 46.6±10.12). An additional 572 healthy persons aged 20-29 (241 men) and 233 individuals aged 66-98 (124 men) were studied. The hs-cTnT assay has a limit of detection (LoD) of 5 ng/L. and a 10% assay CV corresponding to 13 ng/L.

**Results:** Hs-cTnT concentrations (range: <3–64 ng/L) are shown in the Table. Detectable hs-cTnT concentrations (>LoD) were seen in 32.1% of all participants, and higher in men (51.1%) than women (14.7%). In both the older group (<98y) and the younger cohort (<65y) successive inclusion of younger subjects below 50 years resulted in a lowering of the hs-cTnT 99PURL.

**Conclusion:** Younger age decreases the troponin-T 99PURL due to a higher proportion of undetectable (<LOD) hs-cTnT values especially in women as the hs-cTnT assay may not exhibit high sensitivity performance in these subjects. Composition of the reference population, especially age and sex, impacts the determination of the hs-cTnT 99PURL. Separate hs-cTnT cut-points for chest pain evaluation and community screening may be needed. As troponin 99PURL is a key decision metric in cardiology, requisite guidelines for constituting a reference population should be provided.

| AGE GROUP | | 50-98 | 40-98 | 35-98 | 30-98 | 20-98 | 50-65 | 40-65 | 35-65 | 30-65 | 20-65 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Males | n | 465 | 704 | 821 | 940 | 1181 | 341 | 580 | 697 | 816 | 1057 |
| | Mean Age y | 61.2 | 55.5 | 52.8 | 50.2 | 45.1 | 56.9 | 51.7 | 49.2 | 46.7 | 41.8 |
| | TnT>LoD % | 77.6 | 65.8 | 60.8 | 56.7 | 51.1 | 67.4 | 57.4 | 52.9 | 49.4 | 44.6 |
| | 99PURL ng/L | 34.0 | 29.9 | 27.6 | 25.6 | 24.2 | 19.6 | 18.2 | 18.0 | 18.0 | 17.0 |
| Females | n | 458 | 701 | 827 | 952 | 1283 | 349 | 593 | 718 | 843 | 1174 |
| | Mean Age | 60.8 | 55.2 | 52.4 | 49.7 | 43.3 | 56.7 | 51.8 | 49.2 | 46.6 | 40.5 |
| | TnT>LoD % | 35.6 | 25.2 | 21.9 | 19.5 | 14.7 | 24.9 | 16.9 | 14.5 | 12.9 | 9.4 |
| | 99PURL | 21.4 | 19.0 | 16.9 | 16.0 | 16.0 | 15.0 | 12.0 | 11.0 | 11.0 | 10.0 |
| ALL | n | 923 | 1405 | 1648 | 1892 | 2464 | 690 | 1173 | 1415 | 1659 | 2231 |
| | Mean Age | 60.9 | 55.3 | 52.6 | 49.9 | 44.2 | 56.8 | 51.8 | 49.2 | 46.6 | 41.1 |
| | TnT>LoD % | 56.8 | 45.6 | 41.3 | 38.0 | 32.1 | 45.9 | 36.9 | 33.4 | 30.9 | 26 |
| | 99PURL | 25.8 | 23.9 | 23.0 | 23.0 | 21.0 | 18.1 | 17.0 | 16.8 | 16.0 | 14.2 |

## A-090

### A whole blood high-sensitivity cardiac troponin assay for POCT

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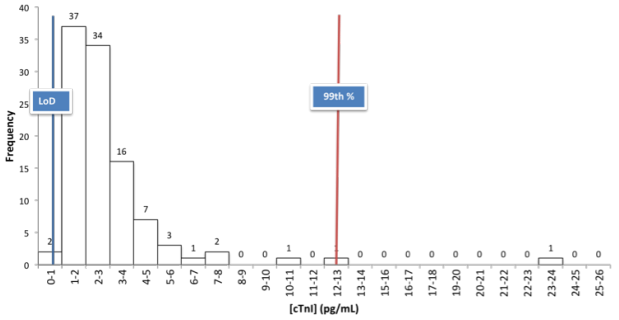
**Background**: The potential life-saving and cost-reduction value of high-sensitivity cardiac troponin (hs cTnI) in the emergency room, in particular as a rule-out, has captured wide attention. We are developing a fully automated hs cTnI assay using the Pylon™ technology designed to deliver central lab performance in the point-of-care setting (abstract submitted). A Pylon test strip with a quartz-glass probe tip, coated with analyte-specific antibody, moves through a test strip as it picks up the sample and goes through the steps of a classic sandwich assay. The assay uses epitopes for capture and signal antibodies common to hs cTnI assays reported in the literature.

