Wednesday, July 30, 2014

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

Cardiac Markers

B-314

One year retrospective cTroponin T observations : Women present themselves at a higher age with ACS.

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Background: Generally presumed women present themselves with Acute Coronary Syndrome (ACS) at higher age than men.

Method: We studied the use of cardiac Troponin T (cTnT) in our patient population with respect to results, age and gender. cTnT was measured with hs cTnT assay (Roche Diagnostics) using a limit of detection of 0.005 µg/l and an upper limit of reference value of >0.015 ug/L. Data on cTnt results from a serially sampled patient population (results, gender and age) were extracted from the laboratory information system over a one year period for those patients with cardiologists involved in their medical treatment. In this patient group only the result of the first blood drawing was incorporated in the dataset investigated.

Results: 1665 patients (703 females (42%), 962 males (58%)) were included. 696 of these patients had a cTnT< 0.015 µg/l and 969 patients a cTnT >0.015 ug/L.

Table1

Conclusion: Our data indicate that women do present themselves at higher age with respect to men with increased cTnT level as indicator of ACS.

cTroponin T >0.015 µg/l				Age (years)	
	N-patients	Mean	25 th percentile	50 th percentile	75 th percentile
Female	364	74	67	76	83
Male	605	67	57	67	79

B-315

Association of Lipid, Inflammatory, Cardiac, and Renal Biomarkers with C-Reactive Protein in Cardiovascular Risk Categorization - A Factor Analysis Approach

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Background. C-reactive protein (CRP) strongly and independently predicts cardiovascular complications, and its use is recommended for risk assessment in primary prevention by several institutions. Also, there is evidence of other factors, contributing to and maintaining the intensity of atherosclerotic processes, which might identify cardiovascular risk contribution not originated from traditional risk factors. The aim of this study was to examine, using factor analysis, the nature of influence of biomarkers of inflammation, lipid metabolism, renal, and cardiac function on cardiovascular risk, and their possible connection and relations to CRP values.

Methods. Principal component analysis was used to investigate clustering of inflammatory markers [serum amyloid A (SAA), fibrinogen, α1-acid glycoprotein (A1AGP), haptoglobin, C3 and C4 complement components], lipid metabolism [total, HDL, non-HDL and LDL cholesterol, triglycerides, apolipoprotein A-I (apo A-I), apolipoprotein B (apo B), lipoprotein (a) (Lp(a))], renal [creatinine, cystatin C (Cys-C), estimated glomerular filtration rate (eGFR)], cardiac function [N-terminal pro-natriuretic peptide type B (NT-proBNP), high sensitivity cardiac troponin T (hs-cTnT)], and traditional cardiovascular risk factors [age, gender, body mass index (BMI), systolic blood pressure (SBP)], obtained from 242 apparently healthy individuals.

Results. Factor analysis identified five clusters, i.e. principal components (factors), which explained 65.3% of the total variance (29.0% factor 1, 13.2% factor 2, 9.0% factor 3, 8.5% factor 4, and 5.6% factor 5). Based on factor loading of ≥ 0.5 clasters were interpreted as 1) „systemic inflammation“ (fibrinogen, SAA, A1AGP, haptoglobin, C3 and C4 complement components); 2) „cardiorenal factor“ (creatinine, uric acid, Cys-C, hs-cTnT and gender); 3) „atherogenic cholesterol“ (LDL and non-HDL cholesterol); 4) „hemodynamic factor“ (age and NT-proBNP); and 5) „metabolic factor“ (triglycerides and HDL cholesterol). The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.75. In multiple regression analysis, five factor model had the best predictive value for CRP concentrations >1 mg/L (OR 6.53, 95% CI 4.06-10.50, P<0.0001 for “systemic inflammation”; OR 1.44, 95% CI 1.04-2.00, P=0.028 for „cardiorenal factor“; OR 1.76, 95% CI 1.23-2.5, P=0.002 for „atherogenic cholesterol”; OR 1.91, 95% CI 1.33 - 2.73, P<0.0001 for „hemodynamic factor“;OR 1.90, 95% CI 1.33 - 2.73, P<0.0001 for „metabolic factor“), while „cardiorenal factor“ and „atherogenic cholesterol“ completely lost their significance (P>0.05) for predicting CRP concentrations >2 mg/L and >3 mg/L. The ability of the factor-based logistic regression model was compared with multivariable logistic model containing all 25 variables in predicting the presence of CRP concentrations >1 mg/L, >2 mg/L, and >3 mg/L. The area under the receiver operator characteristics curve (AUC) of the five factor model was 0.889 and was not statistically significantly different from the 25 variable model (AUC=0.922) (P=0.2113). However, the differences between the two models examined were statistically significant in predicting the values of CRP>2 mg/L and CRP>3 mg/L.

Conclusion. Systemic inflammation, cardiorenal function, atherogenic lipid profile, hemodynamic and metabolic status might independently contribute to the pathophysiology of chronic, subclinical inflammation in atherosclerosis. They might represent underlying dimensions accompanying the elevation of CRP concentration and increased cardiovascular risk.

B-316

Verifying A Cut-Off Value for the Beckman TnI+3 Assay on the DxI 800 and Access-2 Analyzers.

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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 recently re-introduced a Troponin-I (AccuTnI +3) assay for the DxI 800 and Access-2 analyzers. We evaluated whether the stated cutoff determined by the manufacturer was appropriate for our patient population.

Design: We measured plasma cTnI concentrations in 94 patients presenting to our Emergency Department (ED) in whom no history or evidence of cardiac disease was found following a review of each patient’s chart. Our population consisted of 30 Males (ages 16-63 years) and 64 females (ages 5-54 years). Specimens were spun, aliquoted, frozen within 24 hours at -20o C, and analyzed within 30 days of collection. Where a history could not be determined results were not included. cTnI was measured using the AccuTnI+3 assay (Beckman Coulter, Brea, California) on both the DxI 800 and Access-2 Immunoassay Systems. EP Evaluator (Data Innovations, Burlington, VT) and Excel spreadsheet calculations were used to evaluate the data.

Results: cTnI results for the DxI 800 showed (ng/mL): Males range=0.00-0.76, mean 0.03; Females range=0.00-0.36, mean 0.02. For the Access-2: Males range=0.00-0.86, mean 0.04; Females 0.00-0.50, mean 0.02. We determined the cutoff for plasma specimens assayed on the DxI 800 of 0.03 ng/mL and for the Access-2 of 0.04 ng/mL. The ranges (males and females) demonstrate that there were results on non-cardiac patients above the cutoff.

Conclusions: The 99th percentile at a coefficient of variation (CV) of <10% is used to define the cutoff concentration between a non-MI and a MI event. Though the manufacturer determined a cutoff of 0.2 ng/mL, 0.04 ng/mL was selected to provide one consistent cutoff for our hospital. We suggest using non-cardiac specimens that reflect the population to determine an appropriate cutoff. Caution must be used to ensure, as best as possible, exclusion of patients with a history or presenting symptoms of cardiac disease.

B-317

Short Term Variation Important In Evaluating Goodness of Troponin Assays For Diagnosing Myocardial Damage

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Background: While total imprecision is usually used to evaluate the quality of troponin assays, most diagnoses of myocardial necrosis are based on serial troponin changes within 8 to 12 hours. We have used a novel method to isolate the short term variation in sequential, intra-patient results, a mixture of intra-individual biologic variation (sb) and co-existing analytic variation (sa). We have used this method to derive the short term variation of two troponin assays, the Ortho VITROS Troponin I ES and the Beckman Coulter AccuTnI.

Methods: Two different data sets were extracted: all of the patient troponins that were measured by the main Beckman DxI analyzer at University of Alberta Hospital over a 20 month period and serial VITROS troponins of 1271 patients in whom the diagnosis of myocardial infarction (MI) was being considered (measured on one of two VITROS systems over 6 months at Hennepin County Medical Center). For both clinical environments, patient bloods were collected into lithium heparin. Two subsets of patient results were studied and compared, those patients whose serial troponins were all less than 41 ng/L and patients with serial troponins all less than 61 ng/L. We tabulated the pairs of intra-patient troponins that were separated by 2 to 3h, 3 to 4h, 4 to 5h, 5 to 6h, 6 to 7h, 8 to 9h, and 9 to 10h. The standard deviations of duplicates (SDD) of the paired troponins were calculated for each time interval. The graphs of SDD vs. time interval were approximately linear; the y intercept (y0) provided by the weighted linear regression equation represents the sum of sa and sb with y0 = (sa2 +sb2)1/2.

Results: For patients with series of troponins under 41 ng/L (average of 14 and 13 ng/L for Beckman and Ortho, respectively), the average short term variation (including biologic variation) was 3.0 ng/L (SEM=0.8 ng/L) for the Beckman and 1.1 ng/L (SEM=0.3 ng/L) for the Ortho. For patients with series of troponins under 61 ng/L (average of 18 and 15 ng/L for Beckman and Ortho), the short term variation was 3.0 ng/L (SEM=1.3 ng/L) for the Beckman and 2.2 ng/L (SEM=0.6 ng/L) for the Ortho.

Conclusion: Perhaps not surprisingly, the total short term variation (including biologic variation) is approximately equal to the short term variation derived from running low level quality control materials. The biologic variation of troponins for patients with healthy myocardiums is thus very low. For the Beckman DxI, the short term variation (3 ng/L) at a level of 25 or 35 ng/L is considerable. If +/-3SD limits are used (99.7% confidence limits), then variation around these levels is +/- 9 ng/L, variation that can cause both false positive and false negative interpretations. For the Ortho VITROS, there will be fewer misinterpretations. We recommend that serial patient troponin values be collected and "mined" for total variation. The analytical systems that minimize the short term total variation are most useful diagnostically.

B-318

Paraoxonase-1 enzyme activity assay for clinical samples: Validation and correlation studies

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Introduction: Paraoxonase-1 (PON1) enzyme is reported in various types of tissues and linked to numerous pathophysiological disorders; representing a potential biomarker in many pathological conditions such as cardiovascular diseases. Methods: We conducted several small studies to evaluate PON1 performances affected by sample types, storage, and interferences. In addition; we carried out some limited studies to compare the performance of the clinical research widely used PON1 assay to similar commercially available PON1 kit assay method. Results: Type of anticoagulant studies have shown that samples collected in NaF, citrate, ETDA clot activator, and sodium heparin have increased PON1 levels 49%, 24.5% and 19.8%; 11.4% and 8% respectively compared to the serum. Whereas samples in lithium heparin have 10.4% decreases in its PON1 levels compared to the serum. Biological interference such as hemolysis has little effect on PON1 levels; however samples spiked with lipids have shown 13% reduction on PON 1 levels. The method comparison studies between the PON1 method commonly available for PON1 assay and a similar non-ELISA commercially available PON1 kit method have resulted in a weak Spearman correlation displaying a coefficient $R^2 = 0.40$ for the range 104.9- 245.6 U/L. Conclusion: The current study will bridge some of the gaps on our understanding to the enzyme performances. The outcome should encourage additional studies in clinical setting to investigate other missing aspects of the factors known to affect PON1 enzyme function and performance.

B-319

Assessing the incremental value of additional biomarkers versus a high-sensitivity cardiac troponin I assay for predicting a short-term serious cardiac outcome in an early chest pain population

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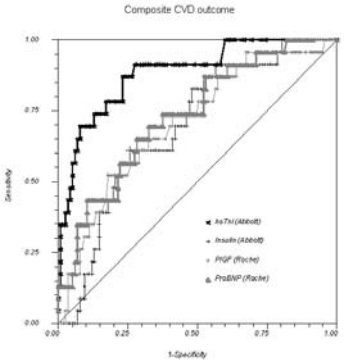
BACKGROUND: Patients presenting with chest-pain to the emergency department (ED) who are at risk for a cardiovascular event in the short-term, must be identified. With the advent of high-sensitivity cardiac troponin (hsTn) assays, most ED patients will have measurable cardiac troponin concentrations. It is unclear whether additional biomarkers of myocardial stress, inflammation, vascular or other endocrine functions can improve prognostic ability when compared to hsTnI alone.

METHODS: After ethics approval, the presentation EDTA plasma sample (stored -80^{0}C) from patients (onset of chest-pain within 6h) in the RING study (Clin Chem Lab Med 2011;49:1915–8) were measured for these analytes (platforms):hsTnI, insulin, myeloperoxidase, 25-OH vitaminD (Abbott:ARCHITECT); IL-6 (Beckman:Access); PlGF, sFlt-1, proBNP (Roche:Elecsys); EGF, NTproBNP, E-selectin, P-selectin, sICAM-3, Thrombomodulin, IL-6, IL-10, MCP-1, bFGF, PlGF, sFlt-1, VEGF (MSD: Multi-Array:); IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFNgamma, TNFalpha, IL-1a, IL-1b, MCP-1, EGF (Randox:Evidence). Using MedCalc, Statsdirect, and R program, we performed ROC curve analyses to select the top three biomarkers with the highest AUC for the composite outcome (percutaneous intervention, coronary artery bypass surgery, significant arrhythmia, refractory ischemic pain, heart failure, myocardial infarction, stroke, cardiac arrest, or death) at 72h and combined these three biomarkers with hsTnI to assess if inclusion of these biomarkers increased the AUC.

RESULTS: Of 140 patients (median age (interquartile range)=60y (49-68); 65% male), 23 had a composite outcome. Abbott hs-cTnI by itself had an AUC of 0.88 (95%CI:0.82-0.93) for prediction of the composite outcome. The top three biomarkers were Roche proBNP (AUC=0.73; 95%CI:0.65-0.81); Roche PlGF (AUC= 0.71; 95%CI:0.63-0.78); and Abbott insulin assay (AUC=0.70; 95%CI:0.61-0.77). The addition of insulin, PlGF and proBNP were not significant (p-values of 0.94, 0.36 and 0.12 respectively) and lowered the AUC (0.86) for the Abbott hsTnI assay.

CONCLUSIONS: The Abbott hsTnI assay alone, provides superior short-term prognostic utility in patients presenting early after chest-pain.



B-320

A high sensitive Homocysteine (hsHCY) assay improves this predictive biomarker's clinical value

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Plasma Homocysteine (HCY) levels predict accelerated development of heart and blood vessel disease and HCY is one of the few tests that predict all cause morbidity

and mortality. A significant practical limitation to HCY testing has been the leakage into plasma of red cell HCY starting within minutes after phlebotomy severely limiting community clinical use of HCY assays. A more stable specimen with reduced variability is needed, especially at lower, more predictive levels. Strategic, clinical, and lab results for an hsHCY assay that addresses this need is reported here.

