**Cardiac Markers**

**Wednesday, July 29, 2015**

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

**Cardiac Markers**

**B-001**

**Heart-type Fatty Acid Binding Protein as a Marker of Ischaemia in Patients Undergoing Percutaneous Coronary Intervention**

R. Bray, P. Collinson, P. Lim, A. Prasad, S. Firoozi, A. Ntalianis, St. George’s Hospital, LONDON, United Kingdom

Objective: To evaluate the potential of heart-fatty acid binding protein (HFABP) as a marker of myocardial ischaemia.

Methods: Percutaneous coronary intervention (PCI) was used as a model for myocardial ischaemia. Fifty patients undergoing planned or elective PCI were prospectively enrolled. Blood samples were taken pre and post balloon inflation and 3 hours after the procedure. Samples were allowed to clot, the serum separated and stored at -20°C until analysis as a batch. Heart-fatty acid binding protein was measured using the Advia 2400 analyser (Siemens healthcare diagnostics). The assay range is 0.747 to 120 ng/mL with a 6.85% CV at 5.47 ng/mL and a 99th percentile of 6.32 ng/mL. Cardiac troponin I was measured on the Advia Centaur with an analytical range of 17 to 50000 ng/mL. The CV is stated as 10% at 30 ng/mL with a 99th percentile of 50 ng/mL.

Results: There was no statistical difference in HFABP pre and post balloon inflation, but HFABP was significantly higher at 3 hours (p<0.05). HFABP was significantly higher in patients who were troponin positive (defined as troponin >50 ng/L or showing a dynamic change) versus those who were troponin negative. There was no significant difference in HFABP in men versus women or those that have had previous PCI procedures. There was no correlation between HFABP and age of patient, number of balloon inflations or total inflation time.

Conclusion: HFABP is not a marker of ischaemia. However, HFABP results were consistent with paired troponin results indicating that it is a marker of cardiac necrosis. This study indicates the ongoing need for cardiac markers for ischaemia and non-MI events.

**B-002**

**High-sensitivity cardiac troponin I in a large community-based population at risk for cardiovascular disease**

A. H. Wu1, J. Estis2, P. Helestine2, K. Bui2, J. Todd2, P. Kavask1, University of California, San Francisco, San Francisco, CA, 1Singulex, Inc., Alameda, CA, 2McMaster University, Hamilton, ON, Canada

Background: With the development of high-sensitivity cardiac troponin assays and the ability to measure cardiac troponin in essentially all healthy individuals, comes the possibility to use this assay for risk stratification for future cardiovascular disease (CVD).

Objective: To determine plasma levels of hs-cTnI and identify the impact of comorbidities on cTnI concentrations in a large at CVD risk population.

Methods: We previously reported the development of a high-sensitivity Single Molecule Counting assay to quantify plasma cTnI. This assay was further developed into a laboratory developed test and offered in a CLIA licensed, CAP accredited laboratory with a LOD of 0.4 pg/mL and the 95th percentile upper limit of normal (ULN) of 4.6 ng/L. Blood samples were measured for hs-cTnI, LDL, HDL and HbA1c in 23,832 (56% female) community-based patients at risk for CVD. Parametric and non-parametric analyses were performed in de-identified data using SAS v9.3.

Results: The distributions of cTnI in the study population are shown in Table. cTnI was quantifiable in 88% patients. Seven percent of the population was above the ULN cutoff. cTnI was significantly higher in patients >50 yrs, males, as well as those with CVD risk factors of pre-diabetes, diabetes and HDL dyslipidemia, with those patients having HbA1c >5.9% having the highest concentrations.

Conclusions: This is the largest community cohort assessing hs-cTn concentrations in patients at risk for CVD, as determined by their primary care physician. Differences exist between gender and age which may represent subclinical disease; as evident by patients with HbA1c >5.9% having the highest cTn concentrations. Based on these results, it may be possible to establish hs-cTnI cutoff concentrations for subgroups that may more accurately define at risk patients than use of a single 99th percentile for a healthy population. Health outcome studies are required for validation of these levels.

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<tr>
<td>Females</td>
<td>0.8</td>
<td>4.6</td>
<td>15.5</td>
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<tr>
<td>Males</td>
<td>1.2</td>
<td>7.6</td>
<td>27.0</td>
<td>9.4</td>
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<tr>
<td>&gt;=50 y</td>
<td>1.1</td>
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<td>HbA1c &lt;=5.9%</td>
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<tr>
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**B-003**

**Changes in the utilization of evidence-based recommendations for the biochemical diagnosis of acute myocardial infarction**

P. O. Collinson1, A. Hammerer-Lercher2, M. van Dieijen-Visser3, K. Pulkki4, J. Suvisaari5, A. Staviljen-Rukavina6, H. Baum7, C. Duñ8, A. Aakre9, M. Langlois10, S. Stankovic11, P. Laitinen12. St George’s Hospital, London, United Kingdom, 1University Hospital Innsbruck, Innsbruck, Austria, 2Maastricht University Medical Center, Maastricht, Netherlands, 3University of Eastern Finland, Kuopio, Finland, 4Helsinki University Central Hospital, Helsinki, Finland, 5University of Zagreb, Zagreb, Croatia, 6Regionale Kliniken Holding RKH GmbH, Ludwigshurg, Germany, 7University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom, 8Haukeland University Hospital, Bergen, Norway, 9AZ St-Jan Hospital Bruges, Bruges, Belgium, 10Clinical Center of Serbia, Belgrade, Serbia

Objective: To assess the progress towards the utilization of evidence-based practice for the diagnosis of acute myocardial infarction in Europe.

Methods: Three consecutive audits in 2006 (pilot study), 2010 and 2013 were performed using a web-based questionnaire distributed to European biochemical societies for circulation to their membership. Questions covered cardiac biomarkers measured, decision thresholds and their derivations, sampling strategies, repeat sample interval and use of decision-making protocols. Results were collated and linked into a central database, the data coded and then analysed using comparative and descriptive nonparametric statistics.

Results. Returns were obtained from 8 (pilot), 39 and 35 respectively by year, countries throughout Europe (220, 303, 442 responses). Central or University hospitals provided respectively (by year) 55%, 58% and 39% of responses with 39%, 35% and 39% from local (community) hospitals (Chi² 2006, 31.4% 2010) Chi² 2006, 31.4% 2010) Chi² 2006, 31.4% 2010) Chi² 2006, 31.4% 2010). In acute units in 2013, 90% continued to offer creatine kinase (CK) or CK MB isoenzyme continue to be offered as supplementary markers either as part of a cardiac marker panel or on request in 72% of hospitals. However, 25.6% continue to offer CK-MB activity. The usual reason cited was by 15.6% of laboratories, (25.5% in 2006, 35.6% in 2010). In acute units in 2013, by only 1.1%. Lactate dehydrogenase or hydroxybutyrate dehydrogenase is offered by 52.7% as part of the cardiac profile in 2006, 34% in 2010 but is now offered by 104.2, p,0.0001). Aspartate transaminase was significantly higher in patients >50 yrs having the highest concentrations. Based on these results, it may be possible to establish hs-cTnI cutoff concentrations for subgroups that may more accurately define at risk patients than use of a single 99th percentile for a healthy population. Health outcome studies are required for validation of these levels.

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S121
Cardiac Markers

B-004 Reference Range Study Using High Sensitivity cTnl Assay in Development for the Sgx ClarityTM Single Molecule Counting Platform

L. Shephard, S. Florey, Y. Cheung, R. Livingston, L. Monsalve, J. Todd, J. Bishop, J. Felberg, Singulex, Alameda, CA

Background: The Research Use Only (RUO) cardiac Troponin I (cTnI) assay developed by Singulex for the ERENNA® instrument provided the first evidence that cTnI is measurable in almost all healthy subjects. With that knowledge, it has become clear that cTnI is not only a valuable marker for diagnosing acute myocardial infarction, but it may also be useful as a prognostic indicator for underlying heart disease. Singulex is now developing the Sgx Clarity System, a fully-automated in vitro diagnostics platform that, similar to the ERENNA system, utilizes Single Molecule Counting technology. The new system will be a clinical diagnostics instrument utilizing a second generation scanning-based detection system which has equal sensitivity to the ERENNA with an improved throughput and an extended dynamic range. The cTnI assay in development for the Sgx Clarity System is based on the 2 + 2 antibody concept introduced by HyTest, with two capture and two detection antibodies. One antibody of each pair recognizes an epitope in the stable central portion of the cTnI molecule, while the second recognizes an epitope in the N- or C-terminal region, thus conferring overall robustness to known cTnI-specific interferences.

Objectives:
To estimate the 99th percentile of cTnl values in apparently healthy subjects on the Sgx Clarity system using a set of reference range samples and to assess preliminary performance characteristics of the assay.

