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/L. The CV is stated as 10% at 30 ng/L with a 99th percentile of 50 ng/L.

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. Wu[1], J. Estis[2], P. Helestine[2], K. Bui[2], J. Todd[2], P. Kavsak[3]. *[1]University of California, San Francisco, San Francisco, CA, [2]Singulex, Inc., Alameda, CA, [3]McMaster 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 co-morbidities 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 LLoQ of 0.4 pg/mL and the (95%tile 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 study assessing hs-cTnI 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 hs-cTnI 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.

hs-troponin results				
	Median, ng/L	95th%, ng/L	99th%, ng/L	%>99th%
Entire cohort	0.9	6.0	21.5	7.0
Females	0.8	4.6	15.5	4.9
Males	1.2	7.6	27.0	9.4+
	0.6	3.4	10.9	3.1
>=50 y	1.1	7.0	24.4	8.5+
HbA1c <=5.9%	0.9	5.1	17.6	5.8
HbA1c >5.9%	1.3	9.1	34.6	11.3+
LDL <= 129 mg/dL	1.0	6.4	22.3	7.6
LDL > 129 mg/dL	0.9	5.8	20.1	6.7
HDL at target*	0.9	5.8	20.1	6.8
HDL less than target*	1.0	7.5	25.4	8.6+
HDL target	>=45 mg/dL females	>-=35 mg/dL males	+P<0.0001	

B-003

Changes in the utilisation of evidence-based recommendations for the biochemical diagnosis of acute myocardial infarction.

P. O. Collinson[1], A. Hammerer-Lercher[2], M. van Dieijen-Visser[3], K. Pulkki[4], J. Suvisaari[5], A. Stavljenic-Rukavina[6], H. Baum[7], C. Duff[8], A. Aakre[9], M. Langlois[10], S. Stankovic[11], P. Laitinen[5]. *[1]St George's Hospital, London, United Kingdom, [2]University Hospital Innsbruck, Inssbruck, Austria, [3]Maastricht University Medical Center, Maastricht, Netherlands, [4]University of Eastern Finland, Kuopio, Finland, [5]Helsinki University Central Hospital, Helsinki, Finland, [6]University of Zagreb, Zagreb, Croatia, [7]Regionale Kliniken Holding RKH GmbH, Ludwigsburg, Germany, [8]University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom, [9]Haukeland University Hospital, Bergen, Norway, [10]AZ St-Jan Hospital Bruges, Bruges, Belgium, [11]Clinical Center of Serbia, Belgrade, Serbia*

Objective. To assess the progress towards the utilisation 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 using 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 50% of responses with 39%, 35% and 39% from local (community) hospitals (Chi^2 2.83 ns).

The measurement of cardiac troponin has now become the preferred cardiac biomarker in 99.5% of hospitals and the first line marker in 97.7% (95.3% in 2006 and 2010) with 37.7% of those hospitals offering troponin alone as cardiac biomarker (28.6% 2006, 31.4% 2010) Chi^2 6.05 p =0.48). The proportion of non-recommended markers offered has fallen significantly (Chi^2 104.2, p,0.0001). Aspartate transaminase was offered by 52.7% as part of the cardiac profile in 2006, 34% in 2010 but is now offered by only 1.1%. Lactate dehydrogenase or hydroxybutyrate dehydrogenase is offered by 15.6% of laboratories, (25.5% in 2006, 35.6% in 2010). In acute units in 2013, 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 clinician familiarity or need for a short term marker.

There has been a statistically significant shift from the use of assay imprecision at the 10% coefficient of variation to the use of the 99th percentile as the decision limit with an increase in use of the 99th percentile from (35.4% 2006, 37.9% in 2010, 52.1% in 2013, $p<0.0001$). There has been an increased use of the manufacturers data sheet as a source of data (51.9% in 2006, 50.2% in 2010, 62.2% in 2013) Chi^2 89.4, $p<0.0001$.

11.1% used values taken from the literature but local validation of the 99th percentile was only reported in 0.9% of those measuring troponin (2013 data).

Conclusion. Although there has been a significant improvement in use guideline-based practice there is still a lack of use of the 99th percentile and lack of independent assessment of the analytical performance claims.

B-004

Reference Range Study Using High Sensitivity cTnI 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 cTnI 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 StatisProTM 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 (0.00005%).

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

E. Nah, H. Cho, J. Choi. *Korea association of Heath Promotion, Seoul, Korea, Republic of*

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, 1594 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) 3.980 (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. Wu[1], J. Estis[2], P. Heseltine[2], T. Dang[2], J. Todd[2], P. Kavsak[3]. *[1]University of California, San Francisco, San Francisco, CA, [2]Singulex, Inc., Alameda, CA, [3]McMaster 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 LLoQ (20%CV) of 0.2 pg/mL and a 95%tile (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.

Endothelin results				
	Median, pg/mL	95th%, pg/mL	99th%, pg/mL	%>95th%
Entire cohort	2.4	3.9	5.5	5.9
Females	2.4	3.8	5.4	5.4
Males	2.5	4.0	5.9	6.6++
	2.2	3.4	4.7	2.1
>=50 y	2.5	4.1	5.8	7.5+
HbA1c <5.9%	2.4	3.8	5.4	5.2
HbA1c >=5.9%	2.6	4.4	6.1	8.8+
LDL <= 129 mg/dL	2.4	4.0	5.7	6.6
LDL > 129 mg/dL	2.4	3.7	5.4	4.9+
HDL at target*	2.4	3.8	5.5	5.7
HDL less than target	4.5	4.2	5.8	7.6++
HDL target	>=45 mg/dL females	>=35 mg/dL males	+p<0.001	++P<0.05

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.

