# Evaluation of an early biomarker panel for the identification of emergency department patients at high risk for a short term cardiac outcome

By

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# ABSTRACT

Patients presenting to the emergency department (ED) with chest pain suggestive of acute coronary syndrome (ACS) often wait long hours before a decision on their care is made. The recommended blood test to aid in diagnosing myocardial infarction (MI) is cardiac troponin I (cTnI) or cardiac troponin T (cTnT). However, other biomarkers representing acute processes and diseases related to ACS might also be useful for early identification. To that end, I evaluated whether a biomarker panel at presentation could improve the diagnostic performance of identifying patients at high risk for MI or any other related cardiac outcome as compared to using cardiac troponin alone. The patient population consisted of 102 patients who presented to the ED with chest pain. Sixteen biomarkers measured in serum obtained at presentation were ranked via receiver-operating-characteristic (ROC) curve analysis for a composite cardiac outcome within the first 72 hours following presentation to the ED. The top four biomarkers (soluble fms-like tyrosine kinase, Creatinine, monocyte chemoattraction protein-1, and NT-pro brain natriuretic peptide) were used to construct the panel test. The ROC derived cutoffs for each of the biomarkers were used to characterize abnormal concentrations with an overall biomarker score incorporating all 4 biomarkers used to classify patients that were either positive or negative for the biomarker panel. When used in conjunction with high-sensitivity cardiac troponin, the panel's sensitivity and specificity were 100% (95%CI: 75-100%) and 54% (95%CI: 43-65%), respectively. This represented an

improvement compared to high-sensitivity cardiac troponin I (hs-cTnI) or hs-cTnT alone which had a sensitivity/specificity of 92% (95%CI: 64-100%)/57% (95%CI: 46-68%) and 85% (95%CI: 55-98%)/55% (95%CI: 44-66%), respectively. In summary, a 4-biomarker blood-based panel used in conjunction with cardiac troponin at ED presentation may identify patients at risk for MI or related outcomes in the short term.

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Emily Gallagher, I treasure your friendship more than words can express. You have been a source of unwavering support for longer than I can care to count. I am grateful to you for all the great laughs, helpful advice, much needed pushes, and nerdy conversations. I selfishly hope that you never stray too far from where I am. Lastly, but certainly not least, I want to thank my parents and other family members. I cannot imagine achieving this milestone without all your help. Special thanks to my dad, I am forever grateful for your never ending support, encouragement, and guidance. You have instilled in me a set of values, morals, and beliefs that will help me succeed throughout all of life's endeavours. You are a very big reason I have succeeded in obtaining my goals thus far. To my mom, thank you for the many ways in which you have supported me. I am particularly grateful to you for always sending me back with more food than I can handle. It has provided me a reminder of home and more time for focus on more productive things. To my grandmother, uncle, aunts, and cousins Audrey and Krystle, thank you for providing me with a home away from home. I am indebted to you all for your constant kindness and support.

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| | |
|---|---|
| ACC | American College of Cardiology |
| ACS | acute coronary syndrome |
| AUC | area under the curve |
| AVP | arginine-vasopressin |
| bFGF | basic fibroblast growth factor |
| BNP | B-type natriuretic peptides |
| CAIII | carbonic anhydrase III |
| CABG | coronary artery bypass graft |
| CK-MB | creatine kinase-MB |
| CRP | C-reactive protein |
| cTnI | cardiac troponin I |
| cTnT | cardiac troponin T |
| CTS | Cardiac Troponin testing in the emergency Setting |
| CV | coefficient of variation |
| CVD | cardiovascular disease |
| ELISA | enzyme-linked immunosorbent assay |
| ECG | Echocardiogram |
| ESC | European Society of Cardiology |
| ED | emergency department |
| EDTA | ethylenediaminetetraacetic acid |
| eGFR | estimated glomerular filtration rate |
| GPBB | glycogen phosphorylase isoenzyme BB |
| hEGF | human epidermal growth factor |
| H-FABP | heart-type fatty acid-binding protein |
| HF | heart failure |
| hs-cTn | high sensitivity cardiac troponin |
| hs-cTnT | high sensitivity cardiac troponin T |
| IL-1 | interleukin 1 |
| IL-6 | interleukin 6 |
| IL-10 | interleukin 10 |
| LoB | limit of blank |
| NT-proBNP | N-terminal pro b-type natriuretic peptide |
| NTSEMI | non-ST-segment elevation myocardial infarction |
| MCP-1 | monocyte chemotactic protein-1 |