Methods:
For the reference range study 120 male and 120 female EDTA plasma samples from self-declared healthy donors were collected from five states across the US. The 99th percentile was calculated using a nonparametric method using StatsProTM software and gender specific results were compared. Additional performance characteristics were assessed according to protocols based on CLSI guidelines where available.

Results:
The 99th percentiles were 13.38 pg/mL and 4.90 pg/mL for male and female donors, respectively. The 99th percentile for the overall population was 11.11 pg/mL. Based on this analysis, the recommendation for gender specific reference ranges should be considered. A precision profile was generated from the duplicate samples less than 1 pg/mL in the reference range study, and the within-run 10% and 20% functional sensitivities were calculated at 0.31 and 0.13 pg/mL, respectively. Troponin values were quantifiable above the 20% functional sensitivity in 100% of samples tested. The reportable range goes up to 25 ng/mL with no high dose hook effect up to 1000 ng/mL, thus giving the assay a 5 log linear dynamic range. No significant impact from common endogenous interferences was observed and the assay formulation was shown to be robust against common cTnI-specific interferences such as heparin, proteolytic cleavage, phosphorylation, and cTnI-C complex. Furthermore, minimal cross-reactivity was observed when tested with 1000 ng/mL of sTnI (0.019%), cTnT (0.03%) and TnC (0.0005%).

Conclusion:
The cTnI assay in development for the Sgx Clarity System demonstrates sensitivity and precision that is equivalent to the ERENNA RUO assay, sufficient to quantify cTnI in 100% of apparently healthy donor samples. This sensitivity allows for the determination of gender specific differences in the 99th percentile of Normal cTnI values.

B-005 Association of Blood Homocysteine levels with Subclinical Coronary Atherosclerosis in Asymptomatic subjects


Background: Atherosclerotic plaques progression has been known to be correlated to elevated circulating homocysteine (Hcy) due to increased thrombogenicity, oxidative stress and endothelial dysfunction. But it remains to be unclear whether the level of Hcy is related with the coronary atherosclerosis in subclinical state. Therefore, we performed this study to investigate the relationship between blood Hcy levels and subclinical atherosclerosis in asymptomatic self-referred subjects.

Methods: We retrospectively enrolled 2,968 self-referred asymptomatic subjects (1,374 men, 1,594 women) who had undergone both coronary CT angiography (CCTA) and coronary artery calcium scoring. The relationships between atherosclerosis, Hcy, and other clinical factors were assessed. The subjects were divided into 4 quartile groups (7.7, 7.7-9.0, 9.1-10.9, >10.9 μmol/L) . We investigated the association of each Hcy quartile with coronary artery calcium score (CACS), coronary plaque, coronary stenosis.

Results: High level of Hcy was related with age (P<0.001), male gender (P<0.001), body mass index (BMI) (P<0.001), waist circumference (P<0.001), Blood pressure (P<0.001), high density lipoprotein (HDL) (P<0.001), Triglyceride (P=0.003), Blood glucose (P<0.001), HbA1c (P=0.01), hsCRP (P=0.006), the number of plaques (P<0.001), extent of coronary stenosis (P<0.001), CACS (P<0.001). The factors associated with CACS were age, Hcy, HbA1c and hsCRP. Logistic regression analysis adjusted for gender and confounding factors showed that the third- and fourth-quartile Hcy level groups had higher odds ratios [odd ratio (OR) 3.990 (1.723-9.195), P=0.001, 7.355 (3.291-16.439), P<0.001, respectively] for high CACS (CACS >400) than did the first quartile group. Coronary plaque was more frequently found in higher Hcy quartile groups (21.3%, 28.8%, 34.4% and 34.3%, P<0.001). Significant coronary artery stenosis (stenosis>50%) was also more frequently found in higher Hcy quartile groups (1.8%, 5.4%, 5.0%, 6.6%, P<0.001).

Conclusion: High levels of blood Hcy were related with an increased risk of the presence and the extent of subclinical atherosclerosis in asymptomatic subjects.

B-006 Characterization of plasma Endothelin-1 in a large community-based patient population at risk for cardiovascular disease

A. H. Wu1, J. Estis2, P. Heszeltine2, T. Dang2, J. Todd2, P. Kavask2.1University of California, San Francisco, San Francisco, CA, 2Singulex, Inc., Alameda, CA, 3McMaster University, Hamilton, ON, Canada

Background: Endothelin (ET-1, (aa1-21)) is a potent physiological vasoconstrictor that plays a pivotal role in vascular dysfunction. Elevated, plasma ET-1 correlates with both the occurrence and severity of atherosclerosis and heart failure; however, no reports characterize ET-1 in a population, judged at-risk for cardiovascular disease (CVD) using traditional biomarkers (lipids, hsCRP, HbA1c).

Objective: To determine plasma levels of ET-1 and identify the effect of co-morbidities on its levels in a large at CVD risk study population.

Methods: We previously reported the development a Single Molecule Counting immunoassay to quantify plasma ET-1. This assay was further developed into a laboratory developed test and offered in a CLIA licensed, CAP accredited laboratory with a LoQ (20%CV) of 0.2 pg/mL and a 95%ile (upper limit of normal; ULN) of a healthy reference population of 3.7 pg/mL. Plasma ET-1 as well as other blood biomarkers including LDL, HDL and HbA1c were measured in a non-selected community-based population at risk for CVD (n=8,422 patients). Identifying information was removed and the data were analyzed via parametric and non-parametric analyses (SAS v9.3, P<0.05 considered significant).

Results: The characteristics of ET-1 in the CVD risk population are shown in Table. ET-1 was quantifiable in >99.9% patients and 5.9% were above the ULN. ET-1 concentrations were slightly higher in patients >50 yrs, males, as well as those with CV risk factors of pre-diabetes, diabetes and HDL dyslipidemia. Of note ET-1 was paradoxically higher in patients with low LDL.

Conclusions: This is the first report of ET-1 concentrations in a large community-based population at risk for CVD, as determined by their primary care physician. Minor differences exist between subgroups; however, an overall ULN of 3.7 pg/mL appears to be appropriate, and unlike high-sensitivity cardiac troponin I, age and gender specific reference ranges may not be required.
**B-007**

Current use of evidence-based recommendations for the biochemical diagnosis of acute myocardial infarction in routine clinical practice - a comparison of European and North American practice.


Objective. To assess current use of evidence-based guidelines for the biochemical markers for the diagnosis of acute myocardial infarction in Europe and North America.

Methods. In 2013/14 a web-based questionnaire was distributed to North American and European biochemical societies for circulation to their membership. Questions covered cardiac biomarkers measured, analytical methods used, decision thresholds and their derivations, sampling strategies, repeat sample interval, use of rate of change and use of decision-making protocols. Results were collated using a central database and analysed using comparative and descriptive nonparametric statistics.

Results. In Europe (E), returns were obtained from 442 hospitals, 50% Central or University hospitals and 39% from local hospitals from 35 countries. 395/442 (89%) provided an acute service and were analysed further. In North America (NA) there were 91 responses (63.7% Central or University hospitals, 19.8% community hospitals) with 76/91 (83.5%) providing an acute service. Cardiac troponin was the preferred cardiac biomarker in 99.5% (E) and 98.7% (NA) and the first line marker in 97.7% (E) and 97.4% (NA). Of those markers no longer recommended, in Europe aspartate transaminase was still offered in 1.0% with lactate dehydrogenase in 15.2%, with only 0% and 2.6% in North America, respectively. Creatine kinase (CK) or CK MB continue to be offered as supplementary markers either as part of cardiac marker panel or on request in 72% (E) and 39.5% (NA).

There were significant differences in the choice of decision limits and their derivations. Decision limits (E vs NA) were: 20% CV - 16.2% vs 24.2%; 10% CV - 2.0% vs 8.1%; 99th percentile - 52.3% vs 45.2%; other - 29.5% vs 22.6%; p = 0.02. The origin of the information was also significantly different, with E vs NA as follows: package insert - 61.9% vs 40%; publications - 17.1% vs 15.0%; local clinical or analytical validation choice - 21.0% vs 45.0%; p = 0.0003.

Conclusion. There are significant differences between European and North American use of cardiac biomarkers. This probably relates to different availability of assays between Europe and North America (such as high sensitivity troponin assays) and different laboratory practices on assay introduction (greater local evaluation of assay performance occurred in North America). Better awareness, agreement, adaptation and adherence with evidence-based guidelines is needed. This survey indicates that more effective strategies for disseminating choice of cardiac marker testing and diagnostic cut-off limits is needed.

**B-008**

An ARCHITECT Assay for Quantitation of Galectin-3 in Human Serum and Plasma


BACKGROUND: Galectin-3 has been implicated in a variety of biological processes associated with heart failure (HF). An assay for galectin-3 on ARCHITECT i series (ARCHITECT Galectin-3) has been cleared in the US for the quantitative determination of galectin-3 in human serum and EDTA plasma and may be used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure (CHF).