P. O. Collinson[1], F. Apple[2], R. Christenson[3], A. Hammerer-Lercher[4], M. van Dieijen-Visser[5], K. Pulkki[6], J. Suvisaari[7], A. Stavljenic-Rukavina[8], H. Baum[9], C. Duff[10], K. Aakre[11], M. Langlois[12], S. Stankovic[13], P. Laitinen[7]. *[1]St George's Hospital, London, United Kingdom, [2]Hennepin County Medical Centre, Minneaopolis, MN, [3]University of Maryland School of Medicine, Baltimore, MD, [4]University Hospital Innsbruck, Innsbruck, Austria, [5]Maastricht University Medical Center, Maastricht, Netherlands, [6]University of Eastern Finland, Kuopio, Finland, [7]Helsinki University Central Hospital, Helsinki, Finland, [8]University of Zagreb, Zagreb, Croatia, [9]Regionale Kliniken Holding RKH GmbH, Ludwigsburg, Germany, [10]University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom, [11]Haukeland University Hospital, Bergen, Norway, [12]AZ St-Jan Hospital, Bruges, Belgium, [13]Clinical Center of Serbia, Belgrade, Serbia*

Objective. To assess current use of evidence-based guidelines for the use of cardiac biomarkers 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

G. D. Thorne[1], A. Vasko[1], E. Roessner[1], K. Smalley[1], K. He[1], Z. Q. Li[1], A. Adorian[2], T. Cappola[3], T. R. Kettlety[1]. *[1]Fujirebio Diagnostics Inc., Malvern, PA, [2]BG Medicine, Waltham, MA, [3]University of Pennsylvania, Philadelphia, PA*

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 combined and incubated in the first step. 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 of galectin-3 were assayed. In 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; $r \geq 0.9$) 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 interferents 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-8, galectin-9, galectin-12, collagen type I, 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[1], A. Man[1], J. E. Hil[1], J. Repass[2], J. Hill[1]. *[1]Spot On Sciences, Austin, TX, [2]ARQ 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 (miRNA) have been identified as potential biomarkers of CVD. miRNA 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.

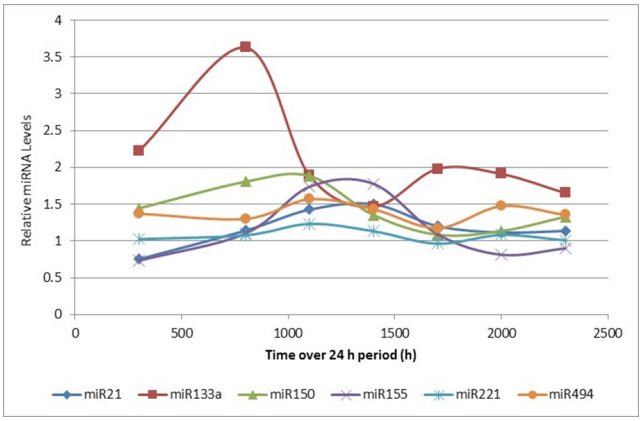
Methods:

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.



B-012

Retrospective analysis of 160,000 BNPs shows that initially low NT-pro BNP is highly predictive of future negative NT-proBNP

Q. Xu[1], J. Cadamuro[2], E. Haschke-Becher[2], G. S. Cembrowski[3]. *[1]University of Alberta, Edmonton, AB, Canada, [2]University Hospital Salzburg, Salzburg, Austria, [3]Alberta Health Services, Edmonton, AB, Canada*

Background:

Low BNP or low NT-proBNP are strong negative indicators of congestive heart failure. Based on our findings of low biologic variation in patients with normal BNPs, we recommended that emergency department patients have no more than 2 consecutive negative BNP's before a computer-generated message is sent to the attending physician stating that another BNP was superfluous, unless there was a change in the patient's clinical status. This encouraged us to study NTproBNP.

Methods:

We analyzed 6.5 years of Salzburg University BNP data (164490 NT-proBNP from 52103 patients). We identified all patients who had repeatedly low results (two, three, or four times consecutively), under 200, 150, or 100 pg/mL. For three age groups, we determined the probability that the subsequent BNP would be positive (> 450pg/mL and >900 pg/mL for ages <55y and >55y, respectively).

Results:

The Table presents the probabilities of a positive test following a series of negative NT-proBNP's as well as the reduction in BNP testing. The greater the number of consecutively low NT-proBNP or the lower the BNP limit, the lower the probability of a positive test but at a cost of increased testing.

Conclusion:

Repeatedly low initial values signify a high probability of another negative test result. In the situation of prior low BNP test results in a newly re-admitted patient, either the laboratory information system or clinical information system should inform the attending physician: "In the absence of new clinical symptoms, another NT-proBNP would offer no new diagnostic

information." Between 1 and 5.5% of NT-proBNP tests would not be done, depending on replicate/cutoff combination and resulting in savings ranging from 34100 € to 452168 €

Number of consecutive BNPs & upper BNP limit	Age < 55y (N=27,119 BNP's)		Ages 55-75y (N=78,248 BNP's)		Ages >75y (N=59,123 BNP's)		
	% missed	BNP's not done	% missed	BNP's not done	% missed	BNP's not done	Total BNPs not done
2 <200pg/mL	5.0	3026	4.0	5115	8.3	852	8993
2 <150	4.0	2505	3.5	3791	7.3	507	6803
2 <100	3.5	1806	2.4	2273	5.8	224	4303
3 <200	3.3	1750	2.7	2922	5.7	436	5108
3 <150	2.3	1409	2.2	2089	4.5	245	3743
3 <100	1.8	979	1.7	1190	2.9	105	2274
4 <200	2.8	1150	2.1	1871	3.2	251	3272
4 <150	2.1	906	1.5	1300	2.8	143	2349
4 <100	1.9	582	1.4	725	3.5	57	1364

B-013

Automated approaches to wrangling wayward troponins

D. S. Herman, G. S. Baird, D. N. Greene. *University of Washington, Seattle, WA*

Background: Quantification of cardiac troponin I (cTnI) serum concentration is a critical part of evaluating patients for acute myocardial infarction. Errors in this quantification can lead to significant clinical mismanagement. To mitigate this risk, we sought to quantify the frequency of false-positive cTnI results and develop strategies to prevent them.