| | |
|---|---|
| MI | myocardial infarction |
| MMP | matrix metalloproteinase |
| MSD | Meso Scale Discovery |
| NVP | negative predictive value |
| PCI | percutaneous coronary intervention |
| PlGF | placental growth factor |
| REB | research ethics board |
| RING | Reducing the time Interval for identifying New Guideline defined myocardial infarction in patients with suspected ACS in the emergency department |
| ROC | receiver operating characteristics |
| sFlt-1 | soluble fms-like tyrosine kinase-1 |
| sICAM-3 | soluble intercellular adhesion molecule-3 |
| SMC | smooth muscle cell |
| SST | serum separating tube |
| STEMI | ST-elevation myocardial infarction |
| TNF-α | tissue necrotic factor α |
| UA | unstable angina |
| VEGF | vascular endothelial growth factor |

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## Chapter 2



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# CHAPTER 1: INTRODUCTION

## 1.1 Acute coronary syndrome

Acute coronary syndrome (ACS) refers to a range of conditions caused by myocardial ischaemia – an insufficient flow of blood to the heart (1). The term encompasses clinical states of varying severity: from unstable angina (UA) to non-ST-elevation myocardial infarction (NSTEMI) to ST-elevation myocardial infarction (STEMI).

The primary event leading up to ACS is the build-up and rupture of atheromatous plaque in the subendothelium of coronary arteries. This interferes with the maintenance of healthy vessel walls which can lead to various complications including ACS. The atherosclerotic process begins with the adhesion and extravasation of leukocytes from the circulation into the vessel wall. Monocytes recruited into the area form lipid laden foam cells as they take scavenge lipids from modified lipoprotein particles (2). These foam cells release cytokines and chemokines that stimulate the migration of smooth muscle cells (SMCs) into the intima from the surrounding muscle layer where these SMCs multiply and synthesize proteins producing a fibrous cap surrounding the foam cell layer (2). These processes effectively reduce the vessel lumen and can cause stable angina, where chest pain occurs with stress or activity but subsides with rest. As lesions progress, inflammatory responses to injury weaken the fibrous protective cap of plaques and fissure of the unstable plaque exposes procoagulant material of the necrotic core to the blood triggering thrombus formation in the lumen (2). The resulting vessel occlusion

interrupts local blood flow preventing myocardial perfusion and depending on the extent of occlusion, these events can lead to different conditions of ACS (UA, NSTEMI, STEMI). The failure to restore blood flow to the heart can lead to necrosis of myocardial tissue and eventually heart failure. Furthermore, the scarred heart tissue can interfere with normal cardiac electrophysiology leading to arrhythmias which in turn, can lead to cardiac arrest and death (3).

### 1.1.1 Treatment

The treatments for ACS vary with the spectrum of clinical presentations and their severity (e.g. extent of lumen constriction). Patients are sometimes prescribed medications that can include antiplatelet treatment (e.g. aspirin and clopidogrel), antithrombin treatment (e.g. low molecular weight heparins), and/or anti-ischaemic treatments (e.g. nitrates and β blockers) (5). Interventional and surgical procedures are often also employed, including percutaneous coronary interventions (PCI; which involves a catheter to place a stent that opens the narrowed vessel) and coronary artery bypass surgery (CABG; where a new route is created, using a graft, and placed around the narrowed or blocked artery to ensure sufficient blood flow to the heart) (5).