METHODS: ARCHITECT Galectin-3 is a chemiluminescent microparticle immunoassay (CMIA) utilizing paramagnetic microparticles coated with mAb M3/38 and an acridinium- labeled mAb 87B5 conjugate. Sample and microparticles are mixed and incubated for 1.5 min. After a wash, the conjugate is added to the mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting signals in relative light units (RLUs) is directly proportional to the amount of galectin-3 in the sample and allows quantitative determination of galectin-3 in serum and EDTA plasma.

RESULTS: The calibration range for the assay is 0.0 to 114.0 ng/mL. The LoB, LoD and LoQ were 1.0, 1.1 and 2.8 ng/mL, respectively. Linearity supports a measurement range of 5.5 to 103.1 ng/mL. There was No High Dose Hook effect when samples containing up to 1420.5 ng/mL galectin-3 were assayed. The sample tube type study, a matrix comparison of > 40 matched serum and EDTA plasma samples from CHF patients demonstrated a linear correlation (Slope: 0.9 - 1.1; r ≥ 0.9) within the measurement range for each tube type when compared to the Red Top serum samples. In the interference studies, 11 endogenous substances spiked individually into sera and plasma were tested at the following levels: (≥) 1000 ng/mL HAMA, 800 IU rheumatoid factor, 40 mg/dL conjugated bilirubin, 40 mg/dL unconjugated bilirubin, 5 g/dL human gama globulin, 5 mg/dL creatinine, 5 mg/mL whole blood lysate, 3000 mg/dL triglycerides, 250 mg/dL hemoglobin, 500 mg/dL cholesterol, and 12 g/dL human serum albumin. The average percent difference between test and control samples for all endogenous interferers was ≤ 10%. Percent difference in the presence of 35 potentially interfering drugs was ≤ 4.0%. The potential cross-reactants (galectin-1, galectin-2, galectin-4, galectin-7, galectin-9, galectin-12, collagen type 1, and collagen type III) were evaluated at a concentration of 500 ng/mL. The percent cross-reactivity of the potential cross-reactants was ≤ 0.3%. The Precision study of 3 controls and 5 panels demonstrated a total %CV ≤ 5.8%. In a clinical performance study of 405 CHF patients, elevated baseline levels of galectin-3 (> 17.8 ng/mL) in CHF patients were shown to be significantly and independently associated with a higher risk of hospitalization due to worsening HF, ventricular assist device placement, cardiac transplantation or all-cause mortality (first to occur).

CONCLUSION: The ARCHITECT Galectin-3 assay is an accurate, precise, and sensitive assay for the quantitative determination of galectin-3 in human serum and EDTA plasma. The ARCHITECT Galectin-3 assay may be used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure.

**B-010**

Circadian Rhythm of Cardiovascular Disease-Related Micro RNAs from HemaSpot™ Dried Blood Samples

R. T. Taylor, A. Man, J. E. Hill, J. Repass, J. Hill. 1Spot On Sciences, Austin, TX, 2ARQ Genetics, Bastrop, TX

Background: Disruption of the body’s circadian rhythm has been linked to numerous chronic cardiovascular diseases (CVD) such as insomnia, and hypertension. Changes in gene expression of cardiovascular pathways by mirco RNAs (miRNAs) have been identified as potential biomarkers of CVD. miRNAs are short (~22 nucleotides), single-stranded, non-coding, posttranscriptional regulators expressed in various tissues including circulating blood. MicroRNAs are ideal biomarkers for disease as they are abundant, relatively stable, and easily quantifiable. Dried blood spot (DBS) sampling...
technology has advanced to provide assessment of numerous analytes and blood-based biomarkers including proteins, DNA and micro RNA (miRNA). In this study we utilize the HemaSpot blood collection device to analyze the expression profiles of miRNAs that have been shown to be differentially regulated in individuals with cardiovascular diseases. By understanding the expression patterns of CVD related miRNA in healthy individuals, we seek to identify changes in expression of these biomarkers on DBS samples from at-risk or CVD patients.

Methods:
2-3 drops of whole blood were collected by finger stick onto the HemaSpot HF device every four hours (8 time points) over a 24 hour period from five healthy individuals. Total RNA was extracted from DBS samples and Taqman ® RT-PCR analysis was performed to determine the expression profile of six miRNAs (miR21, miR133a, miR150, miR155, miR494, and RNU48). The relative quantitation of each CVD-related miRNA was normalized to that of the non-variable small RNA molecule RNU48.

Results:
miR133a shows a cyclic expression pattern with an average early morning peak approximately 9:30 am all five individuals over a 24-hour period. MiR155 displays peak expression at noon (mid-day) of the 24-hour cycle.

Conclusion: The HemaSpot device proves to be an optimal tool for studying circulating miRNA expression profiles from dried blood spot samples. Cyclic expression of miRNAs may prove to be significant biomarkers for regulation of gene expression of cardiovascular diseases.
Circulating myostatin levels in heart failure with preserved ejection fraction
T. B. Dschietzig1, C. Georgi2, U. Fahlenkamp1, J. Ruppert1, F. P. Armbruster1, I. Diaz3, Immunodiagnostik AG, Bensheim, Germany, 3Charité - University Medicine, Berlin, Germany

To date, heart failure with preserved ejection fraction (HFPEF) represents a growing medical and economic challenge since, in contrast to HF with reduced EF (HREF), there is no evidenced-based therapeutic approach that lowers mortality. Hence, a better comprehension of the complex pathophysiology of HFPEF is urgently needed. Myostatin, a negative regulator of skeletal muscle growth, has been implicated in the regulation of myocardial hypertrophy and cardiac cachexia; however, no data are available on myostatin levels in HFPEF. This observational study investigated circulating myostatin levels in femoral artery (A) and vein (V) as well as coronary sinus (CS) of 43 patients (n = 17, controls without HF; n = 19, HREF; n = 7, HFPEF) undergoing ablation for atrial fibrillation.

First, we detected significant differences between myostatin levels measured at A, V, and CS neither in controls, HREF, nor HFPEF; nor were there significant differences between controls, HREF, and HFPEF. In a multiple linear regression analysis including clinical and echo parameters, we determined the following: Relative LV mass and CS myostatin correlated inversely (univariate r = -0.43, P = 0.02) in 28 patients (9 controls, 13 HREF, 6 HFPEF), in which the quality of the procedure-related CT scan allowed for determination of LV mass. In all 43 patients, diastolic dysfunction as measured as E/E' correlated directly with CS myostatin (r = 0.50, P = 0.001).

In conclusion, the circulating levels of myostatin appear not to be altered in HFPEF patients as compared with patients displaying atrial fibrillation without heart failure. Nevertheless, the strong correlations of CS myostatin with E/E' and LV mass point to significant myocardial actions of the peptide: The positive association with diastolic dysfunction may reflect myostatin-induced cardiac fibrosis while the inverse relation to LV mass presumably indicates its anti-hypertrophic effect.

High Sensitivity Cardiac Troponin I and T Assays for Predicting Heart Death in a Hemodialysis Population
F. S. Apple1, R. Ler1, S. A. Love1, Y. Hu1, D. Gilbertson2, M. M. Murakami1, S. M. Brunelli1, A. Young1, C. A. Herzog1, 1Hennepin County Medical Center, Minneapolis, MN, 2Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN, 3DaVita Clinical Research, Minneapolis, MN

Objective: This study determined the prognostic value of hs-cTnI and hs-cTnT assays for all-cause death in 2499 hemodialysis patients. Methods: Hazard ratios of all-cause death were determined using hs-cTnT and hs-cTnI (ARCHITECT i1000). hs-cTnI (Roche Cobas e411) assay concentrations (EDTA plasma) based on quintile distributions from a hemodialysis patient biorepository enrolled between May 2011 and November 2012. Increased concentrations were based on sex-specific 99th percentiles: hs-cTnI male 34 ng/mL, female 16 ng/mL; hs-cTnT male 20 ng/mL, female 13 ng/mL. Unadjusted relative risks of death were compared between dialysis vintage of <1 year (n = 316) vs vintage ≥1 year (n = 2176). Results: 463 deaths occurred during a 3-year follow-up, n = 40 for vintage <1 year and n = 414 for vintage ≥1 year. For vintage <1 year, three-year probabilities of death were 12.4% [CI 8.0%, 19.1%] (n = 236) with increased hs-cTnI (<0.0001); 0% (n = 8) with normal hs-cTnI and 22.2% [14.6%, 32.8%] (n = 308) with increased hs-cTnT (p = 0.26). For vintage ≥1 year, three-year probabilities of death were 20.2% [16.6%, 24.5%] (n = 758) with normal hs-cTnI and 37.1% [30.9%, 44.1%] (n = 1418) with increased hs-cTnI (p < 0.0001); 5.2% [1.4%, 19.1%] (n = 47) with normal hs-cTnT and 26.5% [23.2%, 30.1%] (n = 2129) with increased hs-cTnT (p = 0.023). Unadjusted hazard ratios by quintiles for both hs-cTnI and hs-cTnT by vintage year, with corresponding concentrations, are shown in Table. Both high sensitivity assays showed a greater risk of death over increasing hs-cTn concentrations. hs-cTnI was significantly more predictive of death compared to hs-cTnT in vintage <1 year (p = 0.001) and tended at p = 0.07 at ≥1 year. Conclusions: hs-cTnI and hs-cTnT are both predictors of three-year mortality in hemodialysis patients. However, hs-cTnT appears to be a particularly powerful predictor of death in incident (vintage <1 year) hemodialysis patients.
Cardiac Markers

Evidence-based diagnostic decision limits for cardiac troponin for the biochemical diagnosis of acute myocardial infarction in routine clinical practice.