Specimens (N=30) were collected in tubes containing EDTA (current preferred specimen) and a novel cell preservative EAB. Specimens were stored at 4±1°C until analysis. Plasma was isolated and analyzed at 0.5, 4, 8, 24, 48, 72, and 96 hours after phlebotomy using a Diazyme™ Analyzer 700. The data of HCY values were statistically analyzed by one-way ANOVA.

HCY levels started to increase within 30 minutes in EDTA specimens while the EAB cell preservative sample values were stable for at least 48 hours. Indeed initial HCY levels in EDTA samples averaged 32% higher than ones in the EAB preservative ($p<0.0001$). The initial HCY variance in EDTA is 10.23 ± 2.23, while the variance in EAB is 7.63 ± 1.60. This suggests that HCY is released into plasma from red blood cells in EDTA samples starting minutes after phlebotomy and does not occur in EAB cell preserved specimens over 48 hours.

Initial and more precise HCY can be measured and maintained for at least 48 hours when the EAB cell preservative is used. HCY increase appears to starts within minutes after blood draw in EDTA but not EAB preservative. The EAB cell preservative allows a more stable and accurate HCY assay for this important predictive biomarker. More studies of high sensitivity HCY (hsHomocysteine) are needed to further validate this predictive biomarker clinically and globally.

B-321

Development of a New Latex-Enhanced Immunoturbidimetric Assay for the Determination of Type IIA Secretory Phospholipase A2 (sPLA2), a Biomarker of Increased Cardiovascular Risk

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Background: Secretory phospholipase A2 (sPLA2) enzymes are biomarkers of increased cardiovascular risk and are targets of emerging therapeutic agents. They are associated with incident coronary artherosclerosis in healthy men and women and with recurrent adverse cardiovascular events in patients with acute coronary syndromes. sPLA2-IIA is a member of the phospholipase A2 family and is a potent biomarker for cardiovascular risk assessment. It is widely expressed in hepatocytes, macrophages, platelets and vascular smooth muscle cells and is up regulated in response to pro-inflammatory compounds such as interleukin-1β, interleukin-6, tumor necrosis factor-α, interferon-γ and oxidized low-density lipoprotein (LDL). The objective of this study was to develop a new quantitative, latex-enhanced immunoturbidimetric immunoassay to determine levels of sPLA2-IIA in human serum / plasma which have prognostic value in patients with Coronary Heart Disease (CHD) and can be employed to assess risk of future cardiovascular events. The assay is applicable to a variety of automated, clinical analyser systems, which ensures the reliability and accuracy of the measurements and facilitates the testing procedure.

Methods: The assay is a latex-enhanced immunoturbidimetric assay based on the principle of measuring changes in scattered light. The latex particles are coated with anti-sPLA2-IIA antibodies, which in the presence of sPLA2-IIA rapidly agglutinate. When a sample containing sPLA2-IIA is introduced, the agglutination reaction is initiated and the change in scattered light is measured as a change in absorbance that is directly proportional to the concentration of sPLA2-IIA in the sample. The assay is applicable to a variety of automated systems. A correlation study was conducted using a commercially available enzyme immunoassay. Results:The assay presented a limit of detection of ≤15 ng/mL sPLA2-IIA (analytical range: 0 to 500 ng/mL sPLA2-IIA). The within-run precision and the total precision expressed as %CV, were typically <5% and <8% respectively. In the correlation study, citrate plasma samples, spanning the analytical range, were tested with this immunoturbidimetric assay and a commercially available enzyme immunoassay. Linear regression on the resulting data generated an r-value >0.9.

Conclusion: The data generated indicates that this latex-enhanced immunoturbidimetric assay is applicable to the detection of sPLA2-IIA in human plasma and serum. The assay is of value as a new analytical tool for assessment of cardiovascular risk in clinical settings. Its applicability to a variety of automated clinical analysers ensures the reliability and accuracy of the measurements and facilitates the testing procedure.

B-324

Comparison of hs-cTnT and a conventional cTnI assay for the detection of ischemia induced myocardial injury and type 4 myocardial infarction post PCI.

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Background: comparison of a high sensitive troponin T (hs-cTnT) assay with a conventional troponin I (cTnI) assay, for the follow-up of patients post-percutaneous coronary intervention (PCI), more particular for the detection of ischemia induced myocardial injury (IIMI) and type 4 myocardial infarction (type 4 MI).

Methods: PCI and stenting was performed in 103 stable cardiac patients with significant coronary artery stenosis. hs-cTnT (Modular, Roche) and conventional cTnI (Dimension Vista, Siemens) were measured at 4 time points (0, 90, 180 and 360 min post-PCI). IIMI during stenting was defined as at least one cTn value > upper reference limit (URL = 99th percentile of the reference population) and an absolute rise of >50% of the 99th pctl URL in the 360 min blood sample. Type 4 PCI MI was defined in the 360 min blood sample according to the Third Universal Definition of Myocardial Infarction: elevation of cTn values (>5 x 99th pctl URL) in patients with normal baseline values (=<99th pctl URL) or a rise of cTn values >20% if the baseline values were elevated and were stable or falling (Thygesen et al Circulation 2012;126:2020-2035).

Results: both assays correlated fairly well (r= 0,9081, 95%CI 0,8898 to 0,9235). IIMI during stenting was detected in 68 patients (66%) with the hsTnT assay and in 69 patients (67%) with the TnI assay. The number of patients who answered the criteria for IIMI at 90 min and at 180 min was significantly less (18.2% for TnI and 20.9% for hsTnT at 90 min; 27.3% for TnI and 38.2% for hsTnT at 180 min). A frequency table for IIMI showed agreement between both methods for 31 patients without IIMI and for 65 patients with IIMI (93% concordance). Discrepancies were found for 7 patients (3 positive for hsTnT but negative for TnI and 4 positive for TnI but negative for hsTnT). Chi square test and Fisher's exact test were very significant ($p<0.0001$). The contingency coefficient between both methods was 0.637. Type 4 MI was detected in 33 patients (32%) with the hsTnT assay and in 31 patients (30.1%) with the TnI assay. A frequency table for type 4 MI showed agreement between both methods for 61 patients without type 4 MI and for 22 patients with type 4 MI (81% concordance). Discrepancies were found for 20 patients (11 positive for hsTnT but negative for TnI and 9 positive for TnI but negative for hsTnT). Chi square test and Fisher's exact test were very significant ($p<0.0001$). The contingency coefficient between both methods was 0.465.

Conclusion: myocardial injury caused by a short period of myocardial ischemia during PCI was detected with comparable sensitivity by the hsTnT assay and by the conventional TnI assay. For both assays, values at 90 min and at 180 min underestimated the presence of IIMI as established at the 360 min time point. There were more discrepancies between both assays for the detection of type 4 MI post PCI.

B-325

Quality Assessment and Reagent Lot-to-Lot Consistency in High-Sensitivity and Contemporary Troponin T Assays

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Background: Introduction of high-sensitivity cardiac troponin T (hs-cTnT, Roche Diagnostics) in 2009 outside the US has facilitated and expedited earlier diagnosis of NSTEMI and demonstrated prognostic value in a variety of patient populations. Accompanying this trend are new analytical challenges with hs-cTn assays due to the significant impact small analytical confounds may have in the diagnosis of AMI and interpretation of serial troponin results. Lot-to-lot reagent stability for hs-cTn, particularly at low concentrations, is of utmost importance.

Objective: Assess lot-to-lot performance of the hs-cTnT and contemporary cTnT assays across multiple reagent lots, instruments and targeted at various cTnT concentrations.

Methods: For the period between 2008-2014 (cTnT Gen. 4) and 2013-2014 (hs-cTnT/hs-cTnT-STAT), we reviewed all cTnT reagent lot data (Roche Diagnostics, Penzberg). Acceptability of reagent performance utilized protocols designed to assess variability and bias between reagent lots and instruments. Residual pooled patient specimens were used in all assessments of reagent lot-to-lot performance. Data were analyzed collectively and validated between multiple platforms (Cobas e411/e601/e602; Elecsys 2010; Modular E170).

Results: Unique reagent lot data were available for the hs-cTnT, hs-cTnT-STAT and cTnT Gen.4 assays (n = 3, 5 and 42, respectively). Lot-to-lot reagent performance demonstrated minimal bias across the reportable range for hs-cTnT (0-1%), hs-cTnT-STAT (3-17%) and cTnT (5-7%). Very low bias at the 99th percentile of the hs-cTnT assay (14 ng/L) with the STAT (range: 13.00-13.09 ng/L) and routine assays (13.91-13.98 ng/L) was observed. The largest bias for hs-cTnT was noted at the LoD (13-17% at 5 ng/L).

Conclusion: Excellent lot-to-lot comparability was achieved with the hs-cTnT and cTnT assays, allowing laboratories to confidently integrate the assays into their clinical AMI decision making protocols. Assurance of reagent processes and transparency regarding performance criteria is critical for implementation of troponin and interpretation in the context of serial sampling and biological variability.

Assay (Concentration)	Lots Tested (n)	Target Concentration	Actual Conc. Range (Min – Max)	% Bias at Target Concentration (Low/High)
hs-cTnT (ng/L); 2013-2014	3	5	4.97 – 4.97	-1/-1
		10	9.94 – 9.98	-1/0
		14	13.91 – 13.98	-1/0
		30	29.81 – 29.99	-1/0
		50	49.68 - 50.01	-1/0
		100	99.36 - 100.05	-1/0
hs-cTnT STAT (ng/L); 2013-2014	5	5	4.17 - 4.36	-17/-13
		10	9.08 - 9.21	-9/-8
		14	13.00 - 13.09	-7/-7
		30	28.59 - 28.65	-5/-4
		50	47.97 - 48.31	-4/-3
		100	96.43 - 97.35	-4/-3
cTnT Gen. 4, (ng/mL); 2008-2014	42	0.03	0.0286 - 0.0322	-5/-7
		0.05	0.0503 - 0.0510	-5/-7
		0.1	0.0952 - 0.1073	-5/-7
		0.5	0.4759 - 0.5366	-5/-7
		1.0	0.9518 - 1.0732	-5/-7
		5.0	4.7590 - 5.3660	-5/-7

B-326

Cystatin c as biomarker for Coronary Artery Disease patients in southern India.

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BACKGROUND: Coronary Artery Disease is the leading cause of mortality and morbidity across the globe. Prevalence of Coronary Artery Disease is alarmingly increasing in developing countries. India is also experiencing the same with the increasing urbanization, changing lifestyles and obesity. Present study is focused on estimation of Cystatin-C in Coronary Artery Disease patients in correlation with lipid profile, obesity and other risk factors.

Materials and methods: The study was conducted in the department of biochemistry, Mamata Medical College and General Hospital, Khammam, Andhra Pradesh, India. The patients attending outpatient and wards of cardiology and general medicine departments of hospital and local cardiac centers were included in this study. Study group comprised of 145 patients diagnosed as Coronary Artery Disease with an age group of 30-50 patients with associated risk factors i.e., Diabetes, hypertension, smoking. And alcoholism. 66 sex and age matched subjects were recruited as control group (non Coronary Artery Disease cases) using the same criteria.

All patients and controls were measured cystatin-C and lipid profile by using authentic methods available. These patients were also divided as per their Body Mass Index with associated risk factors.

Results: In present study, there is significant increase in the values of serum cholesterol ($p<0.0001$), Low density lipoprotein cholesterol (0.0001) and significant decrease in High density lipoprotein cholesterol ($p<0.0001$) level was observed in Coronary Artery Disease cases when compared with controls. Cystatin-C ($p<0.0001$) was significantly elevated in people with Coronary Artery Disease when compared with controls. The levels of cystatin-C is correlating positively with total cholesterol, low density lipoprotein cholesterol, Body Mass Index and waist circumference.

Conclusion: Cystatin-C is one of the promising early risk marker for the Coronary Artery Disease patients. Importance of Cystatin-C as one of the biomarker for Coronary Artery Disease patients, positive correlation with cholesterol and body mass index will be discussed.

Mean and standard deviation values of Cystatin-C and Lipid Profile

Parameter	Mean ± (Controls)	Mean ± SD Cases)	t-value	p-value
Cholesterol	162.5 ± 25.9830	201.9 ± 56.8112	5.37	< 0.0001
HDL-C	43.1818 ± .7746	39.0138 ± .6248	-5.22	< 0.0001
LDL Cholesterol	119.3 ± 24.9667	161.9 ± 54.0915	6.10	< 0.0001
Cystatin-C	0.9738 ± 0.2067	1.3883 ± 0.3822	8.27	< 0.0001
BMI	23.3668 ± 3.0605	24.3015 ± 3.1302	2.00	0.0467
WC	87.8939 ± 7.7819	93.1778 ± 8.8080	4.15	< 0.0001

B-327

Diagnostic Accuracy of the Trinity Biotech Meritas® Cardiac Troponin I Point of Care Assay

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Background: The diagnosis of acute myocardial infarction (MI) is based on clinical factors and an increased cardiac troponin (cTn), with a rising and /or falling cTn pattern required. In addition, utilizing point-of-care (POC) technology to measure cTn may assist in a more rapid management of patients presenting to rule in or rule out MI. The goal of this study was to validate the diagnostic accuracy of the Trinity Biotech Meritas POC cTnI assay based on the 99th percentile value (19 ng/L).

Methods: 293 Patients presenting with symptoms suggestive of myocardial ischemia presenting to Hennepin County Medical Center's emergency department with cTnI orders based on clinical indications were evaluated in this study. Plasma (EDTA) was obtained at 0h (baseline), and 6h. cTnI was measured by the Meritas cTnI assay (LoD, 12 ng/L; 10% CV at 24 ng/L). All charts were adjudicated for MI, predicated on the Third Universal Definition of Myocardial Infarction guidelines based on the 99th percentile of the Abbott ARCHITECT cTnI assay used routinely in the hospital.
Results: MI was diagnosed in 8.6% (n=25) patients. MIs were comprised of 15 type 1 and 10 type 2. Clinical sensitivity improved over the time of serial testing, from 64.0% at 0h to 88.0% over 6h. Specificity was 88.8% at 0h and 86.8% over 6h; with ROC AUC values of 0.84 and 0.94 at 0h and 6h, respectively. In a subset of 92 patients in which EDTA whole blood was measured in parallel to plasma, similar ROC AUCs were found, 0.96 vs. 0.97, for maximum cTnI over 6h (p=0.43). Conclusion: Our findings demonstrate that the Trinity Biotech Meritas POC cTnI assay is a diagnostically useful aid in ruling in and ruling out acute MI in an emergency room setting.

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Improved Diagnostic Accuracy for Myocardial Infarction of the Abbott ARCHITECT High Sensitivity Assay Compared to the Contemporary cTnI assay in an unselected US Population

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Background: High-sensitivity (hs)-cTn assays have not yet been cleared for clinical use in the US. This study compared the diagnostic accuracy for myocardial infraction (MI) of the Abbott ARCHITECT hs-cTnI assay to the Abbott ARCHITECT contemporary cTnI assay.