Methods: We reviewed clinical cTnI results (N=22,976; 1/21/2014 - 11/20/2104) performed using the Beckman Coulter AccuTNI+3 assay across two academic medical centers. cTnI results for individual patients were grouped and then serial tests were compared based on their resulted concentrations and collection times.

Results: Retrospective analyses of the temporal patterns of cTnI concentrations for individual patients revealed decay rates that were faster than physiologically expected and not explained by other clinical factors. Focusing on results > 0.04 ng/mL with follow-up testing within 24 hours (N=6,205; 27%), we developed specific criteria ($t_{1/2}$ < 3.7 hours; %Δ[cTnI] ≤ -25%; 1st [cTnI] > 0.05 ng/mL; 2nd [cTnI] 0.03 ng/mL, (B) rapid concentration increases (t_2 < 4.7 hours; %Δ[cTnI] ≥ 35%), and (C) unexpectedly rapid concentration decreases ($t_{1/2}$ < 5 hours; %Δ[cTnI] ≤ -20%). Retrospective analyses indicate that the application of these rules to our study data would have triggered repeat assessment in 15% of cTnI tests and identified all of our suspected false elevations. Additionally, we expect this intervention to identify false elevations that our retrospective analyses could not flag because there was no timely follow-up testing.

Conclusions: Retrospective analyses of serial cTnI concentrations have identified a set of potentially falsely elevated results (~1 in 200) with important clinical ramifications. We are leveraging the power of our middleware system to prospectively identify these potential errors in real-time and automatically trigger repeat evaluation. Ongoing studies, including cross-institution and cross-instrument comparisons of the temporal patterns of cardiac troponin concentrations, will help to understand the sources of these potential false-elevations and enable the refinement of strategies to prevent patient harm.

B-015

Circulating myostatin levels in heart failure with preserved ejection fraction

T. B. Dschietzig[1], C. Georgi[2], U. Fahlenkamp[2], J. Ruppert[1], F. P. Armbruster[1], I. Diaz[2]. *[1]Immundiagnostik AG, Bensheim, Germany, [2]Charité - 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 (HFREF), 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, HFPEF; n = 7, HFREF) undergoing ablation for atrial fibrillation.

First, we detected significant differences between myostatin levels measured at A, V, and CS neither in controls, HFPEF, nor HFREF; nor were there significant differences between controls, HFPEF, and HFREF. 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 HFPEF, 6 HFREF), 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.

B-016

High Sensitivity Cardiac Troponin I and T Assays for Predicting Death in a Hemodialysis Population

F. S. Apple[1], R. Ler[1], S. A. Love[1], Y. Hu[2], D. Gilbertson[2], M. M. Murakami[1], S. M. Brunelli[3], A. Young[3], C. A. Herzog[1]. *[1]Hennepin County Medical Center, Minneapolis, MN, [2]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN, [3]DaVita 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-cTnI (Abbott ARCHITECT $i1000_{SR}$) and hs-cTnT (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/L, female 16 ng/L; hs-cTnT male 20 ng/L, female 13 ng/L. 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 = 49 for vintage <1 y and n = 414 for vintage ≥1 y. For vintage <1 y, three-year probabilities of death were 12.4% [CI 8.0%, 19.1%] (n =236) with normal hs-cTnI and 47.3% [26.2%, 74.0%] (n=80) with increased hs-cTnI (p <0.0001); 0% (n=8) with normal hs-cTnT and 22.2% [14.6%, 32.8%] (n=308) with increased hs-cTnT (p=0.26). For vintage ≥1 y, 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 trended at p=0.07 at ≥1y. **Conclusions**: hs-cTnI and hs-cTnT are both predictors of three year mortality in hemodialysis patients. However, hs-cTnI appears to be a particularly powerful predictor of death in incident (vintage <1 y) hemodialysis patients.

Unadjusted hazard ratios and concentrations by quintile for hs-cTnI and hs-cTnT by vintage year

Quintile	hs-cTnI				hs-cTnT			
	hs-cTnI, ng/L		HR (95%CI)		hs-cTnT, ng/L		HR (95%CI)	
Vintage	<1 y	≥1y	<1 y	≥1y	<1 y	≥1y	<1y	≥1y
Q1	7	8	1.0 (ref)	1.0 (ref)	35	39	1.0 (ref)	1.0 (ref)
Q2	11	14	0.4 (0.1, 2.4)	1.4 (0.9, 2.0)	53	59	1.9 (0.5, 6.4)	1.9 (1.3, 2.9)
Q3	17	22	2.5 (0.7, 8.0)	1.5 (1.0, 2.2)	81	85	2.6 (0.8, 8.2)	2.1 (1.4, 3.2)
Q4	31	38	3.5 (1.1, 10.7)	2.4 (1.6, 3.4)	124	136	2.5 (0.7, 8.1)	3.3 (2.2, 5.0)
Q5	391	10653	5.4 (1.8, 16.0)	3.7 (2.6, 5.2)	1090	3750	3.9 (1.3, 11.9)	4.1 (2.8, 6.1)

B-017

Verification of the Analytical Performance of an Assay to Determine the Enzymatic Activity of Circulating Lipoprotein-Associated Phospholipase A2 (Lp-PLA2)

M. Cerelli, K. Grimm, X. Duan, E. Mulberg, E. Zychlinsky. *diaDexus, South San Francisco, CA*

Objective: Characterize analytical performance of the PLAC Test for Lp-PLA2 Activity Assay for use as a laboratory test to augment clinical risk assessments of coronary heart disease (CHD).