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Objective. To assess current use of evidence-based decision limits for cardiac troponin for the diagnosis of acute myocardial infarction (AMI) in Europe.

Methods. And of current practice in 2013-14 was performed using a web-based questionnaire by distribution to European biochemical societies for circulation to their membership. Questions covered cardiac biomarkers measured, analytical methods used, decision thresholds and their derivations. Results were collated using a central database and analysed using comparative and descriptive nonparametric statistics.

Results. Returns were obtained from 442 hospitals, 50 Central or University hospitals and 39% from District (Community) hospitals from 35 countries. 395/442 (89%) provided an acute service 11% were non acute laboratories. In 98.6% troponin measurement was the preferred biomarker for diagnosis of AMI.

The decision limit for diagnosis was based on assay imprecision in 71/44/16.1% with the 10% CV in 61 (13.8) and the 20% CV in 10 (2.3%). The 99th percentile was used in 196 (44.3%) an optimised decision thresholds from receiver operating characteristic curve analysis in 5 (1.1%) and a local decision in 104 (23.5%). No data was available for 66 (15%). The choice of value for the decision limit was derived from the manufacturers package insert in 244 (55.2%), from peer-reviewed literature, national or international recommendations in 68 (15%), from locally-based consensus review in 80 (18.1%) No data was available for 42 (9.5%) and 3 laboratories reported they did not use a decision limit.

A detailed analysis of the decision limits used was performed for the Roche diagnostics high sensitivity troponin T (n = 183) and the largest single troponin I group, the Abbott cTnI.

The decision limit used varied from 2 ng/L to 700 ng/L with peaks of utilisation at 30 ng/L, 50 ng/L and 100 ng/L. For the 10% CV a value of 14-100 ng/L was reported and for the 99th percentile 14-400 ng/L. For the Abbott assay 99th percentile 28 ng/L, 10% CV 32 ng/L (8.3%) used 28 and 3 (3.6%) 2. The range used was from 25 ng/L to 500 ng/L with peaks at 30 ng/L, 40 ng/L and 300 ng/L. The 10% CV was reported as 28-500 ng/L and the 99th percentile 28-300 ng/L. Conclusion. There is currently a lack of understanding of the decision thresholds and their derivation which should be in routine clinical use for they diagnosis of acute myocardial infarction using cardiac troponin measurement. Recent publications show that this lack of understanding will result in under diagnosis of preventable disease.

Cardiac biomarkers for early detection and prediction of cardiotoxicity in patients undergoing chemotherapy: Can they help?


Background

Cardiotoxicity after anticancer-drug therapy is an important issue in survivors. Left ventricular ejection fraction (LVEF) is used to diagnose it. Tools for early detection and prediction of cardiotoxicity are needed. Cardiac biomarkers can detect myocardial injury and may play a role in subclinical detection of drug-related toxicity.

Methods

331 patients undergoing chemotherapy with potential cardiotoxicity with a 24m follow-up were enrolled. Blood samples were drawn at baseline, 21d, 3m, 6m, 12m, 18m and 24m. Transthoracic echocardiograms (LVEF) were performed at all visits.

Chemiluminescence assays were used for biomarkers: hs-cTnT(Roche Elecsys), Galectin-3 and hs-cTnI(abbott Architect), c-Tnl and NT-proBNP (Siemens Vista) and 99th percentile (P99) was used as cut-off (hs-cTnI: female 13 male 20 pg/mL CV=10%, hs-cTnI female 15.6 male 24.2 pg/mL CV=4% and c-Tnl 27 pg/mL CV=7.7%). Cardiotoxicity was defined as: LVEF decrease >10% without or >5% with heart failure to a value <55%.

Results

Patient’s age: 58±14.3 years; 84.3% women. Diagnosis: 71% breast cancer; 27.7% leukemia or lymphoma; 1.3% other tumours. Incidence of cardiotoxicity was 10.3% (decreased LVEF from baseline 63.8% to 61.8% at 24m). Maximum value for all troponins occurred at 3m (table 1). Using P99 as cut-off, hs-cTnT identified additional patients when compared to hs-cTn and c-Tnl. Percentage of troponins >P99 were higher in patients with cardiotoxicity. Sensitivity for hs-cTn at 3m for early detection of cardiotoxicity was 76.3% and specificity 94.5%. Positive predictive value was 87.3%, and negative predictive 89.1% and hs-cTnT S=70% E=42.7% NPV=91,1% PPV=17.4%. No significant relation was observed between NT-proBNP and Galectin-3 and cardiotoxicity.

Cardiac Markers

Are Heart Failure Recommendations And Guidelines Established In Practical Laboratory Medicine In Europe, US And Canada? The CarDiaC Marker Guideline Uptake in Europe (CARMAGUE)

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Objective and Relevance. The well-established heart failure (HF) markers B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) are widely used by clinicians. Analytical and clinical recommendations for measurement of these biomarkers have been published. The aim of this survey was to investigate how well these guidelines for measurement of NP have been implemented in laboratory practice in Europe and as a comparison in the United States (US) and Canada. Methodology: Member societies of European Federation of Clinical Chemistry and Laboratory Medicine were invited in January 2013 to participate in a web-based audit-questionnaire. Laboratories in the US and Canada were also invited to participate. The survey was implemented using a web-based survey system consisting of a HTML-AJAX interface and a CGI program storing the results in XML files on a database server. Results from raw data XML files were joined into a tabular format and the numbers of different answers for each question were calculated for further analysis with Microsoft Excel. The questionnaire requested information including type of tests performed, reason for method choice, decision limits for HF and laboratory
Cardiac markers. The survey closed in August 2014. Validation: Preliminary results show that participating laboratories consisted of 29% and 41% university laboratories in Europe and US/Canada, respectively. In Europe, 305 responders out of 494 measured NP as well as 64 out of 135 US/Canadian responders. NT-proBNP was most widely used in Europe (67% of NP offering laboratories) and BNP slightly more (55%) in US/Canada. The reason was availability of instrument in both regions, and in Europe additionally stability and clinician preference. The preferred methods were Roche and Abbott in Europe, and in US/Canada Roche, Abbott and Siemens (Advia Centaur). Most laboratories used ng/l or pg/ml with pmol/l only rarely stated as a unit. More than half of the European responders used the guideline recommended BNP rule-out cut-off of 100 ng/l for acute HF. However, cut-off values for NT-proBNP were diverse and only 5% used the guideline recommended rule-out cut-off of 300 ng/l. Several laboratories (18%) used age dependent cut-off values, which were recommended by the European Society of Cardiology to rule-in HF (<50 years: 450 ng/l, 50-75 years: 900 ng/l, >75 years 1800 ng/l). In Europe, age dependent decision limits for chronic HF were reported only in one third of responders and were mostly stated to be the same as for acute HF. In US/Canada the same cut-off levels for acute and chronic HF were used in the majority of laboratories. There were still laboratories not performing external quality assurance in Europe (Europe: EQA 11%; US/Canada: EQA: 1%). In Europe, one third of laboratories were not accredited for NP, whereas in US/Canada almost all were. Conclusion: NP measurement for HF diagnosis was available in more than half of the responding laboratories. However, guideline recommended cut-off values for acute HF were still not adequately implemented. Further, accreditation is an ongoing process in Europe, and full EQA participation for HF biomarkers remains incomplete in European laboratories.

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In vitro Studies of Human Cardiac Troponin I Degradation.

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Background. Cardiac troponin I (cTnI) is a golden marker of myocardial cell death. It was shown that a wide diversity of cTnI forms, including proteolytic fragments could be found in patients' blood. Partial cTnI proteolysis happens from N- and C-terminal parts of the molecule, whereas central part of cTnI remains relatively stable. Though N- and C-terminal degradation of cTnI was described more than 15 years ago, still it is not clear, how it happens - is it a sequential truncation, or relatively big fragments from both ends are cleaved on the very first steps of cTnI degradation.