Methods: Retrospective analysis of 310 unselected patients with symptoms suggestive of acute coronary syndrome (ACS), in which serial cTnI measurements were obtained on clinical indication. Fresh EDTA plasma specimens were simultaneously measured with both the contemporary and hs-cTnI assays. Unique to this study was adjudication of MI [using the 3rd Universal Definition of MI subtype (type1 and type 2) classification] independently predicated on the 99th percentiles of a normal population for a) the contemporary cTnI assay (99th percentile, 30 ng/L), b) the hs-cTnI assay (overall 99th percentile, 26 ng/L), and c) the gender-specific hs-cTnI (99th percentiles: male 34 ng/L and female 16 ng/L). Sensitivity, specificity, and positive and negative predictive values were calculated using baseline and maximum values.
Results: 24% Fewer MIs were identified based on the hs-cTnI assay: 33 (10.7%) using the overall hs-cTnI assay cutoff and 32 (10.3%) using the gender-specific cutoffs

compared to 43 (13.9%) using the contemporary cTnI assay. Using the hs-cTnI assay, type 1 MIs decreased from 14 to 11 and type 2 MIs decreased from 29 to 22. The table demonstrates our diagnostic accuracy findings. ROC areas under the curve showed that baseline diagnostic accuracy was improved using the hs-cTnI assay: overall cutoff 0.734; female cutoff 0.763, male cutoff 0.705; compared to the contemporary cTnI assay: 0.691.

Conclusions: Our data demonstrate that the hs-cTnI assay would not result in an over diagnosis of MI, and with careful adjudication, appears to result in fewer misclassifications. The hs-cTnI assay demonstrated superior specificity, with the best diagnostic accuracy in females. Future outcomes studies will be important.

hs-TnI and cTnI Sensitivity & Specificity				
Troponin Assay (99th percentile)	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV %(95%CI)
Baseline cTnI (>30 ng/L)	55.8 (39.9, 70.9)	65.5 (59.5, 71.2)	20.7 (13.7, 29.2)	90.2 (85.1, 94)
Baseline hs-TnI (>26 ng/L)	54.6 (36.4, 71.9)	73.2 (67.6, 78.3)	19.6 (12.0, 29.2)	93.1 (88.9, 96.1)
Baseline Gender-Specific hs-TnI (M>34 and F>16 ng/L)	56.3 (37.7, 73.6)	71.2 (65.5, 76.5)	18.4 (11.2, 27.5)	93.4 (89.2, 96.3)
Maximum cTnI (>30 ng/L)	100.0 (91.8, 100.0)	58.1 (51.9, 64.0)	27.7 (20.9, 35.5)	100.0 (97.7, 100.0)
Maximum hs-TnI (>26 ng/L)	100.0 (89.4, 100.0)	68.8 (63.0, 74.3)	27.7 (19.9, 36.7)	100.0 (98.1, 100.0)
Maximum Gender-Specific hs-TnI (M>34 and F>16 ng/L)	100.0 (89.1, 100.0)	65.8 (59.9, 71.4)	25.2 (17.9, 33.7)	100.0 (98.0, 100.0)

B-329

Zonulin as a potential biomarker of metabolic inflammation and pulmonary endothelial permeability

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Background: In obesity and metabolic syndrome, disturbed intestinal permeability and low-grade chronic systemic inflammation appear to act in a vicious circle called metabolic inflammation. Zonulin, a tight junctions modulator and key regulator of intestinal permeability, has been shown to be up-regulated in individuals with type-1 diabetes and to play a role in gut-related dysfunctional auto-immunity. In addition, there are some preliminary reports indicating a possible association between zonulin and metabolic inflammation or type-2 diabetes as well. Moreover, zonulin is implicated in the regulation of general endothelial/epithelial permeability, and its association with increased pulmonary permeability has been demonstrated in animal experiments.

Methods: This study aimed at investigating plasma zonulin and its dependence on various clinical and biochemical factors in 225 patients carrying automatic implantable cardioverters/defibrillators (AICD), with 75% of them suffering from systolic heart failure, 69% from coronary artery disease (CAD), and 27% from type-2 diabetes (T2D).

Results: Univariate linear regression analysis showed that zonulin levels were associated with plasma creatinine, plasma nitrotyrosine, severity of CAD, left ventricular ejection fraction, and NYHA functional class, but not with high-sensitivity C-reactive protein (hsCRP), body mass index, weight, height, sex, or age. After multiple linear regression analysis, the negative association with creatinine ($p = 0.006$) and the positive one with NYHA class ($p = 0,013$) remained significant. In the subgroup of individuals with T2D, multiple regression revealed a significant positive affection of zonulin by hsCRP only ($p = 0.025$).

Conclusion: These findings may support reports on zonulin's involvement in the phenomenon of metabolic inflammation in T2D patients. The association of zonulin with NYHA may reflect its newly established role in altering endothelial/pulmonary permeability in heart failure. The robust
negative correlation with creatinine is unexpected and needs further clarification in experimental and clinical studies.

B-330

A Multi-Center Analytical Evaluation of the ARCHITECT *STAT* High Sensitive Troponin-I Assay

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Introduction: Troponin is the preferred biomarker for the diagnosis of acute myocardial infarction in the presence of symptoms of ischemia, with the recommended cutoff at the upper reference limit (URL) or 99th percentile value with precision of <10%. Troponin assays have improved in sensitivity to meet these guidelines.

Objective: This study evaluated the analytical performance of a new high sensitivity troponin-I assay on the ARCHITECT instrument to confirm the data provided by the manufacturer.

Methods: The ARCHITECT *STAT* high sensitive Troponin-I (hsTnI) assay is a double monoclonal, sandwich assay with chemiluminescent detection. Nine laboratories in Europe participated in this study using either ARCHITECT $i2000_{SR}$ or $i1000_{SR}$ instruments in their routine laboratories. Total precision, limit of blank (LoB), Limit of detection (LoD), limit of quantitation (LoQ), linearity and interference were determined following guidance from CLSI documents EP5-A2, EP17-A, EP6-A, and EP7-A,respectively. Method comparison was performed using the ARCHITECT *STAT* Troponin-I assay(contemporary TnI) as the referent method and 2598 samples that spanned the dynamic range of the assays. The 99th percentile URL was determined using 1769 samples from healthy populations (screened with a questionnaire) or blood donors from seven countries. Ethics approval or waiver was received for specimens collected for the reference interval and method comparison studies.

Results: Precision ranged from 1.5 to 8.9% using the manufacturer's controls. The LoB, LoD and LoQ ranged between 0.1-1.4, 0.5-2.1 and 4.6-8.5 ng/L, respectively, confirming the information in the package insert. The lowest concentrations corresponding to a total CV of 10% was 5.6 ng/L. Common interferences did not affect the hsTnI results. The overall 99th percentile URL was determined to be 19.3 ng/L and was higher in men (27.0 ng/L) than in women (11.4 ng/L). Troponin was detectable in between 52.1 and 87.8% of the apparently healthy population depending on the LoD value used. Concordance between the investigated hsTnI assay and the contemporary TnI assay at the 99th percentile cutoff was found to be 95%.

Conclusions: These results demonstrate that the ARCHITECT *STAT* high sensitive troponin-I assay is a precise and highly sensitive method for measuring troponin I on a high throughput analyzer. This new assay meets the criteria of a high sensitivity troponin test with the 10% CV concentration below the 99th percentile URL.:

B-331

Comparison of sCD14-ST (Presepsin) with Eight Biomarkers for Mortality Prediction in Patients Admitted with Acute Heart Failure

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Background sCD14-ST represents a 13 kDa fragment of sCD14 which is released after conversion of CD14++ monocytes into CD14+/16+ monocytes after M-CSF activation. sCD14-ST may play a role in acute heart failure (AHF) as monocyte TLR4 expression has been shown to be increased in this condition and as it was related to disease severity.

Objective To evaluate the diagnostic and prognostic value of sCD14-ST in AHF in comparison with cardiovascular markers (NT-proBNP, hscTnT, GDF-15, sFlt-1,), inflammatory markers (C-reactive protein (CRP) and procalcitonin (PCT)), kidney markers (neutrophil gelatinase-associated lipocalin (NGAL)), and soluble ST2.

Methods The marker concentrations were measured in base-line plasma samples obtained from 60 patients (50 to 90 years old, median 77 years; 26 females, 34 males) with AHF attending the emergency room (ER). Patients with myocardial infarction or sepsis were excluded. Outcome measure was mortality at 2 years. sCD14-ST was determined by using the PATHFAST Presepsin assay (Mitsubishi Chemical), NT-proBNP, hscTnT, GDF-15, sFlt-1, PCT, and CRP by using the ELECSYS tests (Roche

Diagnostics), and ST2 and NGAL using the Presage assay (Critical Diagnostics, San Diego, CA, USA) and the NGAL Rapid ELISA Kit (BioPorto Diagnostics, Denmark), respectively.

Results Baseline NT-proBNP ranged from 361 - 27287 ng/L, median (IQR) = 5773 (2207 - 8488) ng/L confirming the diagnosis of AHF. During the 2years follow up 25 patients (41.7%) died. The results of the biomarker determination are summarized in the table.

Tab. 1: Prognostic validity criteria of 9 markers for mortality prediction in emergency patients with acute heart failure

	Survivors, n=35 Median (IQR)	Non-survivors, n=25 Median (IQR)	p-value	ROC analysis AUC (95% CI)
sCD14-ST, ng/L	763 (601-1144)	1414 (1069-1712)	0.0001	0.789 (0.662-0.885)
GDF-15, ng/L	2885 (1766-4582)	3979 (2725-7717)	0.0392	0.681 (0.546-0.798)
ST2, µg/L	57 (34-83)	79 (50-120)	0.0453	0.675 (0.540-0.792)
PCT, µg/L	0.022 (0.02-0.037)	0.044 (0.02-0.13)	0.0314	0.667 (0.531-0.785)
hscTnT, µg/L	21 (12-33)	31 (20-47)	0.0280	0.646 (0.509-0.767)
CRP, g/L	13.1 (3.8-25.3)	25.1 (7.28-62.0)	0.1227	0.640 (0.504-0.762)
NT-proBNP, ng/L	5453 (1901-6919)	6161 (3518-9664)	0.1609	0.607 (0.470-0.732)
sFlt-1, ng/L	113 (90-179)	145 (106-210)	0.3410	0.605 (0.468-0.731)
NGAL, ng/L	1.60 (0.85-3.0)	1.69 (0.74-2.69)	0.7472	0.505 (0.370-0.639)

Conclusion sCD14-ST, hscTnT, PCT, GDF-15 and ST2 differed significantly between survivors and non-survivors.

Surprisingly, sCD14-ST was found to be the best prognostic marker for mortality prediction in patients admitted with AHF to the ER. The data may provide new information on the pathogenesis of heart failure and may improve therapeutic approaches in the future.

B-332

Validation of a Novel Equation for Estimating Low-Density Lipoprotein Cholesterol

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Background: Aggressive low density lipoprotein cholesterol (LDL-C) lowering strategies are recommended for primary and secondary prevention of cardiovascular events. A newly derived equation for LDL-C estimation was recently reported which addressed limitations in the commonly used Friedewald calculation method (LDL-C_F). The novel method (LDL-C_N) adjusts for the large inter-individual variability in triglyceride (TG) to very-low-density lipoprotein (VLDL-C) ratio using an adjustable factor empirically determined based on patient TG and non-high-density lipoprotein cholesterol (non-HDL-C). LCL-C_N reportedly classified patients with superior concordance to measured LDL-C compared to the Friedewald method, particularly in patients with LDL-C <70mg/dL. We evaluated the performance of the novel method in a data set from patients with LDL-C directly measured by ß-quantification (BQ-LDL-C), the gold-standard reference method.

Methods: Retrospective analysis identified 23,055 subjects, excluding those with TG >400mg/dL. Serum total cholesterol (TC) and TG were measured on a Roche Cobas c501. High-density lipoprotein cholesterol (HDL-C), LDL-C (BQ-LDL-C) and VLDL-C concentrations were determined by ß-quantification. Our analytical performance of these lipid measurements is directly certified by the Center for Disease Control and Prevention Lipid Standardization Program. The Friedewald LDL-C was calculated as (LDL-C_F =[TC] - [HDL-C] - [TG/5]); and the novel method was calculated as (LDL-C_N =[TC] - [HDL-C] - [TG/X]), where X is an adjustable factor based on the 180-cell method described by Martin, *et al (JAMA* 2013;310(19):2061-2068).

Results: Overall, LDL-C_F underestimated BQ-LDL-C, while LDL-C_N tended to overestimate BQ-LDL-C. The median LDL-C_N was 112mg/dL (IQR 88-141), significantly higher than the BQ-LDL-C median of 108mg/dL (IQR 84-136; P<0.0001). The median difference for [LDL-C_F] - [BQ-LDL-C] was -1mg/dL (95%CI -1-1) and the median difference for LDL-C_N was 3mg/dL (95%CI 1-4).

Both estimation methods significantly deviated from the reference LDL-C method when BQ-LDL-C was <70mg/dL (P<0.0001). The median difference for LDL-C_F was -2mg/dL (95% CI -3 - -1) compared to +2mg/dL (95%CI 1-3) for LDL-C_N. Consequently, the LDL-C_N method calculated fewer <0mg/dL compared to LDL-C_F (1 vs. 9, respectively).

Overall, LDL-C_F correctly classified 16,593 (72%) patients and LDL-C_N correctly classified 16,749 (73%) patients according to guideline cutoffs. LDL-C_F was more sensitive at identifying patients with BQ-LDL-C <70 mg/dL at 85% compared to 76% for LDL-C_N. However, LDL-C_F was less specific at 86% compared to 91% for LDL-C_N. The largest discrepancy in classification was observed in subjects with a BQ-LDL-C <70 mg/dL and triglycerides between 200-399 mg/dL, where sensitivity and specificity for LDL-C_F were 88% and 86%, compared to 53% and 99% for LDL-C_N.

Conclusions: We compared both novel and Friedewald estimated LDL-C against the gold standard LDL-C reference method, in contrast to the prior study which relied on validation of a subset of samples by ß-quantification to allow the use of the vertical auto profile method for direct LDL-C measurement. In our patient cohort, the novel method significantly overestimated LDL-C. Conversely, the Friedewald method tended to underestimate LDL-C; however the bias was not statistically significant. We conclude that the novel method has some benefits but whether the improvements are significant enough over the Friedewald calculation to justify making the change in routine clinical practice is unclear.