## 1.2 Cardiac biomarkers

Early identification of individuals with ACS is vital to providing timely interventions and improving patient outcome. Furthermore, early ruling out is also important in reducing

treatment cost and patient burden (i.e. from unnecessary admissions). Here, biomarkers serve as a valuable tool. They are quantifiable biological parameters that reflect a pathological or physiological state. In the ACS setting, a clinically useful biomarker will be easily measured, have cardiac specificity, accurately reflect risk (with high specificity and sensitivity), and provide information independent from existing markers or clinical presentations (6). The wide array of cardiac biomarkers currently discussed in the literature measure various processes of ACS such as inflammation, plaque instability, plaque rupture, and myocardial necrosis. As greater insight is gained into the pathophysiology of atherosclerosis, the potential for discovery of novel cardiac biomarkers increase as well.

### 1.2.1 Cardiac troponin

In 2000, through a joint effort of the European Society of Cardiology (ESC) and the American College of Cardiology (ACC), cardiac troponin (cTn) replaced creatine kinase-MB (CK-MB) as the preferred biomarker in identifying those with a suspected myocardial infarction (MI) (7). Cardiac troponin I (cTnI) and T (cTnT) are proteins released upon myocardial injury and unlike CK-MB; cardiac troponin levels do not increase upon skeletal muscle injury (e.g. after trauma or surgery) and are thus specific to myocardial injury (1). Up to four hours following injury, cTn levels begin to rise and usually peak at 12 hours. These levels can remain elevated for over five days – usually longer for cTnT than cTnI (6,8).

Recent guidelines on the use of biomarkers in ACS established that measurements of cTn should be taken at presentation to the emergency department (ED) and again between six to nine hours following symptom onset (9,10). In order to diagnose a MI, the concentrations must serially increase with at least one measurement above the 99$^{th}$ percentile of a reference healthy population (11). While there is a single assay for cTnT, there are over a dozen cTnI assays making standardization difficult. Since different epitopes of cTnI are being measured by the various assays, cutoff values vary between the different assays which have caused debates on assay interpretation (8).

The need for improved analytical performance of cTn assays has led to the introduction of high-sensitivity cardiac troponin (hs-cTn) assays. These assays are able to measure cTn levels in a greater proportion of the population – including those with levels that were undetectable with previous assays (12).

### 1.2.2 B-type Natriuretic Peptide

There is increasing evidence suggesting that B-type natriuretic peptides (BNP) can also serve as a useful ACS biomarker. It is released by the ventricles in response to myocyte stretch and is significantly up-regulated in regions surrounding the MI (13). It acts to reduce systemic vascular resistance and central venous pressure through vasodilation, renin and aldosterone production and also stimulates cardiac myocyte growth (14). Initially secreted as a 134 amino acid pre-proBNP, a 26 amino acid peptide is subsequently cleaved off to form proBNP (14). Afterwards, the serum protease corin

further cleaves proBNP to form a N-terminal pro b-type natriuretic peptide (NT-proBNP) and the physiologically active BNP (Figure 1.1) (15). While the active BNP has a short biological half-life of approximately 20 minutes, the inactive NT-proBNP circulates for up to 120 minutes (15). The superior stability of NT-proBNP in samples may make it a superior marker in detecting cardiovascular events such as heart failure (HF) and myocardial infarction (MI).