**Methods**: The reference range study used 500 EDTA plasma samples from presumably healthy individuals and calibration values assigned by NIST SRM 2921. To assess analytical performance using whole blood, samples from 105 healthy blood bank donors were tested against corresponding EDTA plasma samples.

**Results**: The Pylon cTnI assay detected more than 95% of the healthy group samples. Preliminary data showed that 99th percentile is estimated to be 12 pg/mL, with precision of <10% CV. LoD is within the range of 0.5-1.0 pg/mL; LoQ is ~2 pg/mL. There is no interference from samples containing human anti-mouse antibodies. Spike recovery of whole blood samples is >90% at 10, 100 and 1000 pg/mL cTnI. There is good correlation between whole blood samples and corresponding plasma samples (R=0.92); precision is comparable. Quantified value of >95% whole blood samples is higher than LoD. Time to results is about 20 minutes.

**Conclusions**: The Pylon cTnI assay met the definition of a fourth-generation hs cTnI assay, with <10% CV at the 99th percentile and detection in >95% of reference population. This will be confirmed by testing the assay using the AACC normal range sample bank when it becomes available.




**Whole blood cardiac troponin I distribution in healthy individuals (n=105)**

## A-091

### Cardiac Marker Test Utilization Strategy for Impacting Change

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**Background**: Cardiac biomarkers and their use in clinical practice have evolved over the years. Specifically, cardiac troponin assays have become the biomarker of choice due to their organ specificity, analytical sensitivity and low imprecision. As troponin assays continue to advance, the need for less specific cardiac biomarkers have been debated, offering an opportunity to affect lab test utilization in the hospital. In many cases, education through peer reviewed medical journals can provide enough evidence for change. In some cases, however, embracing change after years of familiar practice can require different strategies. This poster outlines one such strategy, to provide internal cardiac biomarker results for the patient population served. The strength of this strategy is tied to the high performance and proper use of the cardiac Troponin I test available in the network.

**Methods**: Two ad hoc reports were generated for data analysis. One report was pulled from the laboratory information system, Sunquest v.7.1 and Ad Hoc Report Writer™, and one from the health care information system, EPIC™. The Abbott ARCHITECT Cardiac Troponin and CK-MB testing were performed per the manufacturer's recommendations.

**Results**: Data was summarized around two core questions. 1.) How many patients had negative troponin values (<99%ile), using the Abbott ARCHITET cardiac Troponin I (TnI) assay in combination with a positive CK-MB result on the same blood collection. 2.) How many patients that fit these criteria had discharge diagnosis codes for Acute Myocardial Infarction (AMI). 254 unique results met the criteria for inclusion into the discharge diagnosis assessment. These data represented ~110 unique patient events. After calculating a CKMB Relative Index for each abnormal CKMB result, only 9% of the positive CKMB results were greater than 5.0 indicating cardiac origin. A discharge diagnosis code for AMI was found in 2/ 110 patients identified. Review of these two cases with staff cardiologists confirmed, in each case, the underlying disease state (Drug overdose and Terminal illness), complicated the cardiac biomarker interpretation and had no impact on downstream clinical decision pathways.

**Conclusion**: Data mining for the purposes of lab test utilization projects can help support change. These findings were shared with the Emergency Medicine and Cardiology physicians and both groups agreed to remove CKMB from their order sets currently in place. This compromise effectively reduced automatic ordering during chest pain work-ups but allowed physicians to order CKMB in selected cases. Interestingly, the data also prompted further discussions within the respective departments on individual ordering patterns and the need for standardized protocols. Clinical and laboratory data will continue to drive test utilization improvements. It also serves as an opportunity for labs to demonstrate leadership and effectively collaborate with physicians and hospital administration.