B-333

Elevated Serum Levels of Von Willebrand Factor Antigen (vWF:ag) Predict for Early Death and Shorter Survival in Patients with Primary Systemic Light Chain (AL) Amyloidosis Independently of Cardiac Biomarkers

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Background: Cardiac involvement is the main determinant of prognosis in AL amyloidosis, but the role of endothelium in this disease has not been extensively studied and no marker of endothelial dysfunction has been evaluated, as yet. We aimed to study the prognostic role of vWF:Ag in patients with AL amyloidosis who were treated with novel agents.

Patients and Methods: The analysis included 81 consecutive patients with newly diagnosed AL amyloidosis, with median number of involved organs was 2; heart was involved in 62%, kidneys in 74%, peripheral nerve in 24%, liver in 9% and soft tissue in 21% of patients. Median NTproBNP level was 2,318pg/mL (range 33-75,000pg/mL); 36% had NTproBNP levels ≥4,000pg/mL (Roche Diagnostics, CH) and 28%, 38% and 34% of patients had Mayo stage -1, -2 and -3, respectively. vWF antigen (vWF:ag) levels were measured using a latex particle-enhanced immunoturbidimetric assay (ACL Top 3G, Instrumentation Laboratory, USA).

Results: The median serum level of vWF:Ag in patients with AL amyloidosis was 181U/dL (range 20-557U/dL), significantly higher compared to healthy controls (median: 84U/dL, range 48-124U/dL; p <0.001). There was no significant association of vWF:Ag levels with renal, cardiac, nerve or liver involvement, as well as with the levels of NTproBNP or Mayo stage and there was no significant correlation with the degree of renal dysfunction, serum albumin levels or proteinuria. Levels of involved free light chains did not have any correlation with vWF:Ag levels either. The prognostic significance of vWF:Ag revealed that vWF:Ag levels within the top quartile (≥230U/dL) were associated with a very poor outcome (median survival 4 months vs. 47 months, p=0.001). Because the most important predictor of early death in patients with AL amyloidosis is cardiac involvement, we performed a multivariate analysis, which included NTproBNP levels: vWF:Ag levels ≥230U/dL were independently associated with survival (HR:2.64, 95%CI1.2-5.8, p=0.01), along with NTproBNP levels ≥4,000pg/ml (HR:4.17, 95%CI1.98- 8.8, p<0.001). As survival curves indicated that patients with vWF:Ag ≥230U/dL had a significant probability of early death, we performed an analysis to identify whether vWF:Ag is a significant predictor of early death, adjusting for the levels of NTproBNP ≥4,000pg/mL. Thus, in multivariate analysis, vWF:Ag ≥230 U/dL was independent predictor of death within 6 months from initiation of therapy (HR:15, 95%CI2.6-84, p=0.002) together with NTproBNP ≥4,000pg/mL (HR:16.8, 95%CI3-94, p=0.001). A combination of the two risk factors was able to identify patients at a very high risk of early death: 75% of patients with both risk factors present died within 3 months from initiation of therapy.

Conclusion: vWF:Ag levels are elevated in patients with AL amyloidosis but are not correlated with other features of the disease, such as pattern of organ involvement and cardiac biomarkers. For first time, we found that high levels of vWF:Ag are associated with a high risk of early death and shorter survival in patients with AL amyloidosis, independently of cardiac biomarkers. In addition, vWF:ag levels improve the prognostic ability of cardiac biomarkers in patients with AL amyloidosis. Our data also justify the investigation of the role of endothelial dysfunction in AL amyloidosis.

B-335

Cardiac troponin T and cardiac troponin I in patients with Chronic Renal Disease: A Meta-Analysis.

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BACKGROUND: There are considerable methodological differences amongst studies evaluating the prognostic significance of an elevated cardiac Troponin T and I (cTnT, cTnI) in patients with Chronic Kidney Disease (CKD). This makes it difficult to study the factors involved or the mechanisms responsible for cTn elevations in CKD. The heterogeneity of patients included and the small sample size in many of the studies as well as the use of multiple assays and cut off values has resulted in imprecise estimation of prognostic significance. This systematic meta-analysis reviews the clinical value of measuring cardiac troponin in CKD.

METHODS: Suitable papers were identified from Medline/EMBASE searches. Each paper was abstracted for demographics, clinical outcome, assay used and cut off value. Primary end-point was all-cause mortality. Meta-analysis was performed reporting diagnostic odds ratio (dOR) by the DerSimonian-Laird random effects model. Publication bias was assessed by Funnel plots and Egger's regression asymmetry test; heterogeneity by Q-test. Summary areas under the receiver operator characteristic curve(sROC) were calculated. Meta-regression was used to assess the independent effects of sample size and increasing renal dysfunction on the dOR.

RESULTS: 183 datasets were identified. 107(58%) reported using cTnT and 96(52%) using cTnI. 33% of studies did not include follow up. In total, 22,820 patients were included. Median age=61(38-73)years. 58% were male. Median length of follow up was 24mts. The overall dOR for all-cause mortality was 2.88 (95%CI=2.23-3.71), (Sensitivity=53%,Specificity=65%,sROC=0.749) irrespective of assay used and data were heterogeneic without evidence of publication bias. For cTnT, 18,058(79%) subjects were studied. The dOR was 2.79 (95%CI=2.12-3.70, (Sensitivity=64%,Specificity=60%,sROC=0.943). The 2ndgen and 3rdgen assays had similar dOR. For cTnI, 16,747(73%) subjects were studied. The dOR was 2.38 (95%CI=1.78-4.51, (Sensitivity=22%,Specificity=88%,sROC=0.905). Sample size had a positive influence on the overall dOR(p=<0.0001) which was negated for cTnT(p=0.829) but significant for cTnI(p=<0.0001). 30(15%) reported pre-dialysis creatinine concentrations (median=682.31,95%CI=639-763µmol/L) which was negatively correlated to the dOR irrespective of assay used but of no significance(p=0.09), which was negated for cTnT(p=0.687)but significant for cTnI(p=0.039).

DISCUSSION: cTnT and cTnI are predictors of all-cause mortality in patents with CKD but no evidence of acute coronary syndrome. The evidence base is larger for CKD patients who have measurable cTnT compared to cTnI. The ability to predict all-cause mortality is higher with cTnI compared to cTnT, but the values are similar. cTnI exhibits greater specificity in CKD compared to cTnT for the determination of all-cause mortality, suggesting the overall diagnostic performance of cTnT and cTnI in CKD patients may reflect different associated risk of death possibly due to differences in pathophysiology. These data suggest that the presence of renal dysfunction alone is not related to the ability of cTn to predict all-cause mortality.

B-336

A simple fluorescence total homocysteine microassay for very low sample volume

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Background: Total plasma/serum homocysteine (tHCY) is an independent risk factor for cardiovascular and other diseases. However, the tHCY assays on the current market are limited due to the sample volume which is not suitable for routine screening for newborn or for small animal studies. Therefore, we developed a low sample-size novel portable HCY fluorescence assay to meet those needs.

Methods: The novel portable tHCY fluorescence assay is a single-enzyme two-step assay. Reagent I (RI) is a combination of a reducing reagent, homocysteine α γ-lyase, and a compound consisting of a Schiff-based of N,N-dibutyl-p-phenylenediamine (DBPDA) and pyridoxal 5′-phosphate (PLP). A sample (5 µl) of either plasma or serum is added to RI where reduction of tHCY takes place and enzymatic reaction occurs, producing H2S, which binds to the DBPDA-PLP, producing a pre-chromophore. The first-step takes 5 min. In the second-step, Reagent II (RII), an oxidant [Ferric Chloride (FeCl3) in acid] is added. The oxidation reaction is complete in 10 minutes, and produces a chromophore, which can be measured by fluorescence at Ex 660/Em 710 nm. The total assay time is 15 min. The assay is carried at room temperature. A fluorescence reader, including a small portable device, is the only equipment needed.

Results: This novel portable tHCY fluorescence assay was compared to the A/C Portable tHCY Assay [FDA 510(k) 080851] for 40 plasma samples. The correlation coefficient is 0.95 and the slope is 0.96. The precisions of within and between assay were below10%. The linearity of tHCY in the assay is 4.2-44.8 µmol/L, and the detection limit is 4.2 µmol/L. The interferences of L-CYS, L-MET, lipid and protein were all below 10%.

Conclusion: This new portable tHCY fluorescence assay is a highly-sensitive and simple single enzyme two-step assay that can be used with a very small sample volume. A simple portable fluorescence reader is the only equipment required. This assay only needs a 5 µl sample, which meets the needs for newborn routine tHCY screening and for small animal studies.

B-337

Development of a New Specific and Highly Sensitive Enzyme-Linked Immunosorbent Assay to Detect Heart Type Fatty Acid Binding Protein in Human Serum

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Background: The human genome comprises nine putative fatty acid-binding proteins (FABPs) with an approximate molecular weight of 15 kilodaltons. FABPs are structurally well conserved but interestingly exhibit only moderate sequence homology. FABPs bind and transport intracellular hydrophobic ligands through cellular compartments. Heart-type FABP or FABP 3 (H-FABP) is a member of the FABP family that exhibits high expression in both cardiac and skeletal muscle. Due to its low molecular weight and cytoplasmic location, H-FABP is released quickly into the circulation following myocardial injury. Consequently, this protein has been demonstrated to be an early indicator of acute myocardial infarction. Furthermore, elevated levels of H-FABP have also been reported following acute stroke. The availability of analytical methods allowing the determination of this protein in serum is relevant in clinical settings. This study reports the development of a new specific and highly sensitive enzyme-linked immunosorbent assay (ELISA) for the detection of H-FABP, which represents a useful analytical tool to facilitate clinical research applications.

Methods: A colorimetric sandwich immunoassay was employed. The in-house made monoclonal capture antibody was immobilised and stabilised on the 96-well microtitre plate surface. The analyte, if present in the sample, is bound to the capture antibody and then a second in-house made monoclonal antibody labelled with horseradish peroxidase is bound to the analyte. Absorbances were read at 450nm. The signal is proportional to the concentration of the analyte in the sample. Recognition of native H-FABP was confirmed with analysis of 15 selected stroke samples (6 ischemic stroke and 9 haemorrhagic stroke) compared with 15 healthy controls. Difference was assessed with Kruskal Wallis Test(Medcalc version 12.7.8.0).

Results:The ELISA was specific for H-FABP showing %cross-reactivity values ≤0.05% with the other FABP family members. The assay presented a functional sensitivity of 0.2 ng/ml (assay range of 0 to 50ng/ml). Native recognition of H-FABP in serum samples was demonstrated by comparing H-FABP concentrations in 15 selected stroke samples (Median 5.016ng/ml) versus 15 controls (Median 1.041ng/ml).The stroke samples were significantly higher than the healthy controls (P<0.0001).

Conclusion: The results show that the developed ELISA for measurement of H-FABP in human serum is both highly specific and sensitive. Furthermore, the median concentration values were significantly higher in the selected stroke patient samples when compared with controls. This indicates clinical utility of the developed ELISA in the context of stroke. This represents a new analytical tool for clinical research applications related to this important biomarker.

B-338

Troponin concentrations in young women measured by high-sensitivity troponins T and I

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Introduction: The high-sensitivity troponins (hs-cTn) have a reported limit of detection (LOD) of 5 ng/L (Apple. Clin Chem 2012;58:59) for hs-TnT (Roche) and 1.5 ng/L for hs-TnI (Abbott) (Aw. Clin Chim Acta 2013;422:26-8). Both assays have lower 99th percentile upper reference limits (P99URL) in women than men. The hs-TnI URL in females aged 40-65 is 17.9 ng/L with 11.1% of the hs-TnI results undetectable (below the LOD) (Aw. Clin Chim Acta 2013;422:26-8). Such low hs-cTn levels are more noticeable in younger subjects. Disproportionate representation of

low troponin levels will confound the determination of the P99URL for women. We decided to investigate hs-cTn levels in a large cohort of young women below 40 years.

Methods: We measured serum troponins (hs-TnT and hs-TnI) in 260 apparently healthy (via questionnaire) ambulant female subjects aged 20-39 years. An additional 94 women (aged 30-39) were recruited to determine a robust P99URL for hs-TnI in the entire cohort of 354 women. Pregnant subjects and those with a personal or family history of hypertension, diabetes, renal, heart, or muscle disease were excluded. Statistical analyses were performed using MedCalc 12.0 (Mariakerke, Belgium).

Results: Overall 98.5% (256/260) of the subjects studied had hs-TnT values below the assay LOD while only 27.7% (72/260) of the hs-TnI values were undetectable as shown in the Table below. Undetectable hs-cTn values are most pronounced in the 20-29 age group. The female (age 20-39) P99URL for hs-TnI (n=354) is 7.7 ng/L (90% CI 5.1-12.6), median hs-TnI and interquartile range were1.7 and 0.7-2.4 ng/L respectively.

Table. Proportion of hs-cTn values < LOD

Age Group (n)	hs-TnT < LOD (%)	hs-TnI < LOD (%)
20-29 (72)	100	72.2
30-34 (91)	97.8	7.7
35-39 (97)	97.9	11.3
Overall (260)	98.5	27.7

Conclusion: hs-cTn values are much lower in healthy younger females (age 20-39) than older subjects. These women may not be suitable subjects for inclusion in the reference population for the determination of hs-TnT P99URL while women 30-39 can be included for hs-TnI reference range studies.

B-339

Turnaround time for a sensitive cardiac troponin I assay at Point of Care

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Objective: We compared turnaround times for cardiac troponin I (cTnI) measurement in the adult Emergency Department (ED) from order to analytical system (pre-analytical), system to result (analytical/post-analytical) and Brain-to-Brain (Order to Result) for the Pathfast point-of-care system (Misubushi) and for a Vitros 5600 central lab analyzer and automated track system (Ortho Clinical Diagnostics).

Relevance: Point-of-Care availability has been shown to improve the disposition time for patients with suspected myocardial ischemia in the ED. Both cTnI assays have ≤10% total CV at the 99th percentile of normal subjects. We compared the difference in turnaround times in a busy ED associated with a 750-bed tertiary care medical center. This study helps define turnaround time expectations for in an ED population presenting to a large metropolitan hospital.

Methodology: ED orders for both the Pathfast point-of-care and the Vitros automated systems are placed by clinicians in the hospital information system and barcode labels print; this is '*Order'* time. When the specimen is presented to the point-of-care system the label is scanned; after the sample is tubed to the lab, the specimen is placed on the automated track system and scanned; the time scanned is *'Instrument'* time. Both the point-of-care and automated lab systems are interfaced and auto-verify results if no issues impacting analytic testing quality are detected; this is *'Report'* time. The total turnaround time, is termed *'Brain-to-Brain'.* Results for 73 patients were audited to determine if there were differences in diagnostic results between the Pathfast point-of-care and automated Vitros system. The study was conducted for 100 consecutive days.