Methods. Cardiac proteins were extracted from human cardiac tissue and incubated at 37°C for 3 hours. In the preliminary experiments it was shown that in such conditions cTnI was partially cleaved by co-extracted endogenous proteases. After incubation extracted proteins were separated by gel-filtration (GF) chromatography. cTnI immunoreactivity in GF fractions was measured by sandwich immunoassays utilizing pairs of antibodies specific either to N- or to C-terminal parts of the molecule. On the next step cTnI fragments from GF fractions were purified by means of affinity and reverse-phase chromatography. Purified fragments were analyzed by mass spectrometry.

Results. Both types of immunoassays (specific to N- and C-terminal parts of cTnI) revealed two peaks of immunoreactivity in GF fraction. In both cases the first peak was detected in fractions corresponding to the proteins with molecular masses about 60-80 kDa (most likely cTnI in ternary complex), whereas second peak of immunoreactivity was found in fractions corresponding to the proteins with significantly lower molecular masses. By MS studies we were able to identify only few peptides (27-36 amino acid residues long) truncated from the N-terminal part of the molecule and multiple peptides (20-40 amino acid residues long) from the C-terminal part. Peptide sequence analysis suggested that the N-terminal part of cTnI has a very limited number of cleavage sites, whereas C-termiusn contains much more sites of protease degradation.

Conclusion. Using different biochemical methods and mass spectrometry analysis we were able to purify and identify peptides that are formed in vitro after cTnI cleavage by endogenous proteases. Our studies suggest that relatively big and relatively stable peptide(s) is (are) truncated from the N-terminal part of cTnI. C-terminus of cTnI molecule contains multiple possible sites of proteolysis and protease cleavage results in formation of wide diversity of different size peptides.
Diurnal and Longitudinal Variations of Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) Enzyme Activity

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Objective: Characterize the diurnal and longitudinal variations of serum measurements of Lp-PLA2 enzyme activity, a biomarker of vascular inflammation in coronary heart disease (CHD).

Background: Since clinical interpretation of laboratory values requires comparisons of results within and among individuals, understanding both intra- and inter-individual and also temporal biobraviability of assays is important. To assess the relative variability of Lp-PLA2 activity in the context of tests used in clinical assessments of CHD risk, we compared the biobraviability of Lp-PLA2 activity to lipid profiles and C-reactive protein (CRP).

Methods: To assess diurnal variation, phlebotomy was performed in 15 subjects at baseline (fasting) and at 3 hr intervals during a 24 hr period to obtain serum samples, with measurements of Lp-PLA2, CRP and lipids performed on all specimens. To assess longitudinal variation, phlebotomy was performed on 23 subjects at baseline and at weeks 2, 4, 8 and 12 to obtain serum samples which were stored at -78°C. Assays for Lp-PLA2 activity, lipase, total cholesterol, HDL, triglycerides, ApoA1, ApoB, and lipoprotein (Lp) (a) were performed for all samples at all-time points. Lp-PLA2 activity was measured by the PLAC Test for Lp-PLA2 Activity using the Beckman Coulter AU4000. Variations in intra-individual and inter-individual results were determined for both the diurnal and longitudinal analyses.

Results: An analysis of diurnal variation showed mean percent intra-individual diurnal variation (95% CI) for Lp-PLA2 activity of 4.1 (3.2-5.0), with corresponding results as follows: lipase, 7.1 (5.1-9.2); total cholesterol, 2.7 (2.2-3.2); triglycerides, 28.8 (22.6-35.0); HDL, 4.0 (2.6-5.3); ApoA1, 1.7 (1.2-2.2); ApoB, 2.6 (1.8-3.4); and Lp(a), 5.8 (3.9-9.2). Inter-individual diurnal variation was 28.4% for Lp-PLA2 activity, compared to a range of 12.9 to 33.4% for lipase, total cholesterol, triglycerides, HDL, ApoA1 and ApoB; the mean inter-individual diurnal variation for Lp(a) was 116.8%. An analysis of longitudinal variation over 12 weeks demonstrated a mean percent intra-individual variation of 5.3% for Lp-PLA2 activity, with corresponding results published as follows: LDL cholesterol, 8.3%; total cholesterol, 5.4%; HDL cholesterol, 7.1%; oxidized cholesterol, 21.0%; Lp(a), 20.8%; and CRP, 42.2%. The mean percent inter-individual longitudinal variation for Lp-PLA2 activity was 29.7%, compared to 25.7% for LDL cholesterol, 15.2% for total cholesterol, 19.7% for HDL cholesterol, 50.0% for oxidized LDL, 18.1% for Lp(a) and 76.3% for CRP. The mean index of individuality for Lp-PLA2 activity was less than 0.2.

Conclusions: The diurnal variation of Lp-PLA2 activity was similar to that of lipase, total cholesterol, HDL, triglycerides, Lp(a), ApoA1 or ApoB, and less than that for triglycerides. The longitudinal variation of Lp-PLA2 activity was similar to that for total cholesterol, LDL, HDL, and Lp(a), and less than that for oxidized LDL or CRP. These data suggest that the diurnal and longitudinal variations for Lp-PLA2 activity results are similar to those for lipid biomarkers measured in clinical assessments of CHD risk, suggesting the suitability of measuring Lp-PLA2 activity for patient management.

Fully automated ultrasensitive digital immunoassay for troponin using single molecule array technology

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Background: Ultra-sensitive cardiac troponin measurement offers a promising new tool for early detection and monitoring of cardiovascular disease. With growing interest in exploring as an early indicator of adverse heart health trends, the ability to quantify troponin in healthy control populations is emerging as a highly desirable assay capability. We report analytical data from a fully automated digital immunoassay for cardiac troponin I (cTnl) based on single molecule array (Simoa) technology with a limit of detection 2 logs lower than contemporary high sensitivity troponin assays.

Methods: To assess diurnal variation, phlebotomy was performed in 15 subjects at baseline (fasting) and at 3 hr intervals during a 24 hr period to obtain serum samples, with measurements of Lp-PLA2, CRP and lipids performed on all specimens. To assess longitudinal variation, phlebotomy was performed on 23 subjects at baseline and at weeks 2, 4, 8 and 12 to obtain serum samples which were stored at -78°C. Assays for Lp-PLA2 activity, lipase, total cholesterol, HDL, triglycerides, ApoA1, ApoB, and lipoprotein (Lp) (a) were performed for all samples at all-time points. Lp-PLA2 activity was measured by the PLAC Test for Lp-PLA2 Activity using the Beckman Coulter AU4000. Variations in intra-individual and inter-individual results were determined for both the diurnal and longitudinal analyses.

Results: An analysis of diurnal variation showed mean percent intra-individual diurnal variation (95% CI) for Lp-PLA2 activity of 4.1 (3.2-5.0), with corresponding results as follows: lipase, 7.1 (5.1-9.2); total cholesterol, 2.7 (2.2-3.2); triglycerides, 28.8 (22.6-35.0); HDL, 4.0 (2.6-5.3); ApoA1, 1.7 (1.2-2.2); ApoB, 2.6 (1.8-3.4); and Lp(a), 5.8 (3.9-9.2). Inter-individual diurnal variation was 28.4% for Lp-PLA2 activity, compared to a range of 12.9 to 33.4% for lipase, total cholesterol, triglycerides, HDL, ApoA1 and ApoB; the mean inter-individual diurnal variation for Lp(a) was 116.8%. An analysis of longitudinal variation over 12 weeks demonstrated a mean percent intra-individual variation of 5.3% for Lp-PLA2 activity, with corresponding results published as follows: LDL cholesterol, 8.3%; total cholesterol, 5.4%; HDL cholesterol, 7.1%; oxidized cholesterol, 21.0%; Lp(a), 20.8%; and CRP, 42.2%. The mean percent inter-individual longitudinal variation for Lp-PLA2 activity was 29.7%, compared to 25.7% for LDL cholesterol, 15.2% for total cholesterol, 19.7% for HDL cholesterol, 50.0% for oxidized LDL, 18.1% for Lp(a) and 76.3% for CRP. The mean index of individuality for Lp-PLA2 activity was less than 0.2.

Conclusions: The diurnal variation of Lp-PLA2 activity was similar to that of lipase, total cholesterol, HDL, triglycerides, Lp(a), ApoA1 or ApoB, and less than that for triglycerides. The longitudinal variation of Lp-PLA2 activity was similar to that for total cholesterol, LDL, HDL, and Lp(a), and less than that for oxidized LDL or CRP. These data suggest that the diurnal and longitudinal variations for Lp-PLA2 activity results are similar to those for lipid biomarkers measured in clinical assessments of CHD risk, suggesting the suitability of measuring Lp-PLA2 activity for patient management.