Background: While lipid levels are key parameters in assessing an individual's risk for CHD, the occurrence of myocardial infarction in patients who have achieved targeted levels of LDL, suggests the need for additional clinical tools to improve patient management. The PLAC Activity test uses a synthetic substrate that shares the cleavage site of the endogenous substrate of Lp-PLA2 to determine the enzyme's activity in plasma or serum.

Methods: Testing for Lp-PLA2 activity was done using an open user-defined channel of the Beckman Coulter AU400. Activity was determined by spectrophotometrically monitoring the rate of 4-nitrophenol formed and calibration was achieved using a 5-point calibration curve (0-400 nmol/min/mL). The analytical performance of the test was assessed to characterize its performance for clinical use.

Results: Males had higher Lp-PLA2 activity (median: 176, 95th percentile: 295 nmol/min/mL) compared to that for females (median: 154, 95th percentile: 264 nmol/min/mL), with expected values established using samples from all-comer donors (154 males and 146 females, age range: 35 to 75 yrs). Analyses of Lp-PLA2 activity in EDTA plasma and serum yielded similar results including a slope of 1.00, a y-intercept of 0.05 and R value of 0.988 (n=131). Sample stability for activity determinations was acceptable for different temperatures and storage durations, including ambient for 24 h, 4°C for 2 wks, -20°C for 18 mos, and -70°C 2 yrs, with a mean recovery of 90-103 %. Five freeze/thaw cycles had minimal influence when samples were stored at -20 or -70°C, with a mean recovery of 95-97 % (95% CI). Analytical sensitivity, limit of quantitation (LOQ) was ≤ 10 nmoL/min/mL. Total lab precision with 4 samples and 2 controls assayed in duplicate twice a day over 20 days with 3 reagent lots yielded a coefficient of variation of <3.8% (113-315 nmol/min/mL). Linearity determined in 3 reagent lots with 3 pairs of plasma samples demonstrated an assay measuring range from 10-382 nmol/min/mL. Analysis of endogenous substances, including albumin 60 g/L, unconjugated bilirubin 20 mg/dL, conjugated bilirubin 12 mg/dL, cholesterol 300 mg/dl, triglycerides 400 mg/dL, hemoglobin 1 mg/mL) demonstrated no interference. Similarly, analysis of drugs at low/high concentrations in µmol/L, including acetaminophen 33/1324, aspirin 720/3600, atorvastatin 2/20, Diphenhydramine 2/20, fenofibrate 42/125, lisonopril 0.25/0.74, niacin 480/4800, tolbutamide 400/2300, warfarin 10/33, Metformin 31/310, clopidogrel bisulfate 10/100, vitamin C 14/342, demonstrated no interference. Recovery of Lp-PLA2 tested with spiked solutions and 3 reagent lots, yielded regression slopes of 0.99-1.10, y-intercept -2.87-4.21, and R2 values of 0.997 - 1.000. On-board stability was validated up to 4 weeks for reagents and open bottle stability of 3 months was validated for calibrators and controls.

Conclusions: Key performance parameters of sample stability and assay precision demonstrate the analytical robustness of the assay, and its suitability and convenience for use on automated chemistry analyzers in diverse clinical laboratory settings.

B-018

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

P. O. Collinson[1], A. Hammerer-Lercher[2], M. van Dieijen-Visser[3], K. Pulkki[4], J. Suvisaari[5], A. Stavljenic-Rukavina[6], H. Baum[7], C. Duff[8], K. Aakre[9], M. Langlois[10], S. Stankovic[11], P. Laitinen[5]. *[1]St George's Hospital, London, United Kingdom, [2]University Hospital Innsbruck, Innsbruck, Austria, [3]Maastricht University Medical Center, Maastricht, Netherlands, [4]University of Eastern Finland, Kuopio, Finland, [5]Helsinki University Central Hospital, Helsinki, Finland, [6]University of Zagreb, Zagreb, Croatia, [7]Regionale Kliniken Holding RKH GmbH, Ludwigsburg, Germany, [8]University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom, [9]Haukeland University Hospital, Bergen, Norway, [10]AZ St-Jan Hospital, Bruges, Belgium, [11]Clinical Center of Serbia, Belgrade, Serbia*

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 71/441(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 (15.5%) and 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 diagnostics standard assay (n = 84). For troponin T the 99th percentile is 14 ng/L, 10% CV of 13. Only 92 (50.3%) of laboratories were using the 99th percentile for cardiac troponin T recommended. The decision limit used varied from 2 ng/L to 700 ng/L with peaks of utilisation at14 ng/L, 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 7 (8.3%) used 28 and 3 (3.6%) 32. The range used was from 25 ng/L to 500 ng/L with peaks at30 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.