Results: Data are displayed in the table. There was no diagnostic difference between the Pathfast and Vitros systems ($p<0.001$).

Conclusions: Use of the Pathfast system decreased Brain-to-Brain turnaround time by 30 minutes or more (24% to 60% faster) compared to the automated OCD system.

Testing Phase	Pre-analytical		Analytical/Postanalytical		Brain-to-Brain	
Description	Order to Instrument (time, h:min)		Instrument to Report (time, h:min)		Total Time: Order to Report (time, h:min)	
Instrument System	Pathfast POC TnI	Ortho Vitros cTnI	Pathfast POC cTnI	Ortho Vitros cTnI	Pathfast POC cTnI	Ortho Vitros cTnI
Sample Size	262	829	262	829	262	829
10th Percentile	0:03	0:14	0:21	0:42	0:25	1:02
25th Percentile	0:11	0:21	0:21	0:45	0:33	1:10
Median	0:23	0:31	0:21	0:48	0:45	1:21
75th Percentile	0:42	0:46	0:21	0:52	1:04	1:38
90th Percentile	1:05	1:03	0:21	0:57	1:27	1:57

B-341

Reducing CK-MB Utilization: The Calgary Laboratory Services (CLS) Experience

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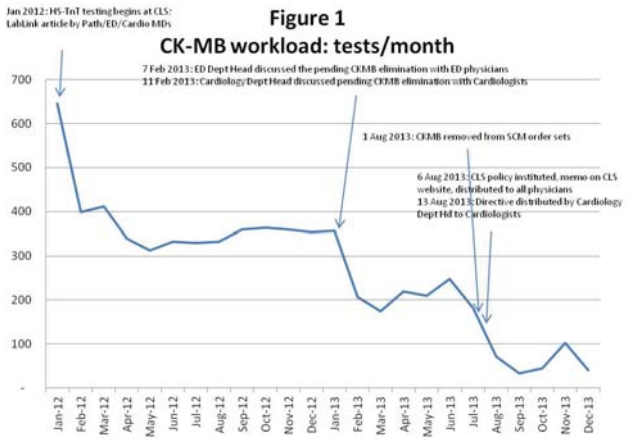
Background: Creatine kinase-MB isoenzyme (CK-MB) was the main cardiac biomarker in Calgary until Troponin T (TnT) testing was implemented in 2001,. However, CK-MB continued to be used not only for acute myocardial infarction (AMI), acute coronary syndrome (ACS) evaluation but also for other indications. High-sensitivity cardiac Troponin T (hs-TnT) testing became available in early 2012. Hs-TnT could be used for virtually every clinical condition where CK-MB had previously been the test of choice. At the time of hs-TnT implementation, CLS was performing >4000 CK-MB tests annually. A laboratory utilization initiative was directed towards significantly reducing CK-MB testing.

Methods: (1) Stakeholder consultation with Calgary Zone Departments of Cardiology, Cardiovascular Surgery, and Emergency Medicine; (2) Stakeholder and lab agreement to restrict CK-MB ordering to Cardiologists and Cardiovascular Surgeons only; (3) Educational article in the CLS newsletter (LabLink) to all medical providers; (4) On an agreed-upon date, all computerized physician order entry (CPOE) order sets containing CK-MB used by non-Cardiology specialists were removed from the CPOE database (Sunrise™ Clinical Manager [SCM]). Monthly CK-MB test workloads were obtained from the laboratory information system (Millennium®, Cerner) pre- and post-intervention; (5) Monthly lists were compiled via SCM of non-Cardiology/non-Cardiovascular Surgery providers who continued to order CK-MB; e-mails were sent to the individual providers as to the superiority of hs-TnT over CK-MB.

Results: Monthly CK-MB workload decreased from a pre-intervention baseline of 646 tests/month (at the time of hs-TnT implementation in January 2012) to a low of 34 tests/month (September 2013) post-intervention (see Figure 1).

Conclusion: The above interventions resulted in a 95% reduction in CK-MB testing in the Calgary Zone. Multiple utilization strategies including engagement of clinical stakeholders, education and limiting CPOE resulted in sustained workload reduction for this test.



B-342

Prognostic Values of Combination of High-Sensitivity Cardiac Troponin I and B-Type Natriuretic Peptide in Outpatients with Chronic Kidney Disease

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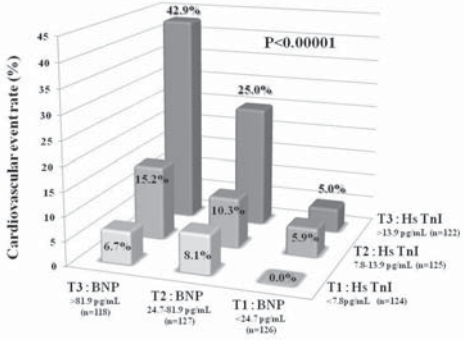
Background: The risk stratification for cardiovascular events is clinically important in patients with chronic kidney disease (CKD: estimated GFR<60mL/min/1.73m^2). We prospectively investigated whether combining cardiac troponin I (hsTnI), measured with a new high-sensitivity assay, and B-type natriuretic peptide (BNP) would be effective for the risk stratification in 371 outpatients (median age of 69 years) with CKD not on dialysis. They were also divided into tertiles of serum hsTnI levels; T1: <7.8 pg/mL, T2: 7.8-13.9 pg/mL and T3: >13.9 pg/mL. They were divided into tertiles according to plasma BNP levels; tertile 1 (T1): <24.7 pg/mL, T2: 24.7-81.9 pg/mL

and T3: >81.9 pg/mL. Results: Median estimated GFR was 24mL/min/1.73m^2. A medical history of cardiovascular disease was present in 32.9%. Log hsTnI levels were positively correlated with log BNP levels (r = 0.43, p < 0.0001). Cardiovascular event rate were 3.2%, 10.4% and 32.0% in T1, T2 and T3 of hsTnI, and were 2.4%, 13.4% and 30.5% in T1, T2 and T3 of BNP, respectively (p < 0.0001 in both). On a multivariate Cox regression analysis, both hsTnI (p = 0.002) and BNP (p = 0.0002) were independently associated with cardiovascular events. Cardiovascular event rates according to combined setting of hsTnI and BNP tertiles were shown in Figure. Conclusions: The combination of hsTnI and BNP may be useful for predicting adverse outcome in this population.

Fig. Cardiovascular event rates according to combined setting of hsTnI and BNP tertiles.



B-343

Presepsin (sCD14-ST) in Acute Coronary Syndromes and Heart Failure

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Background Recently, research activities revealed that TLR4 expression on circulating peripheral CD14+ monocytes may play a pivotal role in the pathogenesis of cardiac diseases. Monocyte activation is triggered by different factors like LPS, ischemia, tissue hypoxia, left ventricular distension, or increasing filling pressures of the heart. It was shown that CD14++ monocytes could be induced by M-CSF (macrophage colony stimulating factor) and changed into CD14+CD16+ monocytes and shed off CD14 into blood circulation as soluble sCD14. Simultaneously, a 13 kDa fragment of sCD14 is formed, named sCD14-ST or presepsin.

Objectives To evaluate the diagnostic value of presepsin in cardiovascular conditions.
Methods 112 healthy volunteers, 40 patients with low grade chronic heart failure (CHF) and 106 patients admitted with chest pain and/or dyspnea were included. Discharge diagnosis was unstable angina pectoris (UAP) in 17, non-ST-elevation myocardial infarction (NSTEMI) in 29, and acute heart failure (AHF) in 60 patients, respectively. Presepsin and cTnI were measured by using the PATHFAST system (Mitsubishi).
Results The control group revealed 95% and 99% upper reference limits of 258 (90%CI: 237-276) and 304 ng/L (90% CI: 288-320) ng/L, respectively.Presepsin differed significantly between controls and patients (Fig. 1). The difference between healthy controls and patients with UAP was more significant with presepsin than with cTnI (p<0.0001 and p<0.0071). This finding could be confirmed by ROC analysis yielding the AUC values 0.992 for presepsin and 0.681 for cTnI, p=0.0001. Detection of NSTEMI revealed AUC values of 0.982 for presepsin and 0.966 for cTnI. Using a threshold of 304 ng/L for the diagnosis of NSTEMI presepsin reached sensitivity/specificity values of 100/98,2% compared to 74,1/100% for cTnI.

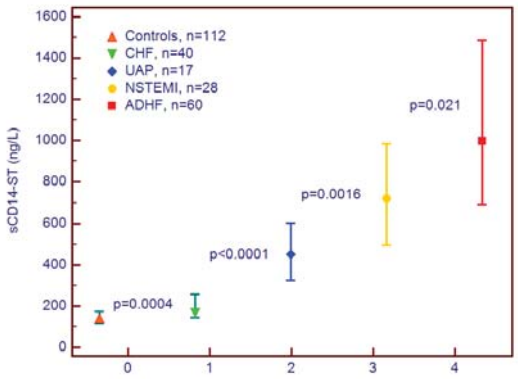

Fig. 1: Presepsin plasma concentrations (Medians, IQR) in healthy controls and patients with cardiac diseases

Conclusion Presepsin concentration showed a strong relationship with the severity of cardiac diseases reflecting inflammatory processes in the pathogenesis of cardiovascular conditions. Our data show that presepsin could provide incremental diagnostic information to distinguish cardiac and non-cardiac chest pain. Further investigation is needed to confirm the possible rule-out diagnosis of myocardial infarction as well as discrimination between cardiac and non-cardiac chest pain at admission in the ER.

B-344

Troponine T results in a patient population: Should women have their own upper reference limit for cardiac Troponin T?

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Background: For a reference population, differences in 99th percentile values for cardiac Troponin T (cTnT) are reported between men and women (1). This study investigates these characteristics in the relevant patient population itself.

Method: Data with respect to cTnT results and gender were extracted from the laboratory information system over a one year period for all patients with cardiologists involved in their medical treatment. cTnT was measured with the hs cTnT assay (Roche Diagnostics, limit of detection 0.005 µg/l). If cTnT was serially sampled in a patient, only the cTnT result of the first drawing was incorporated in the dataset.

Results: The dataset comprised 3515 individual patients (1491 female, 2024 male). The results profile of women and men was as follows:

cTnT<0.005 µg/L: 334 female (22%) and 214 male (10%).

0.005<cTnT< 0.015 µg/L: 519 female (35%) and 733 male (36%).

Elevated levels of cTnT (>0.014 µg/L): 638 female (43%) and 1077 male (53%). So, using 0.014 µg/L as a decision threshold for women as well as for men, elevated levels of cTnT relative to one reference value, are predominantly found in males.

Assuming that the risk for ACS in this patient population is the same for women as for men, in our dataset we established the cTnT decision level for which 53% of the women, i.e. identical to the percentage men, should have an elevated level of cTnT. To reach this target, we found that the cTnT threshold for women should be adjusted to 0.010 µg/L (793 female patients positive instead of 638 female patients).

Conclusion: The results support the use of a lower reference value for women: At cTnT<0.005 µg/L the percentage of females of the whole female patient population is twice that of men. The cTnT decision value for women to reach the same percentage of positive patients as for men was found to be 0.010 µg/L. These decision thresholds coincide with 99th reference limits in a reference population (reference value females <0.010 µg/L; males <0.0145 µg/L) (1).

1. Mitiwala SR, Sarma A., Januzzi JL, O'Donoghue ML. Biomarkers in ACS and heart failure: should men and women be interpreted differently? Clin Chem 2014; 60: 35-43.

B-345

Plasma cotinine is associated with social deprivation and subclinical atherosclerosis

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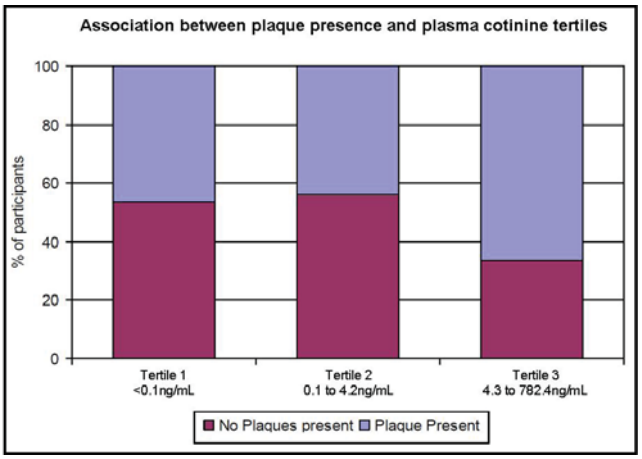
Background: Cotinine is the primary metabolite of nicotine and is the preferred biomarker for verification of self-reported history of cigarette smoking. Cigarette smoking is a well-recognised classical risk factor for cardiovascular disease. The aims of this work were: to use plasma cotinine to verify self-reported smoking history; to study the association between plasma cotinine and social deprivation, and to examine the association between plasma cotinine and subclinical atherosclerosis (ultrasound evidence of carotid plaque).

Methods: Plasma cotinine was measured by an in-house liquid chromatography-tandem mass spectrometry method. Intra-assay Coefficient of Variation (CV) was 4.7% at a cotinine concentration of 1ng/mL; 0.9% at a concentration of 10ng/mL and 1.4% at 100ng/mL. Inter-assay CV was 6.7% at a mean cotinine concentration of 1.8ng/mL and 5.2% at 372.3ng/mL. EDTA plasma samples (n=572) from the Psychological, Social and Biological Determinants of ill-health (pSoBid) study were analysed for cotinine.

Results: Current smokers had higher cotinine concentrations (250 (95% CI 171.1 to 385.8)ng/mL) than ex-smokers (0.1 (0.0 to 1.7)ng/mL), p<0.0001 and non-smokers (0.0 (0.0 to 0.6)ng/mL), p<0.0001. Cotinine was higher in the most deprived group (4.4 (0.2 to 283.2)ng/mL) compared to the least deprived (0.0 (0.0 to 0.5)ng/mL), p<0.001. Cotinine was associated with carotid plaque presence (OR of 1.46 (1.19 to 1.79) per tertile increase in cotinine), p<0.001 (see Figure). After adjusting for self-reported smoking history and area-level social deprivation, this association persisted (adjusted OR 1.40 (1.04 to 1.87)), p=0.025.

Conclusion: Cotinine is independently associated with subclinical atherosclerosis, even after adjustment for self-reported smoking history and social deprivation.



B-346

The value of delta troponin for differential diagnosis of troponin elevation in non AMI patients in the unselected emergency room population.

P. O. Collinson[1], D. C. Gaze[1], S. Goodacre[2]. *[1]St George's Hospital, London, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom*

Objective: To determine if delta troponin allows reliable exclusion of AMI in the non AMI population when more sensitive troponin assays are used for the diagnosis the universal definition of myocardial infarction.