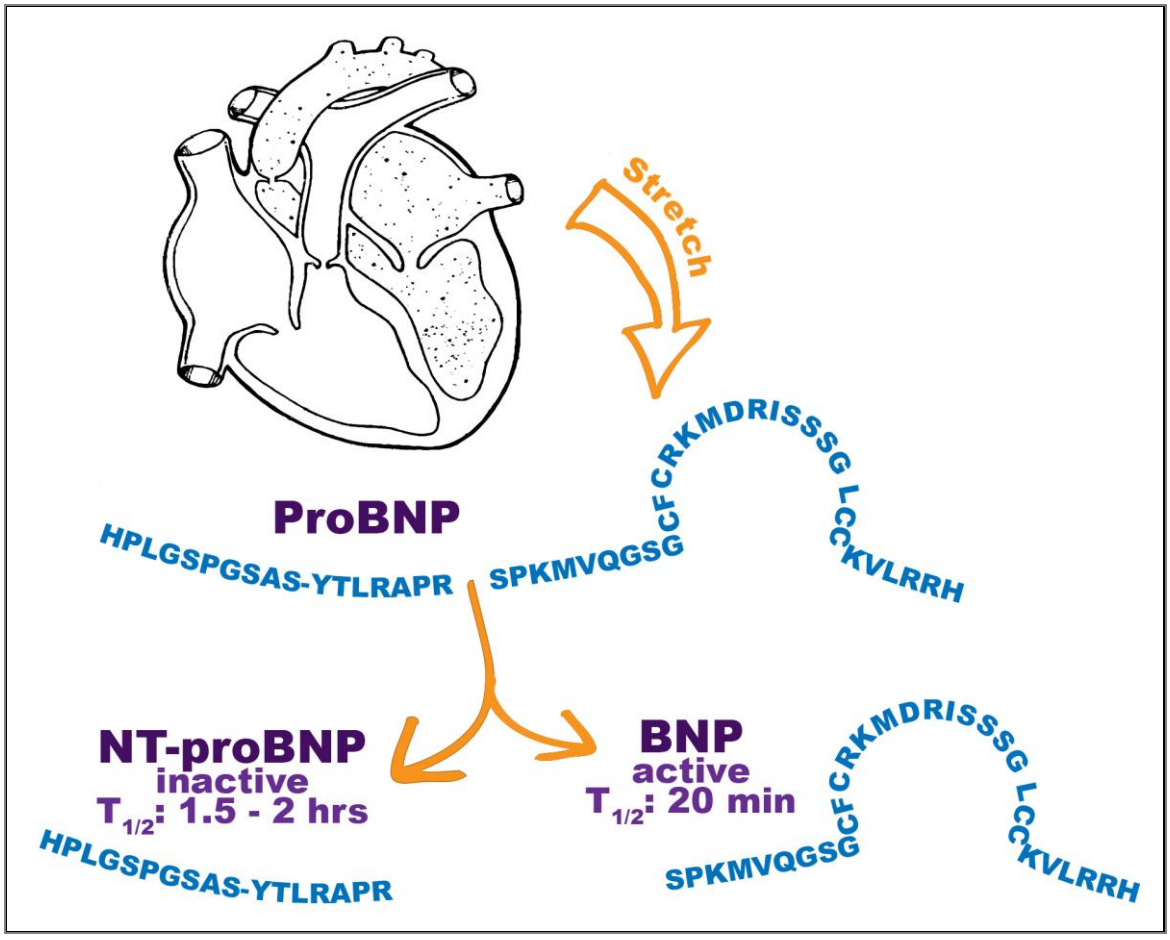



**Figure 1.1 – Secretion and cleavage of proBNP**. proBNP is cleaved by corin to form the inactive NT-proBNP and the active BNP (adapted from Collinson and Gaze, 2007)

The PRISM trial demonstrated that serial measurements of NT-proBNP can be useful in stratifying patients' risk of ACS (16). In a separate study, NT-proBNP levels measured at presentation to the ED were associated with mortality up to 40 months. When NT-proBNP concentration was used in a multivariate analysis which included clinical background factors, ECG and cTn, NT-proBNP remained a significant indicator of risk for ACS (17). Furthermore, in patients with negative cTn values, high NT-proBNP values identified those at higher risk for ACS (18).

### 1.2.3 Cytokines

Atherosclerosis is now known as a chronic inflammatory disorder (19). Inflammation is a fundamental event that occurs throughout all states of the disease from endothelial dysfunction to plaque development, destabilization and rupture. In response to injury, cytokine and chemokines are released by a variety of cells (e.g. endothelial cells, platelets, leukocytes) to mediate these processes. Initially studied to further understand the pathophysiology of atherosclerosis, these markers are increasingly being investigated for their utility in providing unique information of risk for patients with suspected ACS (20). Interleukin 6 (IL-6), interleukin 10 (IL-10), and monocyte chemoattractant protein 1 (MCP-1) are three major cytokines that have been implicated in the ACS setting.