B-019

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

O. Rodriguez Fraga, T. López Fernandez, J. Feliu, M. Canales, E. Ramirez, M. Torrente Regidor, J. López-Sendón, P. Oliver Saez, A. Buño Soto. *University Hospital La Paz, Madrid, Spain*

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. Chemioluminiscence assays were used for biomarkers: hs-cTnT(Roche Elecsys), Galectin-3 and hs-cTnI(Abbott Architect), c-TnI and NT-proBNP (Siemens Vista) and 99th percentile (P99) was used as cut-off (hs-cTnT: female 13 male 20 pg/mL CV=10%, hs-cTnI female 15,6 male 34,2 pg/mL CV=4% and c-TnI 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: 58,1±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). Maximun value for all troponins occurred at 3m (table1). Using P99 as cut-off, hs-cTnT identified additional patients when compared to hs-cTnI and c-TnI. Percentage of troponins >P99 were higher in patients with cardiotoxicity. Sensitivity for hs-cTnI at 3m for early detection of cardiotoxicty 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=14,7%. No significant relation was observed between NT-proBNP and Galectin-3 and cardiotoxicity.

Table1. Media ±SD for troponins

pg/mL	B	21D	3M	6M	12M	18M	24M
Hs-cTnI	2,9±4.5	13,9±33.4	41,1±24.1	10,5±20.8	4±4.4	15,2±7.4	3,7±4.3
Hs-cTnT	7,4±5.6	11,4±5.3	16,5±9.1	15,4±12.8	9,3±4.9	11±4.6	11,1±6.2
cTnI	16,6±15.4	18±18.9	27,5±41.5	25,5±42.7	16,9±9.4	16,1±5.3	16,7±10.3

Conclusions

Increased hs-cTnT and hs-cTnI occurred in the cardiotoxicity group with a maximum concentration at third month.

Hs-cTnT identifies more patients with cardiac injury than hs-cTnI and c-TnI, and has higher NPV (91,1%%) at 3m for early detection of cardiotoxicity.

NT-proBNP and Galectin-3 doesn't seem to add value in this scenario.

B-020

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

A. Hammerer-Lercher[1], P. Collinson[2], M. P. van Dieijen-Visser[3], K. Pulkki[4], J. Suvisaari[5], A. Stavljenic-Rukavina[6], H. Baum[7], C. Duff[8], K. M. Aakre[9], M. R. Langlois[10], S. Stankovic[11], F. S. Apple[12], R. H. Christenson[13], P. Laitinen[5]. *[1]Central Institute for Medical and Chemical Laboratory Diagnostics, Innsbruck, Austria, [2]Departments of Chemical Pathology and Cardiology, St George's Hospital, London, United Kingdom, [3]Maastricht University Medical Center, Maastricht, Netherlands, [4]University of Eastern Finland and Eastern Finland Laboratory Centre, Kuopio, Finland, [5]HUSLAB Laboratories, Department of Clinical Chemistry and Haematology, Helsinki University Central Hospital, Helsiniki, Finland, [6]University of Zagreb, Zagreb, Croatia, [7]Regionale Kliniken Holding RKH GmbH, Ludwigsburg, Germany, [8]Department of Clinical Biochemistry, University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom, [9]Haukeland University Hospital, Bergen, Norway, [10]Asklepios Core-lab, Department of Laboratory Medicine, AZ St-Jan Hospital Bruges and Ghent University, Ghent, Belgium, [11]Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia, [12]Department of Laboratory Medicine and Pathology, Hennepin County Medical Center, Minneapolis, MN, [13]University of Maryland School of Medicine, Baltimore, MD*

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

accreditation status. 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.

B-021

In vitro Studies of Human Cardiac Troponin I Degradation.

A. Kogan[1], I. Katrukha[1], A. Vylegzhanina[2], A. Bereznikova[1], A. Katrukha[1]. *[1]HyTest Ltd, Turku, Finland, [2]Moscow State University, Moscow, Russian Federation*

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 37ºC for 3 hours. In the preliminary experiments it was shown that in such conditions cTnI is 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-terminus 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.

B-022

Clinical Study to Validate the Use of a New Point of Care BNP Test as an Aid in the Diagnosis of Heart Failure

M. M. Murakami[1], K. M. Smith[1], P. R. Stack[1], B. J. Kilburn[1], R. Ler[1], B. Amundson[1], L. Lilja[2], J. Melin[2], F. S. Apple[1]. *[1]Hennepin County Medical Center, Minneapolis, MN, [2]Fiomi Diagnostics, Uppsala, Sweden*

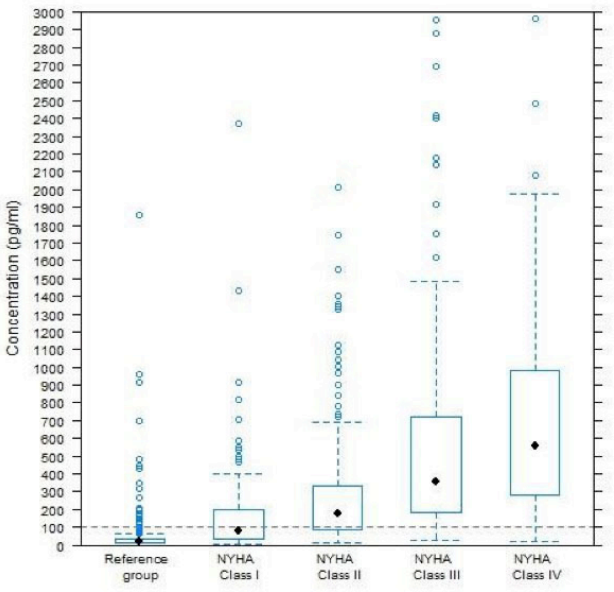
Background: The Trinity Biotech Meritas BNP single-epitope assay for detecting BNP is a Point of Care test used in conjunction with the Meritas Analyzer for quantitative determination of BNP in whole blood or plasma to aid in the diagnosis of heart failure (HF).

Objective: The objective of this study was to validate the clinical performance of the Meritas BNP test (Trinity Biotech) for the quantitative determination of BNP for use as an aid in the diagnosis of heart failure (HF).