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

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

The universal definition of myocardial infarction utilising laboratory measurements of cardiac troponin performed at the participating sites together with measurements performed in a core laboratory was used for diagnosis. All patients were followed up for 3 months for major adverse cardiac events death, myocardial infarction, readmission with unstable angina or need for urgent revascularisation (MACE). Delta troponin was calculated as the difference between the second and first samples. A significant delta was considered as 50% of the reference interval. Only those with a troponin with an initially uncertain diagnosis who had sequential sampling were studied further.

Results: Samples were available from 608/1132 patients enrolled in the study. MACE occurred in 7 patients. The number of patients with at least one elevated troponin for each method was as follows, cTnI CS (>70 ng/L) 8 (1.3%) no MACE, cTnI S (>40 ng/L) 12 (2.0%) 1 MACE, cTnI B (>40 ng/L) 6 (1.0%) no MACE and for cTnT (>14 ng/L) 18 (3.0%) no MACE. Troponin elevation did not predict MACE. Addition of the delta reduced the number of misclassifications as follows cTnI CS to 3/8, cTnI S to 11/12, cTnI B to 5/6 and for cTnT to 9/18.

Conclusion: In this group troponin elevation occurred in 1.0-3.0% of patients. Additional of delta troponin provides only modest further exclusion of alternative elevations of cTnI. Clinicians need to interpret small elevations of cardiac troponin in the appropriate clinical context but they carry a good short term prognosis.

B-347

Absolute or relative deltas for diagnosis of myocardial infarction and how should they be calculated.

P. O. Collinson[1], D. C. Gaze[1], S. Goodacre[2]. *[1]St George's Hospital, London, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom*

Objective: To compare delta values with peak troponin for the diagnosis of myocardial infarction when more sensitive troponin assays are used for the diagnosis using the universal definition of myocardial infarction.

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

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

The universal definition of myocardial infarction (MI) utilising laboratory measurements of cardiac troponin performed at the participating sites together with measurements performed in a core laboratory was used for diagnosis. Delta troponin was calculated for all permutations as follows (where a = 0 minute and b = 90 minute sample). Absolute delta (b - a), absolute positive delta (b - a for b>a and a - b for a>b), relative delta (b - a)/a, relative positive delta (b-a)/a for b>a and (a-b)/b for a>b. Diagnostic accuracy was compared for different diagnostic strategies by receiver operator characteristic (ROC) curve analysis and comparison of area under the curve (AUC) for diagnosis by each delta calculation and for peak troponin value.

Results: Samples were available from 617/1132 patients enrolled in the study, 357 male age 23.7-92.8 years median 53.8 years. Delta troponin was diagnostically equivalent to peak troponin for all four troponin methods. Absolute delta was superior to relative delta for cTnI B (p 0.0007) and cTnT (p 0.0491) and just failed to reach significance for cTnI CS (p 0.064). Absolute and absolute positive delta had equivalent diagnostic performance for all methods. For all methods, expressing relative delta as a positive percentage made diagnostic performance worse when compared to absolute delta or peak troponin or both. Absolute positive delta was superior to relative positive delta for cTnI B and cTnT.

Conclusion: Absolute delta is superior to relative delta for the diagnosis of MI. The calculation should be the subtraction of the two values in temporal sequence.

B-348

Prognostic implications of simultaneous biomarker assessments in patients with type 2 diabetes mellitus - observations from the SAVOR-TIMI 53 Trial

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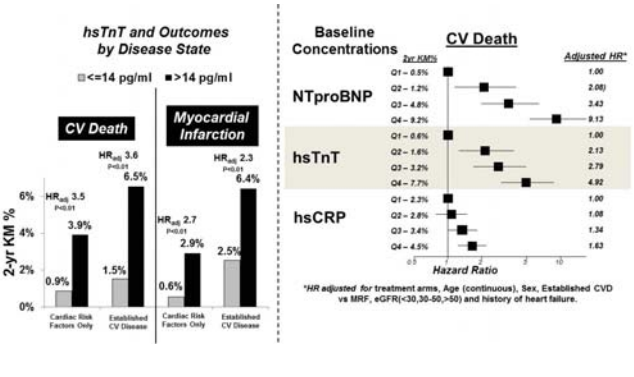
Background: Cardiac biomarkers improve risk stratification and therefore offer an attractive strategy for cardiovascular (CV) screening. We evaluated the incremental prognostic value of multiple biomarkers reflecting different pathophysiologic processes in stable outpatients with type 2 diabetes mellitus (T2DM) and established CV disease (CVD) or cardiac risk factors only to ascertain the benefit of biomarker screening. Methods: Baseline specimens from 12,182 patients enrolled in the SAVOR–TIMI 53 (Does Saxagliptin Reduce the Risk of Cardiovascular Events When Used Alone or Added to Other Diabetes Medications) trial and followed for a median of 2 years were tested using the high sensitivity troponin T (hsTnT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and high sensitivity c-reactive protein (hsCRP) assays (all Roche Diagnostics). hsTnT was categorized according to the 99th percentile (14 pg/ml). Results: hsTnT levels greater than 99th percentile were detected in 25% of patients with risk factors only and 44% of patients with established CVD. Overall, elevated hsTnT was associated with an increased risk of CVD (adjusted hazard ratio, HR_{adj} 3.6, $p<0.01$) and myocardial infarction (MI) (HR_{adj} 2.3, $p<0.01$), with similar results in each risk stratum. (Figure, left) There was a stepwise increase in the rates of CV death and MI with higher quartiles of each biomarker. After adjusting for clinical risk factors and biomarkers, elevated concentrations of NT-proBNP and hsTnT remained significantly associated with both CV death and MI, even at lower level elevations. (Figure, right) Similar results were seen for hospitalization for heart failure and MI. Conclusion: In this study of over 12,000 patients with T2DM, regardless of baseline risk, a substantial proportion of stable patients with T2DM have evidence of structural heart disease or hemodynamic stress, which were strongly associated with subsequent risk of CV death and MI.



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Cardiac Troponin Testing Is Over Utilized in Patient Care to Rule In and Rule Out Myocardial Infarction

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Background: The primary goal of this study was to determine whether clinicians appropriately utilize cardiac troponin I (cTnI) in the assessment of patients at moderate risk of acute coronary syndrome (ACS) / myocardial infarction (MI). Secondarily, we assessed the cost of excessive cTnI orders.

Methods: Following IRB approval, we retrospectively reviewed medical records (EHR) in 100 consecutive patients who had serial cTnI orders in the cardiac renal (CARE) unit, a unit where patients at moderate risk for MI are evaluated. Patients were adjudicated as MI or no-MI according to the 2012 Third Universal Definition of MI guidelines. A cTnI order set consisted of values obtained at 0, 3, 6 and 9 hours (Ortho-Clinical Diagnostics Vitros ES, 99th percentile 0.034 ug/L). Clinicians were not limited to any number of order sets. "Excessive" cTnI measurements were defined as any cTnI beyond those necessary to diagnose MI or rule out MI, according the 2012 definition. All cTnI results during hospitalization were tabulated.

Results: Of the 100 patients studied, 36 were diagnosed with MI (36%). Nine patients had a type 1 MI (25%) and 27 had a type 2 MI (75%). 5 of 36 MIs underwent percutaneous coronary intervention (PCI), while 31 did not. The remaining 64 patients without MI were primarily evaluated for their underlying medical conditions including shortness of breath, chest pain, heart failure, and renal disease symptoms; none underwent PCI. In the MI group (no PCI), 222 cTnIs were measured, with 107 cTnI values (48%) determined to be excessive (measured after the diagnosis of MI was determined). There were 52 additional cTnI order sets beyond the initial one (0, 3, 6, 9 hour), with an average of 7.16 cTnI values per MI patient. Furthermore, 23% of all cTnI measured were from unnecessary 2nd or 3rd order sets. In the no-MI group, 378 cTnIs were measured, with 150 cTnI values (39.6%) determined to be excessive (measured after the diagnosis of MI was excluded). There were 63 additional cTnI order sets beyond the initial one (0, 3, 6, 9 hour), with an average of 6.0 cTnI values per no-MI patient. Furthermore, 18% of all cTnI measured were from unnecessary 2nd or 3rd order sets. Taking into account nursing time for blood draws as well as laboratory time and supplies (reagents, technical FTE, blood drawing, supplies), we conservatively estimate excessive expenditures of approximately $88,000, based on the 32,000 cTnI tests performed per year at our hospital.

Conclusion: Our data show that in a monitored telemetry unit staffed by attending and resident physicians, a substantially, excessive number of cardiac troponin tests are ordered after establishing the diagnosis of both MI and no-MI. The excessive cTnI testing is wasteful. Better education and monitoring of cTn orders in the diagnosis or exclusion of MI is needed. Laboratorians should take the lead in educating their clinical colleagues on the use of cTn monitoring from the 2012 Third Universal Definition of MI guidelines in patients hospitalized with and without ACS.

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Troponin T Identifies Individuals at Higher Risk for Coronary Heart Disease Within Narrow Blood Pressure Categories in The Atherosclerosis Risk In Communities (ARIC) Study

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Background: Although systolic blood pressure (SBP) is a recognized risk factor for coronary heart disease (CHD), there is variable CHD risk for individuals within narrow SBP ranges, even after accounting for other CHD risk factors. We evaluated whether cardiac troponin T (cTnT) measured using a high sensitivity assay identifies individuals at higher CHD risk within narrow SBP categories among 10,754 ARIC study participants without CHD.

Methods: We measured cTnT (Elecsys Troponin-T, Roche Diagnostics®) on an automated Cobas e411 analyzer with a limit of measurement of 3 ng/L. Incident CHD was defined as hospitalization for myocardial infarction (MI), definite coronary death, MI confirmed by electrocardiogram and coronary revascularization. Hard CHD excluded coronary revascularization procedures. Risks for incident CHD by SBP and cTnT categories were calculated using fully adjusted Cox proportional hazard model (table).

Results: The mean age of the participants was 62.8 years (56% women, 22% blacks). Over a mean follow up of 10.95 years there were 1,377 incident CHD events, which included 857 hard CHD events. Approximately half of the events (53%) occurred in individuals with SBP<140 mmHg and cTnT≥3 ng/L. In fully adjusted models, increasing cTnT was associated with increasing CHD or hard CHD events across most SBP categories (table). Of note, individuals with cTnT≥3 ng/L and SBP<140 mmHg had higher hazards for CHD and hard CHD compared to those with cTnT<3 ng/L and SBP 140-159 mmHg.

Conclusion: Risk for CHD increased significantly with higher cTnT levels within narrow SBP categories. Furthermore, individuals with well controlled SBP but elevated cTnT had increased hazards for CHD compared with those with sub-optimal SBP but undetectable cTnT, suggesting that cTnT is an important marker of cardiovascular health with potential value in identifying individuals at higher CHD risk.

Table. Risk of incident CHD across SBP and cTnT categories

cTnT (ng/L) SBP (mmHg)	<3	3- 5	6-8	9-13	≥14	*P* for trend
SBP <120 mmHg and cTnT <3 ng/L as the reference						
CHD						
<120	reference	1.1 (0.8-1.4)	1.2 (0.9-1.7)	1.5 (1.0-2.1)	2.1 (1.5-3.1)	0.093
120-129	1.1 (0.8-1.6)	1.2 (0.9-1.7)	1.4 (1.0-1.9)	1.5 (1.0-2.1)	2.9 (2.0-4.3)	0.001
130-139	1.3 (0.9-1.8)	1.5 (1.1-2.1)	1.5 (1.0-2.1)	1.8 (1.2-2.6)	2.5 (1.7-3.6)	0.008
140-149	1.4 (0.9-2.1)	1.6 (1.0-2.3)	1.7 (1.1-2.5)	1.9 (1.3-2.9)	2.8 (1.9-4.2)	0.011
150-159	1.1 (0.6-2.0)	1.4 (0.9-2.3)	2.2 (1.3-3.5)	2.1 (1.4-3.4)	3.5 (2.2-5.8)	0.001
≥160	1.6 (0.9-2.7)	1.1 (0.6-2.2)	2.4 (1.6-3.7)	3.5 (2.4-5.1)	3.6 (2.3-5.6)	0.001
Hard CHD						
<120	reference	1.0 (0.7-1.5)	1.1 (0.7-1.6)	1.5 (1.0-2.2)	2.6 (1.7-4.0)	0.014
120-129	1.1 (0.7-1.6)	1.0 (0.6-1.5)	1.5 (1.0-2.2)	1.4 (0.9-2.2)	3.3 (2.1-5.2)	<0.001
130-139	1.1 (0.7-1.7)	1.2 (0.8-1.9)	1.3 (0.8-2.0)	1.6 (0.9-2.6)	2.4 (1.5-3.9)	0.008
140-149	1.0 (0.6-1.8)	1.2 (0.7-2.0)	1.5 (0.9-2.4)	1.3 (0.7-2.2)	3.0 (1.8-4.8)	0.001
150-159	0.8 (0.3-1.8)	1.0 (0.5-2.1)	2.2 (1.2-4.0)	2.1 (1.2-3.7)	4.8 (2.9-8.2)	<0.001
≥160	1.5 (0.8-2.8)	1.7 (0.9-3.3)	1.7 (1.0-3.1)	3.0 (1.8-4.9)	3.4 (2.0-5.8)	0.079
SBP 140-159 mmHg and cTnT <3 ng/L as the reference						
CHD						
<120	0.8 (0.5-1.1)	0.8 (0.6-1.2)	1.0 (0.7-1.4)	1.1 (0.8-1.7)	1.6 (1.1-2.5)	0.093
120-129	0.9 (0.6-1.3)	0.9 (0.6-1.4)	1.1 (0.7-1.5)	1.1 (0.7-1.7)	2.3 (1.5-3.5)	0.001
130-139	1.0 (0.6-1.4)	1.2 (0.8-1.7)	1.1 (0.8-1.7)	1.4 (0.9-2.1)	1.9 (1.2-2.9)	0.008
≥160	1.2 (0.7-2.2)	0.9 (0.4-1.7)	1.9 (1.2-3.0)	2.7 (1.7-4.1)	2.7 (1.7-4.4)	0.001
Hard CHD						
<120	1.1 (0.6-1.7)	1.1 (0.6-1.8)	1.2 (0.7-2.0)	1.5 (0.9-2.7)	2.7 (1.6-4.7)	0.014
120-129	1.1 (0.7-2.0)	1.0 (0.6-1.8)	1.5 (0.9-2.6)	1.5 (0.8-2.6)	3.5 (2.0-6.1)	<0.001
130-139	1.1 (0.6-2.0)	1.3 (0.8-2.3)	1.3 (0.8-2.4)	1.7 (0.9-3.0)	2.6 (1.5-4.6)	0.008
≥160	1.5 (0.7-3.2)	1.8 (0.8-3.8)	1.8 (0.9-3.5)	3.2 (1.8-5.7)	3.6 (2.0-6.7)	0.079

Data presented as hazard ratio and 95% confidence interval as calculated using Cox-proportional hazards model
Model adjusted for age, race and gender, anti-hypertensive medication use, estimated glomerular filtration rate, diabetes, fasting glucose, total/high density lipoprotein cholesterol, body mass index and current cigarette smoking.
P for trend was calculated based on the results of Wald chi-square test on linearity hypothesis of ordered cTnT categories.
Results were similar when the reference was SBP <120 mmHg and cTnT ≤5 ng/L
cTnT: high sensitivity cardiac troponin T, SBP: systolic blood pressure, CHD: coronary heart disease

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Analytical Correlation Between Abbott ARCHITECT High Sensitivity and Contemporary Cardiac Troponin I Assays During Evaluation of Acute Myocardial Infarction within an Unselected Urban Hospital Population

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Background: The Abbott high-sensitivity (hs) cardiac troponin I (cTnI) assay is available for clinical use outside the US, while only the contemporary cTnI assay is used in the US; awaiting FDA clearance. The goal of this study was to examine the correlation between the hs and contemporary cTnI assay results at 1) baseline and 2) over serial measurements during the course of ruling in/out patients suspected of myocardial infarction (MI).