## A-092

### Serial Changes in High-Sensitivity Cardiac Troponin I in Emergency Department Patients Without Acute Myocardial Infacrtion

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**Background:** Limited studies have reported serial changes of cardiac troponin (cTn) in patients presenting to the emergency department (ED) in the absence of acute myocardial infarction (AMI) according to cTn concentrations above or below the 99th percentile. High sensitivity (hs) cTn assays have improved total imprecision (%CV) compared to contemporary assays, allowing a better assessment of serial cTn changes with minimal analytical influence.

**Methods:** The objective of our study was to examine serial changes in hs-cTnI measured by a research assay (Abbott ARCHITECT; gender-specific 99th percentile cutoffs (GSCs): F 16 ng/L (%CV=5.3), M 34 ng/L (%CV=3.5); analytical limit of detection (LOD): 1.9 ng/L). 1159 patients presenting to the ED who underwent serial cTnI measurements on clinical indications (UTROPIA, NCT02060760) at presentation (baseline) and at 2 to 6 hours and had both concentrations above the LOD were included. Log normal percent reference change values (RCVs, %) based on the median of within-individual cTnI variations were assessed among patients with or without AMI and with baseline cTnI concentrations above or below the GSC.

**Results:** Patients with AMI (74 female, 83 male) had greater absolute and percent hs-cTnI changes compared to non-AMI patients (368 female, 634 male), independent of the baseline cTnI concentration (Table). RCVs were smaller in non-AMI patients with hs-cTnI at presentation above GSC (F -21 to +26%; M -21 to +27%), compared to non-AMI patients presented with below GSC hs-cTnI concentrations (F -34 to +51%; M -26 to + 35%).

**Conclusion:** In patients without AMI, RCVs were smaller among those with increased hs-cTnI concentrations above the GSC at presentation in comparison to those with concentrations below the GSC. Future studies assessing serial hs-cTn changes according to the presence or absence of increased cTn concentrations and its clinical implications needs to be explored.

| Changes in troponin levels in ED patients diagnosed with AMI or non AMI. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Female | | | | Male | | | |
| | Baseline hs-cTnI < GSC | | Baseline hs-cTnI > GSC | | Baseline hs-cTnI < GSC | | Baseline hs-cTnI > GSC | |
| | AMI[2] | Non AMI | AMI | Non AMI | AMI[2] | Non AMI | AMI | Non AMI |
| Number | 3 | 260 | 71 | 108 | 2 | 516 | 81 | 118 |
| hs-cTnI at presentation (ng/L) [1] | 13.4 | 5.0 (3.0-7.1) | 29.5 (15.8-78.5) | 24.1 (17.9-48.5) | 26.6 | 6.7 (3.8-12.3) | 59.8 (22.5-155.8) | 50.5 (37.7-94.9) |
| Changes in hs-cTnI within 2-6 hrs (ng/L) [1] | 21.8 | 1.1 (0.5-2.0) | 24.4 (4.7-227.2) [3] | 3.5 (1.3-10.9) [3] | 148.3 | 1.2 (0.5-2.6) | 47.0 (11.0-283.9) | 8.2 (3.7-20.4) |
| Changes in hs-cTnI within 2-6 hrs (%) [1] | 163 | 21 (10-46) | 51 (15-304) | 12 (5-26) | 574 | 15 (7-34) | 101 (14-324) | 12 (6-25) |
| Percent RCV limit (%, non AMI group) | | (-34-51) | | (-21-26) | | (-26-35) | | (-21-27) |
| [1] Values are expressed as median (interquartile range, IQR). | | | | | | | | |
| [2] IQRs not shown and comparisons with the non-AMI group not performed due to limited sample size in the group. | | | | | | | | |
| [3] $P < 0.05$ from 2-tailed unpaired T test indicates significant difference between the AMI and non-AMI group. | | | | | | | | |

## A-093

### Regional distribution of 99th percentile of high sensitivity troponin I in China

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**Background and Objective**

Currently, the 99th percentile value of serum high sensitivity troponin I(hs-ThI) of the healthy population is used as a critical criteria for acute chest pain diagnosis. However, little information has been reported in Chinese population. Thus, the aim of this study is to establish the 99th percentile value for hs-ThI in a healthy Chinese population in different regions.