Development of an Enhanced Chemiluminescent High Sensitivity Troponin I assay* on VITROS® 5600 Integrated and VITROS® 3600 and EC/ECIQ Immunodiagnostic Systems


Background: The Joint European Society of Cardiology/ American College of Cardiology guidelines state that cardiac troponins are the preferred biomarkers for the detection of myocardial injury, for risk stratification in patients diagnosed with acute coronary syndrome and for the diagnosis of myocardial infarction. Because of the demand for accurate precise measurement of low troponin levels, there is an increased need for assays with improved analytical performance. We are developing a rapid, fully automated high sensitivity assay for the measurement of cardiac Troponin I (cTnl) in human serum and plasma for use on the VITROS® Systems.

Methods: A prototype assay was developed which uses an immunometric technique in which the cTnl present in the sample reacts simultaneously with one biotinylated antibody and two horseradish peroxidase labeled antibodies. The antigen-antibody complexes are captured by a streptavidin coated well. Unbound materials are removed by washing. VITROS® Immunodiagnostic Products Signal Reagent is added and light emission is measured. The light signal generated is directly proportional to the concentration of cTnl present in the sample.

Results: The following results were generated using the prototype assay. The assay range is 1 pg/mL to 50,000 pg/mL. In a CLSI-EP-15-A2 precision study the results for four patient pools were: (mean cTnl pg/mL, with-in run %CV, with-in laboratory %CV, respectively): 12.3 pg/mL, 3.2%, 5.5%; 28.4 pg/mL, 1.4%, 1.8%; 60.6 pg/mL, 1.6%, 6.3% and 183 pg/mL, 1.5%, 4.4%. The LoD, LoQ and LoQ (established according to CLSI-EP-17-A2) were 0 pg/mL, 1 pg/mL and 2.9 pg/mL (20%CV), respectively. The concentration at 10%CV was 6.5 pg/mL. The 99th percentile was determined by measuring cTnl in samples from 412 individuals with values within reference ranges for cEfr and NT-proBNP. The gender independent 99th percentile was 23 pg/mL. The heart failure samples had significantly higher cTnl concentrations than the healthy subjects (p<0.0002). Evaluation of the predictive value of the cTnl concentrations is ongoing.

Conclusion: The results show the digital Simoa cTnl assay exhibited good general analytical properties and cTnl levels from healthy subjects were above the sensitivity limits. The assay represents a new enabling tool for ultra-sensitive cTnl measurement.
Relative contribution of high sensitivity cardiac Troponins I and T in cardiovascular risk stratification in patients with OSA after treatment with CPAP

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Background: Obstructive sleep apnea (OSA) is a common condition caused by intermittent airway collapse during sleep that results in repetitive hypoxia, arousal, poor quality of sleep and excessive daytime sleepiness. OSA is a risk factor for various cardiovascular conditions and in recent decades, OSA was associated with increased cardiovascular mortality in patients with the severe form of the disease without treatment. Therefore, adequate treatment with CPAP (Continuous Positive Airway Pressure) may improve survival. With the advent of new high-sensitivity markers, there was an increase in the sensitivity of the method consequently increased interest from professionals in the use these high-sensitivity cardiac troponin T and I (hs cTnT and hs cTnI) in risk stratification of cardiovascular diseases since they are released in the cardiac lesions. The aim of our study was to evaluate the (hs cTnT and hs cTnI) methods and evaluate of cTnI conventional by two methods in patients with severe OSA before and after one year of effective treatment with CPAP.

Methods: 36 patients of the Sleep Institute in Sao Paulo, with moderate and severe OSA, 22 men, with mean BMI = 30.20 ± 9.12 Kg/m² and age = 65.4 ± 5.8 years, without other diseases were randomized and treated effectively with a year with CPAP and using average of 5 hours each night, nonsmoking and sedentary. Patients collected 10 ml of venous blood before treatment and after treatment with CPAP, which were frozen at -80°C and was thawed on laboratory measurements. Between several biochemical parameters will be analyzed the hs cTnT by two methods and cTnI conventional by two methods too, before and after one year treatment to apnea with CPAP. The hs cTnT was quantified with a Electrochemiluminescence (hs cTnT) Elecsys ®-Roche/Elecsys) third generation method. The detection limit is 0.5 pg/mL. Other method was used hs cTnT immunoassay (Abbott/ARCHITECT system). It was measured cTnI ES conventional by chemiluminescence (Vitro ES ®- Ortho Clinical Diagnostics) 12 pg/mL is the limits of detection to this methods and cTnI by AccuTnDx (Access/Beckman Counter) with detection limit of 10 pg/mL for this method. Paired samples statistics were performed for correlations and comparisons of results between methods before and after treatment and Wilcoxon test was performed to methods with significance differences.

Results: Based on the results presented there was a statistic significant effect of treatment with CPAP on the hs cTnT presented in the nonparametric statistical test Wilcoxon (Z=−1.955, p= 0.05 and Z=−1.634, p=0.04). However there was not significant difference of treatment with CPAP on the values presented by cTnI conventional dosages to both methods.

Conclusion: The hs cTnT and hs cTnI methods showed significance differences between before and after treatment with one year of CPAP in patients with apnea. This method use monoclonal antibodies with high sensitivity and specificity for cardiac injury. However there is a need of definition about the real importance of these low levels found in the condition of obstructive sleep apnea for cardiac injuries. The cTnI ES and cTnI Dx did not show sufficient sensitivity on condition of OSA.

Levels of Galectin-3 in samples of patients with abnormal values of Brain Natriuretic Peptide

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Acute cardiac conditions such as acute myocardial infarction and heart failure (HF) are associated with significant morbidity and mortality. The prognostic significance of natriuretic peptides (BNP and NT-proBNP) in patients with myocardial ischemia is well established, and their measurement is endorsed by the most important guidelines and recommendations for diagnosis and management of heart failure (HF). Numerous novel biomarkers, such galectin 3 (Gal-3), have been identified to predict outcomes and show potential in assessing prognosis beyond the established natriuretic peptides. In this study, we determined levels of Gal-3 in samples of patients with abnormal values of BNP and in control subjects and analyzed if there is a relationship between the values of BNP and Gal-3. Methods: Plasma Gal-3 concentrations were measured in 10 samples of patients with high BNP concentrations (>100 pg/ml) (Group I) and in 40 asymptomatic subjects, without a family history of cardiovascular disease, who had normal BNP levels (Group II). Gal-3 levels were determined using an automated test (VIDAS® Gal-3 kits, BIO MÉRIEX, France) using the ELFA (Enzyme-Linked Fluorescent Assay) technique. Calibration of the assay was performed according to the manufacturer’s recommendations and values were normalized to a standard curve. The McNemar test was used to test the differences between the results of the paires proportions of BNP and Gal-3. The tests were coded as 0 = “not altered” and 1 = “altered”. The BNP cutoff value was 100 pg/ml. There were two Gal-3 cutoff values: 17.8 ng/mL (results of moderate to high risk of mortality and hospitalization) and at 25.9 ng/mL (results of high risk of mortality and hospitalization). Results: Levels of Gal-3 were 26.57 ± 11.20 ng/mL in Group I and 8.30 ± 1.80 ng/mL in the control group, respectively. Differences in levels of Gal-3 between the two groups were significant (independent samples t-test P < 0.0001). Using a Gal-3 cutoff value of 17.8 ng/mL, 7 out of the 10 patients of the Group I and Zero out of the 40 subjects of the Group II had high Gal-3 concentrations. In the Group I, 2 out 10 patients were classified as moderate risk (Gal-3 > 17.8-25.9 ng/mL) and 5 out 10 patients were classified as high risk (Gal-3 > 25.9 ng/mL). There was concordance between the proportion of altered BNP and Gal-3 results, using the cutoff value of 17.8 ng/mL and discordance when using the cutoff value of 25.9 ng/mL. Conclusion: An increased concentration of galectin-3 was found in all the patients with high BNP concentrations. The patients with BNP concentration above 100 were classified as moderate or high risk.

Gender Wise Correlation of Serum Homocysteine and Gensini Scores with Anthropometric Indices in Coronary Artery Disease

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Background: Obesity is an emerging public health problem throughout the world especially in our country (India) where it is on the rise even during childhood. Obesity and elevated serum homocysteine levels both have been independently considered as risk factors for coronary artery disease (CAD). But, limited data available as to how these factors correlate gender wise with respective Gensini scores used for grading coronary artery disease.
**B-031**

**Characteristics of the New Beckman Coulter High-Sensitive Cardiac Troponin I Assay (hs-cTnI)**


**BACKGROUND:** In order to meet new IFCC guidance new Troponin I assays should exhibit increased sensitivity (LoB, LoD and LoQ), precise measurement of cTnI concentrations in the range seen in healthy individuals and the capability to accurately detect changes in cTnI concentration within this range.