Methods: During the fall of 2013, fresh EDTA samples were collected during the evaluation of patients with symptoms of ACS as clinically indicated. Patients were included if they had at least a baseline and one additional serial sample. Fresh specimens were measured simultaneously for both the contemporary and hs-cTnI Abbott ARCHITECT assays (Abbott ARCHITECT $i1000_{SR}$/$i2000_{SR}$).

Results: 1021 Specimens from 310 patients were analyzed. Correlation data for baseline and all specimens, subdivided by concentration ranges, are shown in the table. For all samples, there was a negative bias with the hs-cTnI assay at baseline, mean -6 ng/L. Focusing at concentrations above and below the 99th percentile values, concordance below 100 ng/L, based on the contemporary cTnI value, was generally poor, 0.77 at baseline and 0.80 for all samples. Slopes varied substantially across the dynamic range of the assays.

Conclusion: Our data show that cTnI results are 1) lower using the hs-cTnI assay, b) show variable correlations depending on specimen concentration range and c) showed poor concordance. Therefore, results are not interchangeable between the contemporary and high-sensitive cTnI Abbott ARCHITECT assays.

Correlation Data				
Specimen	Range	N	Slope	r
Baseline only	all cTnI results	310	0.983	0.98
Baseline only	cTnI < 1000 ng/L	306	0.756	0.94
Baseline only	cTnI < 100 ng/L	271	0.863	0.81
All Specimens	all cTnI results	1021	1.24	0.99
All Specimens	cTnI < 1000 ng/L	967	0.868	0.91
All Specimens	cTnI < 100 ng/L	812	0.829	0.84

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Myocardial injury in cancer patients - are there differences between women and men?

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BACKGROUND: The prevalence of cardiac injury in a general cancer population is not well documented; despite the fact that there are cancer treatments that may elicit cardiotoxicity. Currently, cardiac imaging is the main approach to assess cardiotoxicity; however, laboratory tests may serve as a complementary tool in this regard. New commercially available cardiac troponin assays have obtained regulatory approval (outside of the United States) with preliminary data from healthy individuals suggesting different thresholds in women versus men. These new tests are termed "high-sensitivity cardiac troponin" assays. We conducted an observational study measuring high-sensitivity cardiac troponin I (hs-cTnI) in a convenient non-select group of ambulatory cancer patients assessing whether hs-cTnI differs in male vs female cancer patients.

METHODS: Following ethics board approval, we measured cardiac troponin levels (Abbott hs-cTnI assay) in two cohorts (A and B) attending a regional cancer center with the ordering physicians blinded to the test results. Cohort A consisted of all cancer center patients with clinical lithium heparin plasma samples available for hs-cTnI measurement over a period of 40 consecutive days. Cohort B consisted of cancer center patients whose serum samples collected for measurement of clinical tumor markers and subsequently were measured for hs-cTnI over a period of 20 consecutive days. We used the sex-specific upper limits of normal (i.e., the manufacturer's reported 99th percentiles) to designate myocardial injury (i.e., women >15.6; men >34.2 ng/L) in both cohorts. We used Statsdirect and Medcalc software for non-parametric testing (e.g., Mann-Whitney, spearman correlations) and to compare positivity rates between men and women and correlate with CEA, CA 125, and total PSA in cohort B.

RESULTS: We measured hs-cTnI levels in 4,757 lithium heparin and 285 serum samples. Cohort A comprised 2,272 women of median (IQR) age= 67y (57-76) and 2485 men of median (IQR) age= 62 y (52-70); p<0.01 for the between-sex difference). In cohort A, the prevalence of myocardial injury was higher in females (8.0%; 95%CI:6.9-9.3) compared with males (3.1%; 95%CI:2.5-3.9) (p<0.01). Cohort B comprised 137 women of median (IQR) age= 64 y (54-73) and 148 men of median (IQR) age= 68y (59-76); p=0.03 for the between-sex difference). The prevalence of myocardial injury remained significantly higher in the female population (8.8%; 95%CI:4.5-15) compared with the male population (1.3%; 95%CI:0.2-4.9) (p<0.01) despite the lower age of women in cohort B. There was no difference in hs-cTnI positivity rates in females or males between cohort A and B (p>0.20) nor was hs-cTnI correlated to CEA (rho=0.10 p=0.27), CA 125 (rho=0.12 p=0.33), or total PSA (rho= -0.10 p=0.40) in cohort B (p>0.25).

CONCLUSIONS: Applying sex-specific ULN the prevalence of myocardial injury in female cancer patients is higher than male cancer patients. Additional studies are required to assess what are the contributing causes for this marked difference in this population and importantly, if these differences portend a worse outcome for women.

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On the 99th percentile reference interval determination for the Beckman-Coulter Cardiac Troponin I assays.

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BACKGROUND: The 99th percentile concentration of an apparently healthy population is used define a positive cardiac troponin to aid in the diagnostic and prognostic assessment of chest pain patients with suspected acute coronary syndrome. We sought to determine the 99th percentile upper reference limit of the newly released AccuTnI+3 and pre-commercial hs-cTnI assay.

METHODS: Serum samples (n=1000) were obtained following routine testing from apparently healthy donors. Subjects were selected on the basis of the following criteria: >40 years old with normal urea and electrolytes, liver function tests, glucose and N-terminal pro-B-type natriuretic peptide. Age and gender was also recorded. Serum samples were stored frozen at -70ºC until batch analysis of cTnI using the AccuTnI+3 and pre-commercial high-sensitivity cTnI (hs-cTnI) assay for the Access2 and AccuTnI+3 on the UniCel DxI Immunoassay system (Beckman-Coulter, Chaska, MN). The manufacturers report a total %CV of 5 to 7% in the range 7 to 56,360ng/L

with a European 99th percentile of 40ng/L and a US 99th percentile of 20ng/L for the AccuTnI+3 assay.

RESULTS: Two subjects were removed from the study due to inadequate sample volume leaving a population of 998 comprising 433(43%) males and 565(57%) females. The median age was 59.0 years, interquartile range 57.4 to 60.3 years. There was no significant difference in age between males and females (p=0.4063). There was a good correlation between the AccuTnI+3 concentrations obtained on the two instruments (r=0.90, 95%CI=0.89 to 0.91). However, the 99th percentile upper reference limit were statistically different at 41 and 34ng/L (P=<0.001) for the Access II and DxI AccuTnI+3 respectively. Detectable concentrations were observed in 878 (88%) samples on the Access II but only in 578 (58%) samples on the DxI instrument. Concentrations in males were significantly higher than females using both instruments. Using the prototype assay, the hs-cTnI 99th percentile was 27ng/L on the Access II with detectable concentrations in 98% of subjects. Concentrations of cTnI in males were significantly higher than females using both assay formats.

CONCLUSIONS: The AccuTnI+3 and prototype hs-cTnI 99th percentile concentrations are similar to other contemporary and high sensitive commercial cTnI assays. Differences were seen in the 99th percentile using the AccuTnI+3 on the Access II and UniCel DxI instruments. As gender differences were observed in the reference population, confirming findings for other hs-cTn assays; further prospective studies in chest pain patients are required to assess the clinical utility of gender specific 99th percentile concentrations.

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A step towards D-dimer assays' standardization: Antibodies with Equal Specificity to D-dimer and High Molecular Weight Fibrin Degradation Products

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D-dimer is an acknowledged marker of blood clotting. It has been shown that D-dimer concentration is elevated (higher than 0.5 µg/ml) in plasma of patients with pulmonary thromboembolism, deep vein thrombosis and disseminated intravascular coagulation of different etiology. Despite the long history of D-dimer use in clinical practice, there are some problems concerning its quantitative determination.

D-dimer as well as its precursor - fibrin degradation products (FDPs) are present in blood of patients to varying ratios. Different monoclonal antibodies (Mabs) utilized in different D-dimer assays recognize D-dimer and FDPs with unequal efficiency and that causes significant discrepancy in results obtained by such assays. In addition, differences in Mabs specificities make it impossible to use D-dimer as a standard or a calibrator in current generation of D-dimer assays.

The aim of this work was to obtain monoclonal antibodies with the same specificity to D-dimer and FDPs and to design an assay prototype that equally recognizes D-dimer and FDPs in patient plasma.

Hybridomas producing D-dimer-specific MAbs were generated using standard techniques. Mabs that did not show cross-reactivity with fibrinogen were tested with D-dimer and FDPs in different two-site combinations. To obtain preparations of D-dimer and FDPs with equal concentrations, a fibrin clot was initially partially and then completely digested by plasmin. The partially digested fibrin was used as a source of FDPs, whereas the completely digested product was utilized as a source of D-dimer. Thus, the FDPs and D-dimer preparations contained the same amount of cross-linked fibrin-derived materials. Two-site antibody combination with capture antibody DD189 and detection antibody DD255 (labeled with stable Eu3+-chelate) gave an equal response with FDPs and D-dimer in the range of the antigen concentrations from 20 to 1000 ng/ml and was selected for the further analysis.

DD189-DD255 assay was used to analyze plasma protein profiles obtained by gel filtration on Superdex 200 column. It was shown that plasma samples from different patient groups contained different ratios of D-dimer and FDPs. D-Dimer levels in blood of three patients who had gone through a surgical operation were comparable with the level of FDPs or exceeded it. In contrast, FDPs were the main products of fibrin degradation in samples from two thrombotic patients. In two septic patients' blood different ratios of D-dimer and FDP were observed.

Our results show that the ratio of D-dimer and FDPs varies between patients with different diseases. This fact means that a precise determination of fibrin-derived cross-linked products in patients' blood requires the use of antibodies with equal specificity to D-dimer and FDPs. Use of antibodies with the same reactivity to D-Dimer and FDPs would also allow the use of D-Dimer as a calibrator. This would have a significant impact on standardization of different D-Dimer assays and would benefit number of practicing clinicians who currently need to deal with the variability of different D-Dimer assays.

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An Immunoturbidimetric Assay for the Detection of Thromboxane Metabolites in Urine

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Background: Activated and aggregated platelets play a key role in the pathogenesis of cardiovascular disease. Activated platelets produce thromboxane A2 (TxA2), a potent vasoconstrictor and inducer of platelet aggregation. TxA2 is generated by thromboxane synthase from molecules derived from arachadonic acid by cyclooxygenase-1 (COX-1). TxA2 has a short half-life in plasma and is rapidly hydrolyzed to thromboxane B2 (TxB2). TxB2, in turn, is metabolized to 11-dehydro thromboxane B2 (11dhTxB2), 11-dehydro 2,3 dinor thromboxane B2 (11dh2,3dTxB2, a truncated form of 11dhTxB2), and a number of other minor TxB2 metabolites which are excreted by the kidney. Thus, 11dhTxB2 is a stable metabolite of TxA2 and an *in vivo* indicator of platelet activity. Acetylsalicylic acid (ASA) has been known for many years to have anti-platelet activity. ASA functions by acetylating and irreversibly inhibiting COX-1, thus inhibiting the production of TxA2 and its metabolites. Low dose ASA blocks more than 95% of platelet COX-1 activity. The measurement of stable metabolites of TxA2, such as urinary 11dhTxB2, is a means of quantitating TxA2 production *in vivo* and thus a direct way to analyze ASA's effect post ingestion. Urinary 11dhTxB2 can be quantitated using and ELISA method, but recently an immunoturbidimetric format has been developed. Here we describe the performance and utility of the immunoturbidimetric format (TxBCardio®) on the Selectra ProM chemistry analyzer.

Methods: An R1 reagent/reaction buffer was developed containing a monoclonal antibody specific for 11dhTxB2 and the 2,3 dinor form of 11dhTxB2. The monoclonal antibody facilitates the agglutination reaction with the 11dhTxB2 coated microparticles in a competitive manner based on the amount of 11dhTxB2 present in the urine sample. An R2 reagent/coated polystyrene microparticle buffer was developed containing functionalized polystyrene microparticles coated with 11dhTxB2. These reagents were tested in several iterations to optimize assay performance characteristics specific to the Selectra ProM analyzer.

Results: The linear range was determined to be 225-6000 pg/mL with an LOQ of 400 pg/mL. A 1:10 post-dilution allows the linear range to be extended to 60,000 pg/mL. Within run precision is <10% and total precision is less than <20% at all levels. Control recoveries over 5 days and 2 instruments (n=20) were within labeled ranges. Correlation to an 11dhTxB2 ELISA resulted in an (r) of 0.987, slope of 0.887 and y-intercept of 291 between the two methods. The 2x2 analysis of creatinine normalized results from both assays results in a positive predictive value of 94%, a negative predictive value of 100%, and an overall predictive value of 97%. Of many common urinary components, interference was observed only with high levels of hemoglobin and protein. Reagents are stable for at least 35 days once opened.

Conclusions: The data presented here suggest that the TxBCardio® reagents can be used on the Selectra ProM analyzer to adequately detect levels of **11dhTxB2 in urine samples.**

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Evidence of Inappropriate Utilization Of Cardiac Troponin Order Sets After Initiation Of A Provider Alert Prompt In Electronic Health Record

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Background: Two new, hospital-wide practices were implemented. First, a cardiac troponin I (cTnI) order set was implemented based on serial cTnI testing at 0, 3, 6, and 9 hours, for the diagnosis of myocardial infarction (MI). Second, when any provider placed additional cTnI orders, a pop-up alert was triggered in the electronic health record (EHR) alerting for possible excessive ordering. This study determined the frequency and rationale for providers ordering additional cTnI testing after an initial order set. Further, we determined by patient diagnosis and provider subspecialty the scope of additional cTnI testing beyond the initial order set.