IL-6 is released by a large variety of cells to promote the production of acute-phase reactants such as C-reactive protein (CRP) and the differentiation of myeloid cells

(20). Expressed particularly at the shoulder region of plaques, it also contributes to plaque instability by increasing the expression of matrix metalloproteinase (MMP) which play an important role in degrading the extracellular matrix and other inflammatory cytokines (e.g. MCP-1 and tissue necrotic factor-α [TNF-α]). Plasma concentration of IL-6 in healthy individuals has been shown to predict risk of future cardiovascular events (21). Furthermore, in the FRISC-II study, patients with high levels of IL-6 were at higher risk for mortality at both 6 and 12 months – the marker was independent of cTnT and CRP concentrations (22).

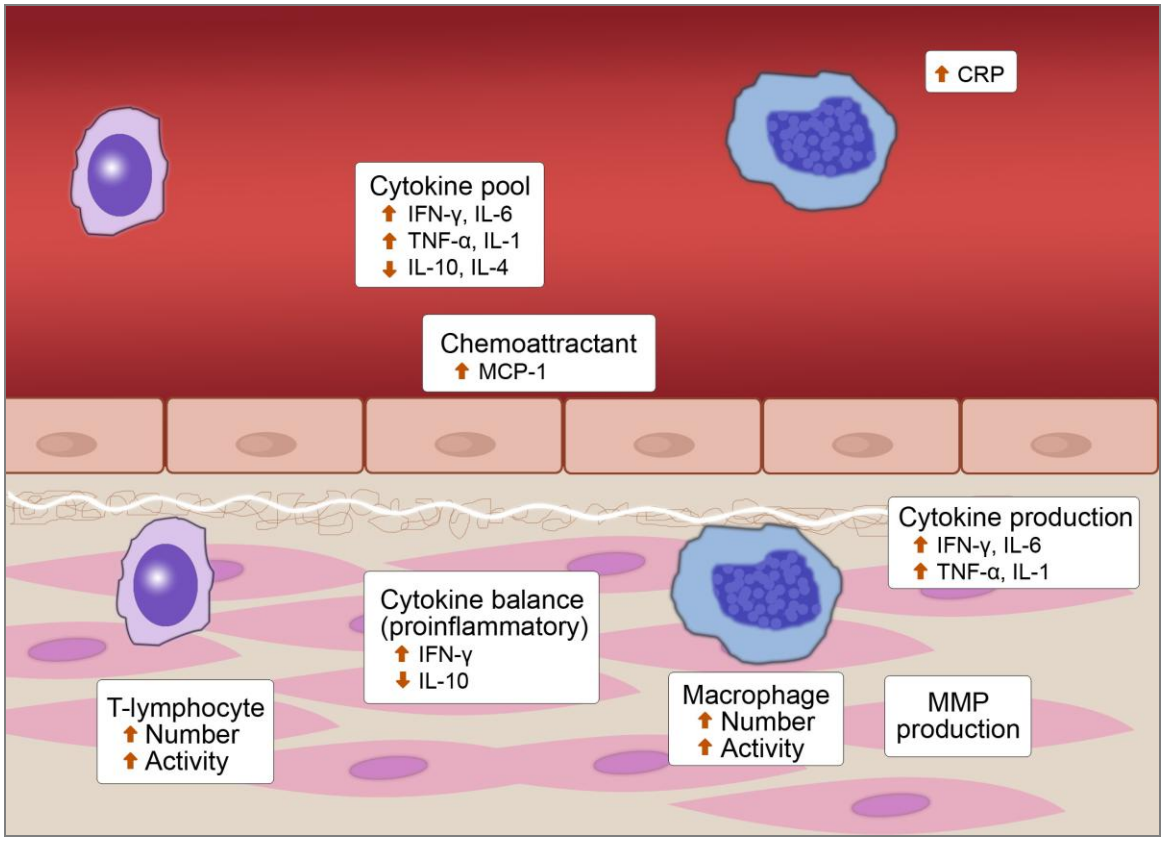



**Figure 1.2 – Effects of and changes in inflammatory hormones involved in atherosclerosis.** MCP-1 activates monocytes and facilitates their migration into the vessel wall (adapted from Ray and Cannon, 2005)(23).