Methods: The study was designed as a retrospective study of banked EDTA plasma from 665 eligible adult subjects (281 females, 384 males) with a diagnosis of HF. The diagnosis was based on the NYHA classification I-IV. The normal range was determined using banked EDTA-plasma from 1424 non-HF patients (822 females, 602 males), including individuals with comorbidities such as diabetes, hypertension, chronic obstructive pulmonary disease (COPD) and renal disease.

Results: A box and whiskers plot of the clinical study population, classified according to NYHA, is presented the Figure. A progressive increase in BNP concentrations with increasing NYHA classifications shows a relationship between the severity of the clinical signs and symptoms of HF and the median BNP concentrations of each NYHA class. The diagnostic sensitivity and specificity using a decision threshold of 100 pg/mL (ng/L) for various age groups (< 45, 45-54, 55-64, 65-74, 75+ years) within each gender were as follows: male: sensitivity, range 64 to 79%, specificity, range 81 to 100%; female, sensitivity, range 63 to 85%, specificity, range 75 to 100%; The ROC curve area for HF based on BNP was 0.938 (95%CI 0.927-0.949).

Conclusion: The data indicate that BNP measurements provide objective information for use in the diagnosis of heart failure. The sensitivities and specificities were determined to be acceptable according to the performance claims.



B-023

Diurnal and Longitudinal Variations of Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) Enzyme Activity

M. Cerelli, P. Sekella, H. Cox, E. Mulberg, E. Zychlinsky. *diaDexus, South San Francisco, CA*

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- or inter-individual and also temporal biovariability 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 biovariability 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 wks 2, 4, 8 and 12 to obtain serum samples which were stored at -70°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 AU400. 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 (2.3-9.2). 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 wks demonstrated a mean percent intra-individual variation of 5.3% for Lp-PLA2 activity, with corresponding published data 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.

B-024

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

P. Patel[1], D. Shan[1], L. Styskal[1], L. Chang[1], D. H. Wilson[1], M. Conrad[2], P. Jarolim[2]. *[1]Quanterix Corporation, Lexington, MA, [2]Brigham and Women's Hospital, Harvard Medical School, Boston, MA*

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 quantitate 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 (cTnI) based on single molecule array (Simoa) technology with a limit of detection 2 logs lower than contemporary high sensitivity troponin assays.

Method: Reagents were developed for a paramagnetic bead-based ELISA for use in the Simoa HD-1 Analyzer. Anti-cTnI capture beads were prepared by covalent coupling of antibody to carboxy paramagnetic microbeads, detector antibody was biotinylated by standard methods, and an enzyme conjugate was prepared by covalent coupling of streptavidin and [[Unsupported Character - Symbol Font β]]-galactosidase. The HD-1 Analyzer first performs a 2-step sandwich immunoassay using 42 µL of serum or plasma sample, then transfers washed and labeled capture beads to a Simoa disc where the beads are singulated in 50-femtoliter microwells, sealed in the presence of substrate, and interrogated for presence of enzyme label. A single labeled cTnI molecule provides sufficient fluorescent signal in 30 seconds to be counted by the HD-1 optical system. At low cTnI concentration, the percentage of bead-containing wells in the array with a positive signal is proportional to the amount of cTnI present in the sample. At higher cTnI concentration, the total fluorescence signal is proportional to the cTnI in the sample. The concentration of cTnI is then interpolated from a standard curve (range to 300 pg/mL). Time to first result is 45 minutes. The assay was evaluated for sensitivity, recovery, linearity precision and normal range. Discrimination of healthy subjects from those with mild to moderate heart failure was also preliminarily assessed.

Results: Limit of detection (2.5 SD) was 0.010 pg/mL across 26 runs. Limit of quantification (20% dose CV from diluted serum samples) was 0.079 pg/mL across 16 runs and 144 determinations. Recovery of cTnI spiked into normal serum averaged 80.5%. Mean linearity was 89.5%. Precision per EP5-A guideline included two serum-based panels, 1 plasma-based panel and two cTnI controls assayed in replicates of three twice per day for five days using a single calibration curve. ANOVA gave CV's <10% for all levels. Serum cTnI values from 97 healthy control samples ranged from 0.072 to 8.40 pg/mL, with a mean and 99th percentile of 1.01 and 8.40 pg/mL. Serum cTnI values from 375 patients with mild to moderate heart failure (NYHA classification II and III) ranged from 0.440 to 1770 pg/mL, with a median of 15.1 pg/mL. The heart failure samples had significantly higher cTnI concentrations than the healthy subjects (p=0.0002). Evaluation of the predictive value of the cTnI concentrations is ongoing.

Conclusion: The results show the digital Simoa cTnI assay exhibited good general analytical properties and cTnI levels from healthy subjects were above the sensitivity limits. The assay represents a new enabling tool for ultra-sensitive cTnI measurement.

B-025

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

S. Sullivan, M. Steinmann, E. Oliver, D. Freyler, K. Smith, K. Michael, M. Cleary. *Ortho Clinical Diagnostics, Rochester, NY*

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 (cTnI) 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 cTnI 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 cTnI 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 cTnI 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 LoB, LoD 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 cTnI in samples from 412 individuals with values within reference ranges for eGFR and NT-proBNP. The gender independent 99th percentile was 23 pg/mL. Correlations between the VITROS® High Sensitivity Troponin I assay and both a commercially available high sensitivity assay and the VITROS® Troponin I ES assay were obtained using 111 patient samples from a variety of clinical categories. The comparison of VITROS® Troponin I ES to VITROS® High Sensitivity Troponin I resulted in a slope of 1.00 pg/mL, an intercept of -20 pg/mL and a R of 1.00.