**Patients and Methods**

A total of 1,537 cases of healthy subjects were recruited from different regions of China. All patients were carefully scanned for any diseases or factors that might influence cardiac troponin levels. The measurement of hs-ThI was standardized and analyzed by ARCHITECT *i2000*. Quality Controls were monitored to ensure that the differences in regional centers were not due to the analytical system.

**Results and Discussion**

There were significant differences in the regional distribution of the 99th percentile as seen in Table 1, wherein for the Harbin region, the total value, the value for males and the value for females were 29.8pg/ml, 35.8pg/ml and 27.5pg/ml respectively. It was higher than the value of the corresponding groups in other regions($P < 0.05$). The total value, the value for males and the value for females in Beijing were 13.5pg/ml ,23.9 pg/ml and 8.8pg/ml respectively, which were lower than in other regions ($P < 0.05$). The overall value for Shanghai, Zhejiang, Chongqing and Xi'an showed no statistical significance. The 99th percentile was higher in males as compared to females which was also noted in many other studies conducted abroad.

**Conclusion**

The present study may provide a preliminary basis for the differentiation of cut-off values of 99th percentile in the Chinese population.

**Table 1. Regional distribution of 99th percentile of hs-TnI in China**

| Region | Study Subjects | 99th percentile (pg/ml) | | |
|---|---|---|---|---|
| | | Total | Male | Female |
| Beijing | 238 | 13.46 | 23.85 | 8.8 |
| Fujiang | 208 | 26.62 | 28.40 | 13.60 |
| Harbin | 144 | 29.76 | 35.80 | 27.50 |
| Lanzhou | 206 | 22.15 | 29.60 | 19.20 |
| Shanghai | 191 | 19.84 | 22.00 | 14.40 |
| Zhejiang | 210 | 19.72 | 29.07 | 17.20 |
| Chongqing | 164 | 16.50 | 17.20 | 13.60 |
| Xi'an | 176 | 16.16 | 16.17 | 13.74 |
| Total | 1537 | 26.42 | 29.40 | 19.46 |

## A-094

### Comparison of Diagnostic Accuracy of B-Type Natriuretic Factor and CA125 Levels in Patient with Congestive Heart Failure

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**Background:** B-type natriuretic peptide (BNP) is important biomarker for diagnosis of congestive heart failure (CHF), which is secreted by cardiac ventricles in response to volume and pressure overload, and regulates the extravascular volume and vascular tone by renin-angiotensin system. CA125 is a glycoprotein that is generally used for diagnosis and follow up of ovarian malign and benign pathologies. Recently, it was reported that serum CA125 levels are increased in CHF. The purpose of this study to compare diagnostic accuracy of BNP and CA125 for CHF in adult population

**Methods:** The patients (n=251) admitted to Hacettepe University Hospitals, suspected with CHF and underwent into ECHO in 2015, were included into the study. Plasma BNP was measured by chemiluminescent microparticle immunoassay method (CMIA) and serum CA125 was measured by chemiluminescent method.

**Results:** In samples with higher than 39 U/mL of CA125, the median BNP values were 171 pg/mL with 68.5- 593 pg/mL of IQR. In that patients the median CA125 values were 132 U/mL with 70.5-299 U/mL of IQR. In samples with lower than 39 U/mL of CA125 the median BNP values were 108 pg/ml with 51.5-208 pg/mL of IQR. In samples with lower than 80 pg/mL of BNP, the median CA125 values were 130 U/mL with 74.8-235 U/mL IQR.. In ROC analysis, AUC was found as 0.981 for BNP for the first group whereas 0,964 for the second group.

**Conclusion:**

We concluded that CA125 and BNP can be used effectively together in diagnosis of CHF.