IL-10 may be an informative marker despite its anti-inflammatory role. As the balance between pro-inflammatory and anti-inflammatory mediators is central to plaque progression, IL-10 might be important in reducing the progress of atherosclerosis. It is said to reduce the expression of pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6, interferon-γ, and TNF-α (24). Moreover, it inhibits plaque progression and rupture; however the exact pathophysiological role of IL-10 is unclear. Through murine studies, it was proposed that IL-10 may represent an anti-atherogenic role by increasing the removal of cholesterol from foam cells and by reducing both inflammation and apoptosis in atherosclerosis (25). In clinical studies, high levels of serum IL-10 has been associated with acute coronary events and reduced risk of death and recurrent MI (26).

Monocytes, which differentiate into macrophages, play an integral role in the initiation and progression of atherosclerosis. Chemokines, such as MCP-1, are secreted by various cells (e.g. monocytes, smooth muscle cells, endothelial cells) to provide a chemoattractant signal initiating the recruitment and migration of monocytes into the subendothelium (27). Other roles of MCP-1 include proliferation and migration of SMCs, stimulating neovascularisation in plaque, and causing vascular inflammation (27,28). Similar to IL-6, MCP-1 has been shown to up-regulate the expression of MMPs thus implicating the biomarker in plaque destabilization (28). In a study evaluating individuals with ACS, levels of MCP-1 were correlated with a twofold increase for MI and mortality (29). However, a separate study of individuals with coronary heart disease determined

that MCP-1 was not an independent predictor of risk for coronary heart disease (30). The utility of MCP-1 is, therefore, yet to be delineated; further research in evaluating this biomarker is still required.

### 1.2.4 Vascular injury

An early pro-atherosclerotic event involves the migration of circulating leukocytes from the blood to areas of vascular injury (11). This process occurs through a multistep cascade beginning with the tethering of leukocytes to the endothelium, leukocyte rolling along the endothelium via weak adhesive interactions, and finally, transmigration across the vascular endothelium into the vessel wall (31). As a response to the endothelial cell injury that occurs during atherosclerosis, the damaged cells express selectins and molecules of the immunoglobulin superfamily to facilitate these events (31).

P-selectin and E-selectin are cell adhesion molecules that are involved in the tethering and rolling interactions of leukocytes with the endothelium (20,32). They are expressed following platelet or endothelial cell activation to facilitate platelet aggregation and leukocyte recruitment in high-shear-stress environments thus promoting the formation of thrombi (32). In terms of utility in the ACS setting, there has been limited consensus with the association of the cell adhesion molecules and risk (33-37).

In the hypercoagulable state of atherosclerosis, thrombomodulin plays a role in regulating the thrombus formation that occurs at unstable plaques. It is expressed on

endothelial cells and binds thrombin, a coagulation factor, to decrease its activity (38). Furthermore, the binding of thrombin to thrombomodulin activates Protein C which initiates a sequence of reactions that inhibit other important factors of hemostasis (39).

### 1.2.5 Growth factors

The major role of growth factors in atherosclerosis is the initiation of neovascularisation and repair of cardiac tissue (32). Within atheromatic plaques, these biomarkers play a role in the recruitment of macrophages into the vessel wall and plaque progression.