**RESULTS:** The new hs-cTnI assay under development at Beckman Coulter exhibits superior sensitivity in comparison to other devices currently marketed in the US with an estimated LoB of ~0.3 pg/ml and 20% CV LoQ of 1.5 pg/ml. The estimated 99th percentile URL of a random healthy population is 21 pg/ml determined with < 3% intra-assay and 5% total imprecision. In addition, this new hs-cTnI assay is capable of accurately measuring 10 pg/ml changes in TnI concentrations. This hs-cTnI assay accurately measures TnI in comparison to a currently validated device (correlation between Access AccuTnI+ and hs-cTnI within 5%) and exhibits < 5% bias between sample types (serum, plasma). The assay does not exhibit cross-reactivity to cardiac TNF, skeletal TnI or skeletal TnT and is robust against common interferences (400 mg/dL hemoglobin, 40 mg/dL bilirubin, 3000 mg/dL triglyceride 60 mg/mL albumin, 1000 mg/dL fibrinogen, 28.8 U/mL heparin).

**CONCLUSIONS:** The new high sensitivity TnI (hs-cTnI) assay under development at Beckman Coulter is highly sensitive and sufficiently accurate to precisely measure TnI in > 85% of the normal population. This new assay also meets new IFCC guidance to accurately detect changes in cTnI concentration within healthy subjects.

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**B-032**

**Increased Incidence of Cardiac Troponin I Abnormalities in Women Using a High Sensitivity Assay**


**Objective:** To determine if sex-specific 99th percentiles with a high sensitivity cardiac troponin I (hs-cTnI) assay versus a single non-sex-cut-off using a contemporary cTnI assay leads to more frequent increases in cTnI levels indicative of acute myocardial infarction (MI).

**Methods:** The data presented are the first results from our ‘clinical trials.gov identifier: NCT02060766’ study. Patients, 18+ years of age, presenting to the emergency department where providers used cTn to rule-in or rule-out MI, were included in the study. Serial cTnI measurements were obtained on clinical indication between February 4 and March 13, 2014. Clinical decisions were based on the contemporary cTnI results, with hs-cTnI measured simultaneously (both on the Abbott ARCHITECT i1000, or 2000, etc.). 99th percentiles were as follows: contemporary cTnI 30 ng/mL (0.030 μg/L); hs-cTnI 16 ng/L for females and 34 ng/L for males.

**Results:** 792 patients presenting for MI rule-in or rule-out were enrolled, of which 45% were female. Over the course of serial cTnI measurements (0.3-6.9h) baseline and maximum values were examined by gender. At presentation mean (95% CI) values were: cTnI assay 128 (0-266) ng/L for males and 60 (31-189) ng/L for females; hs-cTn assay 90 (0-190) ng/L for males and 45 (18-72) ng/L for females. Maximum values were also examined by gender: cTnI male 532 (126-938) ng/L, female 241 (52-430) ng/L; hs-cTnI male 457 (135-781) ng/L, 236 (29-444).

**Conclusion:** Based on sex-specific hs-cTnI assay 99th percentiles we observed a significant decrease in the incidence of cTnI increases and a significant difference in increased rates between sexes. The increased incidence of cTnI increases for women using an hs-cTnI assay could have important implications for improving treatment and outcomes for women presenting with symptoms of acute coronary syndromes.

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**B-033**

**The relationship of Inflammatory cytokines (IL-6, IL-17a and TNFa) to co-morbidities of cardiovascular disease in a large community-based patient population.**

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**Background:** Interleukins 6 and 17-A (IL-6 and IL-17a) and Tumor necrosis factor-alpha (TNFa) are inflammatory cytokines that may play a role in the pathogenesis of atherosclerosis. Previous studies have shown these cytokines to be relevant in many areas of cardiovascular disease (CVD) including acute myocardial infarction, heart failure and coronary artery disease. We determined plasma levels of IL-6, IL-17a and TNFa and identify the effect of co-morbidities on their levels in a large at CVD risk study population.

**Methods:** Immunoassays for IL-6, IL-17a and TNFa were developed using Single Molecule Counting technology and validated in a CLIA licensed, CAP accredited laboratory (Singulex). The 95th percentile upper limit of normal (ULN) were 4.5, 1.9 and 2.5 pg/mL; and the precision profiles were 12% (2.3 pg/mL), 17% (5.3 pg/mL) and 15% (9.2 pg/mL) for IL-6, IL-17A and TNF respectively. Blood samples were measured for the cytokines, LDL, HDL and HbA1c in >21,000 community-based patients at risk for CVD. Parametric and non-parametric analyses were performed in de-identified data using SAS v9.3 (p <0.05 considered significant).

**Results:** The characteristics of the inflammatory cytokines in a population at risk for CVD are shown in the Table. All three cytokines showed higher concentrations in older patients as well as those with CV risk factors of pre-diabetes, diabetes and HDL dyslipidemia.

**Conclusions:** In a population at risk for CVD, IL-6, IL-17 and TNFa concentrations are higher than those observed in healthy populations and coincide with risk factors for CVD.

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**B-034**

**Validation of lithium heparin tube for Cardiac markers in a Clinical Laboratory and its benefits**

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**Background:** Accurate laboratory testing requires an understanding of the complex interactions between collection devices and blood specimens. Clinical laboratories must consider
the pre-analytical challenges in laboratory testing. Proper blood collection and timely processing are critical pre-analytical steps required for the integrity of laboratory results. Although the influence of blood collection devices on laboratory tests is often overlooked, correct pre-analytical handling is essential. In this study, we discuss the use of plasma to chemistry analysis with an emphasis on heparin tube.

**Objective:**
Currently the clot activator with separator gel tube is the first option for biochemical analysis, the objective of the study is to evaluate the possibility and benefits of the introduction of lithium heparin tube for holding some biochemical studies.

**Methods:**
Twenty paired samples using the two tubes were carried out simultaneously. Both tubes were centrifuged before analysis with the difference that the clot activator tube with gel separator needs a time for the formation of clot before the centrifugation procedure. Samples were analyzed for CK mass and Troponin by two different methods: electrochemiluminescence (ECL), in the E411 Roche platform and fluorescence enzyme immunoasay (ELFA), in the Vidas 30 bioMerieux instrument.

**Results:**
For the ECL CK mass analysis, the Test F, T and the Pearson correlation showed up as expected. 5.14% of systematic constant was observed for level 1 and 0.82% for Level II that proved to be insignificant. Proportional systematic error was -3.37% with no impact on levels of clinical decision. The comparative test conducted with clot activator tube with gel separator and plasma from lithium heparin tube showed satisfactory results with total error obtained 7.39% and 8.17% for levels 1 and 2, respectively, less than the total allowable error of 30.6%. The troponin, F test and Pearson correlation showed results as expected. Constant systematic error was 4.06% for level 1 and 0.37% for level 2 which were considered not relevant. Proportional systematic error was -6.57% with no impact on levels of clinical decision. The comparative test conducted from gel x lytic plasma serum samples showed satisfactory results with total error obtained 7.63% and 11.32% for levels 1 and 2 respectively less than the total allowable error 48.9%.

For ELFA, Ck Mass showed a correlated and Kappa index within the references, F test and Pearson correlation as expected. We observed constant and proportional systematic error of zero for the two levels. Comparative tests conducted between serum samples x lytic gel showed satisfactory results with plasma total error of 8.39% obtained for the two lower levels of total allowable error 27.91%.

**Conclusion:**
We observed that the use of both tubes had good performance in the evaluation of cardiac markers, CK Mass and troponin. The final result for the doctor had a shorter TAT, with high quality and efficiency, as the tube with plasma showed the benefit to be processed before the tube with the clot activator and separator gel.

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**B-036**

**Endothelin Assay in Development for the Sgx ClarityTM Single Molecule Counting Platform Detects ET in Plasma and Discriminates Chronic Heart Failure from Healthy Donor Samples**


**Background:**
The Sgx Clarity System, a fully-automated in vitro diagnostics (IVD) platform that utilizes Singulex’s Single Molecule Counting (SMCTM) technology, is in development. The new system will be a clinical diagnostics instrument which uses the same detection concept as the widely used and accepted Research Use Only ERENNA® instrument. The ERENNA has been shown to identify low-abundance biomarkers with unparalleled sensitivity, precision, and accuracy. The Sgx Clarity System utilizes a second generation scanning-based detection system which maintains the exquisite sensitivity of the ERENNA while improving upon the throughput, dynamic range, and usability of that system. Endothelin (ET) is a low abundance biomarker in plasma, and since its discovery in 1989, plasma ET has been studied as a biomarker for CVD risk stratification and for developing heart failure (HF). However, the very low endogenous concentration of the biologically active ET peptide in plasma has made such studies difficult, and to date, there is no IVD platform that tests for ET in plasma.

**Objectives:**
To assess the preliminary performance characteristics of the ET assay in development for the Sgx Clarity System, and to compare ET values obtained from the plasma of chronic heart failure (CHF) patients compared to those obtained from the plasma of apparently healthy donors (normals).