Conclusion:

In conclusion, the prototype VITROS® High Sensitivity Troponin I assay has a 10%CV at a concentration that is significantly lower than the 99th percentile (medical decision limit) and the assay has the ability to measure cTnI above the LoD in 93% of a reference population.

* Under Development

B-026

Relative contribution of high sensitivity cardiac Troponins I and T in cardiovascular risk stratification in patients with OSA after treatment with CPAP

M. C. Feres[1], C. S. Silva[2], L. Mello-Fujita[1], M. C. De Martino[3], M. A. Machado[4], R. Bini Jr.[1], A. A. Lino de Souza[1], D. Poyares[1], S. Tufik[1]. *[1]Universidade Federal de Sao Paulo- Unifesp, Sao Paulo, Brazil, [2]Associacção Fundo de Incentivo a Pesquisa- AFIP, Sao Paulo, Brazil, [3]Associação Fundo de Incentivo a Pesquisa- AFIP, Sao Paulo, Brazil, [4]Laboratorio Central do Hospital Sao Paulo, Sao Paulo, Brazil*

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/m^2 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 -80ºC 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 cTnI immunoassay (Abbott/ARCHITECT system). It was measured cTnI ES conventional by chemiluminescence (Vitros ® - Ortho Clinical Diagnostics) 12 pg/mL is the limits of detection to this methods and cTnI by AccuTnIDx (Access/Beckman Counter) with detection limits 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.

B-028

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

V. Shenoy[1], V. Mehendale[2], P. Shettigar[2], K. Prabhu R V[1], R. Shetty[1]. *[1]Kasturba Medical College Manipal, Udupi, India, [2]Welcome Group School of Hotel Management Manipal, Udupi, India*

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.

AIM: To measure and correlate anthropometric indices with respective serum homocysteine and Gensini scores in male and female patients with CAD.

MATERIALS AND METHODS: Institutional Ethics committee permission was taken prior to the study. Total 70subjects (males and females) in the age group of 30-70years presented to Cardiology clinic who consented to participate were included. All of them had undergone angiogram evaluation and risk stratification using Gensini score as standard of care. Out of 70 subjects, 19 had no evidence of CAD on angiogram (controls) and remaining 51 (Male: Female= 42:9) were considered as cases. Subjects taking vitamin supplements, oral contraceptives, pregnant women were excluded from the study. Height and other anthropometric indices were measured to the nearest 0.1 cm, weight to nearest 0.5 kg in light clothing and without shoes. Body mass index (BMI) was calculated as weight (kg)/height (m^2).Two ml of fasting blood sample collected in plain vacutainers and serum was separated and stored at -70°c until use. Serum levels of homocysteine were determined by using commercially available ELISA kit. Data was analyzed using SPSS version 16.0.Correlation of Gensini scores and serum homocysteine with anthropometric indices was done using Pearson coefficient correlation.

RESULT: There was no significant difference in BMI among cases and controls, but homocysteine was significantly increased in cases. Homocysteine and Gensini scores were correlated with different anthropometric indices in cases also did not show any significant correlation. But when these comparisons were done gender wise, we found a significant correlation of waist to hip ratio with Gensini scores (p = 0.047) and homocysteine (p = 0.012) only in males.

CONCLUSION: Both homocysteine and Gensini scores had a significant correlation with waist to hip ratio only in male gender with CAD indicating that we have to use different strategies in males and females for risk stratification of coronary artery diseases using these markers.

B-030

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

C. NOBRE[1], L. F. A. NERY[1], R. J. HENRIQUES[1], S. F. FONSECA[2], A. DIAS[1], A. RODRIGUES[1], S. S. S. COSTA[1]. *[1]SABIN LABORATORY, BRASILIA, Brazil, [2]UnB, BRASILIA, Brazil*

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 MÉRIUEX, 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 are 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.

B-031

Characteristics of the New Beckman Coulter High-Sensitive Cardiac Troponin I Assay (hsTnI)

M. Szabo, D. Arockiasamy, E. Cicirelli, J. Granger, M. Thomas. *Beckman Coulter, Chaska, MN*

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 hsTnI 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 hsTnI assay is capable of accurately measuring 10 pg/ml changes in TnI concentrations. This hsTnI assay accurately measures TnI in comparison to a currently validated device (correlation between Access AccuTnI+3 and hsTnI within 5%) and exhibits < 5% bias between sample types (serum, plasma). The assay does not exhibit cross reactivity to cardiac TnT, cardiac TnC, 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 (hsTnI) 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.

B-032

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

S. A. Love, Y. Sandoval, S. W. Smith, K. M. Schulz, M. M. Murakami, J. Nicholson, F. S. Apple. *Hennepin County Medical Center, Minneapolis, MN*

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: NCT02060760' study. Patients, 18+ years of age, presenting to the emergency department where providers used cTnI to

rule-in and 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 $i1000_{SR}$ or $i2000_{SR}$). 99th percentiles were as follows: contemporary cTnI 30 ng/L (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 (31-89) ng/L for females; hs-cTnI 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). The hs-cTnI assay resulted in a 15% decrease (p=0.01) in patients with at least one value greater then the sex-specific cut-off. The number of women with an increase above the 99th percentile cut-off was significantly different (p=0.003) vs. males. Further, the hs-cTnI assay sex-specific cut-offs resulted in a 29% decrease in males with an increased value and a 5% increase for females with an increased value.

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.

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.

A. H. Wu[1], J. Estis[2], P. Heseltine[2], J. Todd[2], P. Kavsak[3]. *[1]University of California, San Francisco, San Francisco, CA, [2]Singulex, Inc., Alameda, CA, [3]McMaster University, Hamilton, ON, Canada*

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 95%tile 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) 15% 92 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.