Methods: Over 2 months, data was collected on the alert, triggering provider, provider selected clinical indication, and provider follow up action for each alerts. Providers were not limited to any number of order sets. Alert initiated inclusion of the patient in the study. We reviewed the EHR for patients associated alerts, and collected: patient stay (duration, location), ICD-9 diagnosis, cTnI orders, and timing of cTnI result with measured values (Abbott Architect; 99th percentile 0.025 µg/L).

Results: 1477 alerts were generated by 423 providers. 181 (42%) were resident physicians, of which 42% were 1st year residents, double that for either 2nd or 3rd year

residents (22% each). Alerts were associated with 3045 cTnI results for 702 patients during 833 encounters. 50% of all the cTnI testing (6044 tests) was associated with an alert. Alert-triggering providers acknowledged and overrode the alert 1440 times (97%). For overridden alerts, the provider described the override reason from a pre-determined prompted list or by free text. 929 (65%) alerts were from prompted list, including: 519 (35%) acute coronary syndrome concern (ACS; ST- and non-ST-elevation MI); 249 (17%) demand ischemia; 50 (3%) non-ACS myocardial necrosis. 71% of all free-text overrides gave no indication. The remaining 149 (29%) reasons included: chest pain (N = 23); retiming (N = 11); ACS (N = 10); trauma (N =7); or ED visit (N = 6). In 714 encounters (85%) providers placed a second order set when there were ≤ 2 cTnI results available at the alert time. The most commonly associated primary ICD-9 code was 786.5,chest pain (N = 231, 27%). Using all ICD-9 designations, 1368 alerts (92%) or 779 encounters (93%) were non-ACS related. Alerts were predominately (33%) generated by providers treating in-patients within the cardiac/renal unit (CARE), double the rate for all other units within the hospital. The 52 ACS patients generated 106 alerts.

Conclusion: Our data show that a) visual alerts did not result in a decrease in orders by providers, resulting in excessive cTnI testing after a diagnosis was determined, b) the largest number of ignored alerts was in non-ACS patients, and c) even providers treating patients diagnosed with ACS practiced excessive cTnI ordering. Our observations highlight the need for better education regarding the use and ordering of cTn testing to rule in and rule out the diagnosis of MI.

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Evaluation of standardization capability of current cardiac troponin I (cTnI) assays by a correlation study: results of an IFCC pilot project

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Background: The International Federation of Clinical Chemistry and Laboratory Medicine Working Group on Standardization of Cardiac Troponin I (IFCC WG-TNI) performed a pilot study in collaboration with industry to investigate the feasibility of preparing a commutable and stable cTnI reference material (RM). The study aimed to test whether serum pools prepared from patient sera could be used as an RM to standardize cTnI measurement.

Methods: cTnI-positive serum samples from 90 patients presenting to the emergency department with suspected acute myocardial infarction were used to prepare seven pools in the range, 200-10,000 ng/L. Samples and pools were assessed for method comparison, commutability, and stability, and a normal pool was screened for interference from cTnI autoantibodies and heterophile antibodies by 16 cTnI commercial systems according to predefined testing protocols.

Results: Each assay was assessed against median cTnI concentrations measured by 16 systems using Passing-Bablok regression analysis of 79 patient samples with cTnI values above each assay's declared detection limit. An 8- to 9-fold difference in cTnI concentrations was observed among assays, with Pathfast giving lowest values and Immulite 1000 TPI highest. After correction by a mathematical recalculation using slope and y-intercept values, between-assay variation was re-assessed. At 190 ng/L cTnI concentration, average variation of pools reduced from 49% (range, 43-55%) to 16% (range, 14-19%), at medium concentrations (814, 1634 and 1845 ng/L) from 35% (range, 34-36%) to 13% (range, 11-15%), and at high concentrations (4155 and 7517 ng/L) from 25% (range, 24-27%) to 7% (range 7.0-7.4%). For patient samples at low cTnI concentration, average variation reduced from 40% (range, 11-65%) to 22% (range, 11-38%), at medium concentration from 37% (range, 16-63%) to 20% (range, 7-58%), and at high concentration from 29% (range, 13-63%) to 14% (range, 7-42%). Overall, the 16 assays demonstrated negligible bias after realignment; however, a few samples showed substantial positive and/or negative differences for individual cTnI assays that contributed to larger inter-assay variability than for the serum pools.

Conclusion: cTnI values for pooled samples were equivalent within acceptable limits after straightforward assay realignment. This evidence indicates that pools are a viable alternative for providing large volumes of commutable sample for use as a surrogate matrixed RM for cTnI standardization.

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Preliminary Concentration and Density Analyses of Candidate Reference Material SRM 2924 C-Reactive Protein Solution

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The National Institute of Standards and Technology (NIST) has procured recombinant C-reactive protein (CRP) to generate SRM 2924, C-Reactive Protein Solution. This candidate material is intended to serve as a "pure substance" reference material traceable to SI units for use as a calibrant in the analysis of future reference materials containing CRP in biological matrices such as serum. The material was received from the manufacturer in 1200 vials each containing 1 mL of aqueous buffer with a target concentration of 0.49 g/L to 0.51 g/L of CRP. Certification of this candidate material will require the determination of purity, density, molar mass, structure and accurate concentration with homogeneity across the material space. The analysis of concentration will be determined by amino acid analysis (AAA) via liquid chromatography isotope dilution tandem mass spectrometry. A sampling plan was devised to determine concentration homogeneity by analyzing in four independent groups selected by stratified random sampling. AAA was performed on the first group by vapor phase hydrochloric acid hydrolysis (105˚C for 28 hr) of dried samples spiked with isotope labeled free amino acids (isoleucine, leucine, phenylalanine, proline and valine) and calibrated by similarly processed unlabeled amino acids in a five point linear regression curve. NMIJ 6201-b No. 2, C-reactive Protein Solution, was analyzed in triplicate as a control material to assess accuracy. The five calibrants, six samples and three controls were hydrolyzed within the same hydrolysis vessel. Density measurements were determined for the same samples by the Lang-Levy method with a 500 µL pipet calibrated using pure water. The regression coefficient for calibrants of the five individual amino acids ranged from 0.9996 to 0.9999 indicating excellent linearity within the measurement range and produced similar slope and intercept coefficients. The results of the first group (n=6) indicated that the concentration of the material by AAA was 20.0 µmol/kg (CV = 1.7 %). The value for the control material was found to be 39.2 µmol/kg (CV = 0.2 %) matching well with the certified value of 40.0 µmol/kg (1.6 µmol/kg expanded uncertainty). The higher variation of the sample results vs control is expected because the sample values include bottle-to-bottle variation while the control has only within-bottle variability. The density was determined to be 1.0050 g/mL (n = 6) which was used to convert the concentration to a mass/volume basis with a value (0.46 g/L) that is close to that based on UV absorbance as determined by the manufacturer (0.49 g/L). Although the concentration is slightly below expectations, the material remains a candidate for certification. These results do not include the full set of planned sampling and are uncorrected for calibrant purity and water content. However, they do indicate that preliminary concentrations and homogeneity are sufficient for further analysis leading to the future certification of this candidate material as a NIST Standard Reference Material.

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Growth differentiation factor-15 levels predict recurrent cardiovascular events in patients with an acute coronary syndrome in MERLIN-TIMI 36

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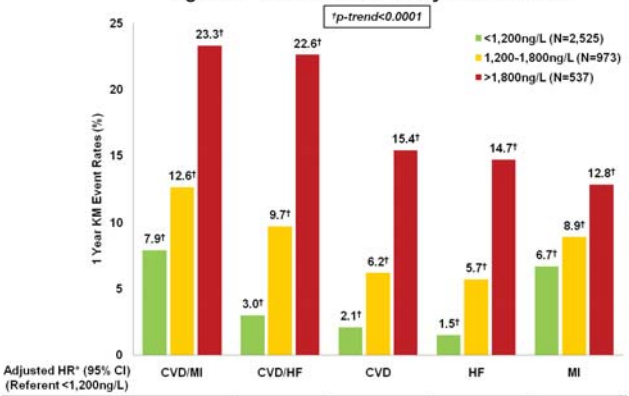
Background: Growth differentiation factor-15(GDF-15), a marker of myocardial stress, has demonstrated a clear association with mortality. However, the association with myocardial infarction (MI) has been less consistent. We assessed the prognostic performance of GDF-15 for recurrent cardiovascular (CV) events in patients with non-ST elevation acute coronary syndrome (NSTE-ACS). Methods: GDF-15 (R&D Systems) was measured at enrollment in 4,035 subjects from MERLIN-TIMI 36, a randomized, placebo controlled trial of ranolazine in patients with moderate to high risk NSTE-ACS. Patients were classified according to the previously established cutpoints for GDF-15 of <1,200 (n=2,525), 1,200-1,800 (n=973) and >1,800 ng/L (n=537). Endpoints of CV death (CVD), MI and heart failure (HF) were adjudicated by a central blinded events committee. Results: Patients with higher GDF-15 values tended to be older and more likely to have hypertension, diabetes and prior CV disease. The rates of the composite of CVD/MI and CVD/HF, as well as the individual endpoints through one year were higher in patients with higher baseline levels of GDF-15 (p-trend<0.0001 for all endpoints; Figure 1, top). This relationship was

also apparent as early as at 30 days for CVD/MI (3.6% vs 5.5% vs 8.7%, p<0.0001) and CVD/HF (1.2% vs 3.7% vs 10.1%, p<0.0001). Higher GDF-15 levels remained significantly associated with CVD and HF, but not MI, after adjustment for multiple factors (Figure 1, bottom). Conclusions: Following NSTE-ACS, GDF-15 provided prognostic value for CVD and HF, but not MI.

Figure: 1 Year Event Rates by GDF-15 Level



Adjusted HR* (95% CI) (Referent <1,200ng/L)	CVD/MI	CVD/HF	CVD	HF	MI
1,200-1,800	1.1 (0.9, 1.4)	1.9 (1.4, 2.6)	1.7 (1.1, 2.5)	2.3 (1.5, 3.6)	1.0 (0.7, 1.3)
>1,800	1.6 (1.3, 2.1)	3.6 (2.6, 4.9)	3.2 (2.2, 4.8)	4.7 (3.0, 7.2)	1.1 (0.8, 1.5)

*Adjusted for prior HF, CrCl, BNP and TIMI risk score components, including troponin

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The influence of eGFR on high-sensitivity troponins T and I in asymptomatic non-dialysis-dependent patients

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Introduction: Troponins are the preferred cardiac biomarkers. High-sensitivity troponins (hs-cTn) has enabled earlier diagnosis of acute myocardial infarction and better definition of cardiac risk. Troponins are frequently elevated in chronic kidney disease (CKD). CKD is associated with increased risk of cardiovascular morbidity and mortality. It remains unclear if troponin elevation in CKD is consequent to increased troponin release from myocardial injury or due to impaired renal clearance. We decided to investigate the influence of renal dysfunction, as reflected by the estimated glomerular filtration rate (eGFR), on serum hs-cTn in asymptomatic CKD patients not on dialysis.

Methods: We measured serum hs-TnT (Roche) and hs-TnI (Abbott) in 530 ambulatory outpatient subjects (265 women) across a range of eGFR (CKD-EPI) [Stage 1-4 CKD]. Pregnant subjects and those from the emergency, renal and cardiac departments were excluded as were those recently hospitalized (< 30 days). The gender-specific 99th percentile upper reference limits (99PURL) for hs-TnT (Giannitsis. ClinChem2010;56::254) and hs-TnI (Aw. ClinChimActa2013;422:26) were – M: 14.5, 32.7 ng/L and F: 10, 17.9 ng/L respectively. An additional 60 subjects (31 females) with stage 5 CKD (eGFR < 15 ml/min) who were not on dialysis were also studied.

Results: Overall hs-TnT was above the 99PURL in 57.5% (305/530) of subjects with CKD stage 1-4 while hs-TnI was increased in 10.6% (56/530). While hs-TnT and hs-TnI values were 45.2% concordant in the normal range, only 9.6% of patients had both hs-TnT and hs-TnI elevation. eGFR impacts hs-TnT more markedly than hs-TnI (< 60 versus < 45 mL/min respectively) (see Table).

Table. Distribution of Elevated hs-troponin values by CKD stage

CKD stage (eGFR ml/min)	1 (90)	2 (60-89)	3a (45-59)	3b (30-44)	4 (15-29)	5 (<15)	Sub-total
N	104	117	102	100	107	60	590
Hs-TnT elevation - n (%)	23 (22.1)	28 (23.9)	55 (53.9)	80 (80)	99 (92.5)	39 (65)	344
Hs-TnI elevation - n (%)	3 (2.9)	8 (6.8)	8 (7.8)	18 (18)	19 (17.8)	22 (36.7)	78

Conclusion: hs-TnT concentrations are more susceptible to renal dysfunction than hs-TnI. When evaluating patients with chest pain, diagnosis and risk stratification needs to take into account the influence of eGFR especially in those with stage 3 CKD and beyond.

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Magnitude of short- and long-term intra-patient BNP variation in low BNP patients: Implications for more rational BNP utilization

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Introduction: Edmonton hospitals have offered BNP testing to the emergency department (ED) for the last 6.5 years and to other wards (nonED) for 5 years. Despite proactive approaches to BNP ordering, BNP usage is increasing in both settings. We used a unique data mining approach to evaluate biologic variation in patients with initial BNP's less than 101 pg/mL (clearly normal). We discovered significant patient repeats as well as very low biologic variation of BNP.

Methods: We tabulated paired intra-patient BNP's repeated within 365 days of each other. The initial BNP had to be under 101 pg/mL and the second BNP could be of any value up to 20,000 pg/mL. We calculated the standard deviation of duplicates (SDD) of the intra-patient pairs grouped in 5 day intervals : 0-5d, 5-10d, 10-15d, and so forth. Using weighted linear regression, the SDDs were regressed against the time intervals. The extrapolation to zero time interval in these regressions represents the sum of the biologic variation (s_b) and the short-term analytic variation (s_a): $\mathbf{y_0}=(s_a^2+s_b^2)^{1/2}$

Results: A total of 2178 patients had initial BNP's that were under 101 pg/mL (1742 ED, 471 nonED). The figure shows SDDs for the intervals of separation between the first and second test. The y-intercept corresponds to a s_b of 14.3 pg/mL (27%) for an initial average BNP of 53.3 pg/mL.

Conclusion: The small biologic variation of patients with initial BNP's under 101 pg/mL implies that such patients have very little risk of congestive heart failure and that repeat testing will result in another low BNP. Therefore, a BNP less than 101 pg/mL should not be followed with a repeat test unless there is a clinical change in the patient.

BNP Results < 101 pg/mL