**Methods:**
44 CHF vs. normal donor plasma samples were tested with the Sgx Clarity ET assay on an Sgx Clarity prototype instrument. Protocols based on CLSI guidelines were followed for analytical performance assessment. Results were compared for statistical significance using the Wilcoxon rank sum test and were displayed in Box-and-Whiskers plots.

**Results:**
The ET assay had an LoB of 0.25 pg/mL and an LoD of 0.44 pg/mL as calculated using StatisProTM software. The 20% functional sensitivity was determined to be 1.0 pg/mL and the 10% functional sensitivity was 1.8 pg/mL as determined using precision profile analysis. Both Within-Run and Total Precision were ≤ 8% CV at concentrations of 1.2 pg/mL and above. The reportable range up to 25 ng/mL demonstrated > 5 logs of dynamic range, and the assay was linear down to the lowest concentration tested (0.3 pg/mL). When tested for hook effect, none was observed up to 500 ng/mL. No significant impact from common endogenous interferences was observed when tested at concentrations recommended in EP-7:28. ET was detected in 100% of normal and CHF plasma samples tested and a significant difference (p<0.0001) was observed between normal and CHF samples.

**Conclusion:**
The ET assay in development for the Sgx Clarity System demonstrates >5 logs of dynamic range, has sufficient sensitivity to detect ET in 100% of samples from apparently healthy donors, and effectively discriminates CHF from normals.
B-037

BNP Assay in Development for the Sgx ClarityTM Single Molecule Counting Platform Demonstrates Increased Clinical Sensitivity Relative to a Conventional BNP Immunossay

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Background:
Singulex is developing the Sgx Clarity System, a fully-automated in vitro diagnostics platform that uses Singulex's Single Molecule Counting technology and has sensitivity equivalent to the widely used and accepted Research Use Only ERENNA® immunoassay while improving upon the throughput, dynamic range, and usability of that system. The BNP assay in development for the Sgx Clarity System uses the single epitope sandwich (SES) concept introduced by HyTest - where a capture antibody recognizing an epitope in the stable ring structure is paired with a detection antibody that binds only to the complex of the detection antibody-bound BNP molecule. The SES concept is hypothesized to confer a higher apparent stability of BNP in patient plasma, a fact which of the circulating BNP is truncated at the N- and C-terminus, which are the epitope targets of most conventional commercially available BNP immunoassays.

Objectives:
To assess the preliminary performance characteristics of the Sgx Clarity BNP assay and to compare the clinical performance to a conventional BNP immunossay.

Methods:
Forty plasma samples spanning the reportable range were tested on the Sgx Clarity BNP assay as well as the Siemens ADVIA Centaur BNP assay. Analytical performance studies followed protocols based on CLSI guidelines.

Results:
An LoB of 0.6 pg/mL, an LoD of 1.4 pg/mL, and a 10% functional sensitivity (LoQ) of 3.4 pg/mL were obtained. Within-Run Precision was 3 - 8% and Total Precision 4 - 10% with plasma samples from 4.5 to 1310 pg/mL. The assay was linear throughout the reportable range, which extends to 5000 pg/mL, and there is no high dose hook effect up to 100,000 pg/mL. No notable impact from common endogenous interferences and minimal cross reactivity was observed when other natriuretic peptides were tested. Passing-Bablok regression demonstrated good agreement between Sgx and Siemens methods (slope 1.09; Pearson correlation 0.97).

A similar finding was observed in a subsequent study, with a small but significant percentage of discordant samples being observed. In all cases, the discrepant results were higher on the Sgx Clarity BNP assay. To further investigate this discrepancy, all samples were assayed for NT-proBNP (Roche). Those results showed clinical agreement with the Sgx Clarity BNP assay results relative to the established clinical cutoffs for the two molecules. This observation supports the hypothesis that some percentage of circulating BNP is not detected by conventional immunoassays whose antibodies bind to epitopes near the unstable termini of the BNP peptide. If true, the clinical relevance of this hypothesis requires further investigation.

Conclusion:
These results support the hypothesis that the Sgx Clarity BNP assay using the SES antibody concept may be more clinically sensitive than conventional BNP assays to circulating forms of BNP in patient plasmas.

B-039

Validation and Correlation study of the Values for the Beckman cTnI and TnI+3 Assay on the DxI 800 and Access-2 Analyzers


Background: Cardiac troponin (cTn) assays have been available in clinical laboratories for nearly two decades and considered a highly sensitive marker for myocardial damage. An elevation of cTn is used, together with other diagnostic criteria, to rule in/out a myocardial infarction (MI). Laboratories measure either cTnI or cTnT isoforms of troponin. Following a recall of cTnI reagents from the DxI Immunoassay analyzer in October 2010, Beckman Coulter (BC) recently reintroduced a Troponin-I (AccuTnI +3) assay for the DxI 800 and Access-2 analyzers.

Design: Method validation studies (correlations, linearity, inter- and intra-precision studies) were performed by comparing the following cTn results (new reagent vs old reagent) on the following analyzers: 1. The Access-2 analyzers in the Emergency Department (ED) and the Main Laboratory (ML); 2. Access-2 in the ED and DxI 800 in the ML; 3. Access-2 ML and DxI 800 ML. A total of 115 patient specimens, presenting to our Emergency Department (ED) with history or evidence of cardiac disease or cTnl ordered following a review of patient’s chart, were used for correlation studies. Specimens were spun, aliquoted, frozen within 24 hours at -20°C, and analyzed within 30 days of collection. Precision was performed using BioRad Cardiac Marker Plus Quality Control (Levels 1, 2, 3). Linearities were performed using BC calibrators.

Results:
Table 1

<table>
<thead>
<tr>
<th>Analyzers</th>
<th>No.</th>
<th>Mean x/y</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access-2 ED/ML</td>
<td>109</td>
<td>0.17/0.108</td>
<td>0.981</td>
<td>-0.008</td>
<td>0.9939</td>
</tr>
<tr>
<td>Access-2 ED/DxI800</td>
<td>115</td>
<td>0.12/0.111</td>
<td>0.791</td>
<td>0.015</td>
<td>0.9776</td>
</tr>
<tr>
<td>Access-2ML/DxI800 ML</td>
<td>115</td>
<td>0.131/0.111</td>
<td>0.735</td>
<td>-0.089</td>
<td>0.9589</td>
</tr>
</tbody>
</table>

Linearities: 1) DXI: 0.000 to 67.240 ng/ml; 2) Access-2 ML: 0.000 to 96.857 ng/ml 3) Access-2 ER: 0.003 to 97.877 ng/ml

Conclusions:
The validation studies show that the new assay demonstrated good precision and correlation between the old and new reagents. The results also showed an extended reportable range (previously reported), allowing our laboratory to report results as low as 0.04 ng/ml (previously reported using the old reagents only down to 0.4 ng/ml). By increasing the sensitivity of the assay, earlier detection of an MI may be potentially achieved.

B-040

Matrix Metalloproteinase 9 as an Early Detector of Coronary Bare-Metal Stenting Restenosis

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Background: In-stent restenosis (ISR) is the major limiting factor of coronary bare-metal stenting (BMS). Establishing a reliable predictor for ISR risk would be an important factor for the decision making process to optimize patient management. Matrix metalloproteinase 9 (MMP9) was formed to be a predictor of cardiovascular mortality in patient with coronary artery disease (CAD). Aim of this work was to evaluate MMP 9 serum level to identify patient who are at high risk for development of ISR after BMS in a sample of Egyptian patients. Methods: Blood samples were collected prospectively from 60 patients previously diagnosed as stable CAD patients who were scheduled for elective BMS. Another 15 healthy volunteers were enrolled as a control group. All subjects were evaluated for diabetic status’ kidney function’ liver function (including hepatic viral markers)’ lipid profile & MMP 9 level was measured by ELISA . After 6 months the cooperative asymptomatic patients were subjected to coronary angiography & stress electrocardiogram (ECG). Results: Among our patients: 4 died within 6 months [Kaplan Meier survival = 93.3% with mean estimate 5.652]. Fourteen patients were proved as clinical restenosis,(5 by angiography & 9 by positive stress test). Measuring the area under ROC curve ,the figure 139.9 µg/L was proved to be the best discriminating figure : sensitivity was 74.2% &specificity was 52.4% . The proved free asymptomatic patients were 43 patients (73.9%) with ≤139.9µg/L MMP9 while 12 (26.1%)had higher levels. Patients with clinical restenosis were 4 (=28.6%) with MMP9 ≥ 139.9 µg/L and 10 (= 71.4%) with higher levels This difference was statistically significant (P = 0.002). Only diabetes mellitus among other risk factors (age, sex, hypertension dyslipidemia ,smoking &positive family history was significant between the two groups with dwithout restenosis . Conclusion the results suggest that serum MMP9 level may play an important role in the prediction of in-stent restenosis in patients with coronary bare metal stenting. Stop