Summary of cytokine results						
	IL6		IL17a		TNFa	
	Median	%>95th of ref. population	Median	%>95th of ref. population	Median	%>95th of ref. population
Entire cohort	1.3	8.9	0.4	4	2.5	32.2
Females	1.4	8.9	0.4	3.9	2.4	31.2
Males	1.3	5.1	0.4	4.2	2.5	33.4
	1.1	5.1	0.4	2.3	2.1	18.3
>= 50 y	1.5	10.6	0.4	4.7	2.6	37.8
HbA1c <=5.9%	1.3	7.3	0.5	3.4	2.4	28.4
HbA1c >5.9%	1.9	15.4	0.4	6.1	2.8	46.5
LDL <=129 mg/dL	1.4	10.2	0.4	4.7	2.5	23.6
LDL >129 mg/dL	1.3	6.7	0.3	2.7	2.4	29.6
HDL at target*	1.3	7.7	0.4	3.7	2.4	29.1
HDL less than target*	2.0	17.2	0.5	6.1	2.9	47.1
*HDL target	>=45mg/dL females	>=35 mg/dL males				

B-034

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

S. Cardoso, N. Silva, K. K. Silva, E. A. Lima, J. D. Cruz, A. C. Almeida, L. R. Almeida, L. B. Faro, M. L. Garcia, M. Akiyama. *DASA, São Paulo, Brazil*

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 immunoassay (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 I 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.

B-035

Biomarkers of Cardiovascular Risk Assessment (High C-Reactive Protein, Homocysteine and Lipoprotein (a)) in First- Degree Relatives of Individuals with premature coronary disease

M. G. CASTELO[1], R. Santos Filho[2], T. Sousa[1], F. Furtado[1], A. Pereira[2], A. Dorea[1], M. D. Freire[3], A. Melo[1], O. P. Denardim[3]. *[1]DASA, FORTALEZA, Brazil, [2]USP, São Paulo, Brazil, [3]DASA, São Paulo, Brazil*

Background: The use of biomarkers that can identify people about the risk of developing cardiovascular disease (CAD) is the subject of many studies. Several individual biomarkers have been linked to the risk of CAD including the levels of high-sensitivity C-Reactive Protein (a marker of inflammation), Homocysteine (a marker of endothelial function and oxidant stress) and Lipoprotein (a) that is involved in the adhesion of inflammatory cells and migration and up take of macrophage foam cells into the arterial wall. The aim of this study was to evaluate the profile of cardiovascular risk biomarkers (C-Reactive Protein, Homocysteine and Lipoprotein (a)) in first-degree relatives (FDR) of young individuals diagnosed with acute myocardial infarction (age < 45 years). **Methods:**It was a cross-sectional study that recruited 167 FDR of both sex of patients with premature coronary disease in period between 2010-2015. We measured high-sensitivity C- Reactive Protein, homocysteine and lipoprotein (a). Framinghan risk score was performed in among the patients. **Results:** The median age was 43,7±12,7 years. The laboratory analysis expressed in median showed homocysteine 9,7 (5,4 - 23,4)µmol/L; lipoprotein (a): 20 (2,9 - 106) mg/dL and high-sensitivity C Reactive Protein: 0,2 (0,01 -1,9) mg/dL. Sixty six percent of patients presented risk of developing cardiovascular disease in 10 years less than 10% by the criteria of Framingham score. **Conclusion:** There are few data available regarding these biomarkers of cardiovascular disease in FDR of patients with premature coronary artery disease. Despite the family history is an indisputable cardiovascular risk factor was not observed in the evaluation of biomarkers such as homocysteine and lipoprotein (a) a significant change in this group of higher risk for cardiovascular disease. However when the biomarker of cardiovascular risk used was the high-sensitive C Reactive Protein most of these patients were classified as medium risk even those described as low risk by the Framingham criteria.

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

A. Bartolome, S. Biscocho, J. Stiles, U. Enkhbayar, R. Ramirez, R. Sweetwood, T. Dang, H. Morrell, K. Bui, A. Mukherjee, J. Estis, R. Livingston, L. Monsalve, J. Bishop, J. Felberg, J. Todd. *Singulex, Alameda, CA*

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-7A2. 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 Immunoassay

X. Wang, S. Tjon-Kon-Sang, V. Torres, R. Livingston, L. Monsalve, J. Todd, J. Felberg, J. Bishop. *Singulex, Alameda, CA*

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® instrument 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, since much of the circulating BNP is truncated at the N- and C-termini, 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 immunoassay.

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 1130 pg/mL BNP. The assay was linear throughout the reportable range, which extends to 5000 pg/mL, and there is no high dose hook effect up to100,000 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). Two samples, which had reported BNP concentrations of 220 and 297 pg/mL by the Siemens assay, had much higher results (1343 and 926 pg/mL) with the Sgx assay. 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 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.

V. R. PATEL, A. Aneja, J. Qiu, J. Wang, A. Khan, J. F. Ekpe, M. Szydlowska, B. Goldsmith. *TEMPLE UNIVERSITY HOSPITAL, PHILADELPHIA, PA*

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 re-introduced 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 cTnI 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 cTnI 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

Analyzers	No.	Mean x/y	Slope	Intercept	Correlation
Access-2 ED/ML	109	0.117/0.108	0.981	-0.008	0.9939
Access-2 ED/DxI800	115	0.12/0.111	0.791	0.015	0.9776
Access-2ML/DxI800 ML	115	0.131/0.111	0.775	0.009	0.9589

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

S. A. Marzouk, M. A. Sadaka, D. N. Younan, B. B. Ali. *Faculty of Medicine ,Alexandria, Alexandria, Egypt*

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 subject 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 ug/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 &without 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 .