Vascular endothelial growth factor (VEGF) is involved in multiple roles which support the progression of atherosclerosis. The growth factor mediates endothelial cell proliferation, permeability, and migration. Placental growth factor (PlGF), another member of the VEGF family of proteins, is involved in initiating the inflammatory process and contributes to plaque instability. It plays a role in the recruitment of macrophages into atherosclerotic lesions, intima thickening via the growth of smooth muscle cells, stimulates angiogenesis, and increases the expression of TNF-α and MCP-1 by macrophages (40). In the CAPTURE study, ACS patients with higher levels of both VEGF and PlGF were shown to have a higher risk for death or MI within 72 hours (41,42). Furthermore, Markovik et al. (2009) demonstrated that PlGF was clinically useful in detecting NSTEMI patients that were at higher risk for a cardiovascular event within 30 days of presentation to the ED (43). Both VEGF and PlGF bind the vascular endothelial growth factor receptor-1, also known as fms-like tyrosine kinase-1, which is expressed

by endothelial cells and macrophages (44). The soluble form of the receptor, sFlt-1, circulates and primarily binds PlGF to reducing its ability to bind to the membrane-bound receptor ultimately inhibiting its actions. In a multicentre study involving ED patients with suspected acute MI, sFlt-1 improved the diagnosis of MI when paired with cTnT and also served as a significant predictor for mortality within a year (45).

### 1.2.6 Fatty acid-binding protein

Heart-type fatty acid binding protein (H-FABP) is a novel early cardiac biomarker that is rapidly released into the circulation in response to myocardial injury and can be detected within 2-3 hours of symptom onset (46). The cytoplasmic protein is found in high concentrations in the myocardium and plays a role in the transportation of long-chain fatty acids in cardiomyocytes (46). Thus, as a very early marker in high concentration, it has been suggested that H-FABP may well improve the assessment of patients presenting early with chest pain. Chan et al. (2004) have shown that H-FABP had better sensitivity and negative predictive value (NPV) when measured at presentation (72% and 67%, respectively) than cTnI (51% and 51%, respectively) (47). While a group recently concluded that the use of H-FABP alone is inferior to cardiac troponin as an early predictor of MI, it may hold promise when used in conjunction with cardiac troponin or other cardiac biomarkers (48).

## 1.3 MULTIPLEX TESTING

### 1.3.1 Multiple biomarker panel

Several studies have investigated the value of using multiple biomarkers in addition to cardiac troponin to determine patient risk for cardiovascular events. The idea is founded on the basis that adding markers of different pathophysiological indications will only increase the accuracy of the assessment.

In the MERLIN-TIMI 36 trial, NT-proBNP measurements used in combination with cTnI levels, provided investigators with prognostic information independent of clinical risk factors (49). In a separate study, NT-proBNP in addition to the cytokines IL-6 and MCP-1 were also found to be independent long-term predictors of heart failure and death (50). Other investigations implementing a multimarker approach have also incorporated hs-CRP, PlGF, IL-10, and myeloperoxidase in their biomarker panels (51-53).

### 1.3.2 Multiplex versus singleplex testing

Multiplexing immunoassays are offered in both bead-based formats and planar formats – both based on enzyme-linked immunosorbent assay (ELISA) technology. Suspended bead-based systems uses distinctly coloured microsphere sets conjugated with a reagent (e.g. antigens, antibodies, enzyme substrates) specific to the analyte of interest (54). In the case of antibodies, two are often used in a sandwich arrangement, one attached to a

set of identically-coloured beads and the analyte, and the second, a labeled antibody specific for the analyte of interest (54). This format allows for the use of various coloured bead sets for the simultaneous detection of multiple analytes. The planar format incorporates high-density microspots containing different capture ligands affixed to separate spots (54). Here, multiplex testing is possible when multiple spots are placed in a single well. Analytes are differentiated based on their geographic location on the planar surface. Detection methods often use chemiluminescence where the addition of a reagent initiates a chemical reaction which ultimately generates light (54). More recently, electrochemiluminescence has been employed where the label affixed to the second antibody emits a signal only when physically close to a stimulated electrode surface (54). While bead-based formats use flow cytometry to detect analyte-specific signals, planar formats will often use a charge-coupled device camera which detects the amount of light emitted and correlates this light detection to analyte concentration (54). The availability of highly specific monoclonal antibodies and sophisticated detection methods allow for analyte detection with high sensitivity (55).

While incorporating multiple biomarkers in patient assessment may provide great value, a major technical obstacle involves assessing a large number of potential markers in a large population. With the introduction of multiplex assays, several

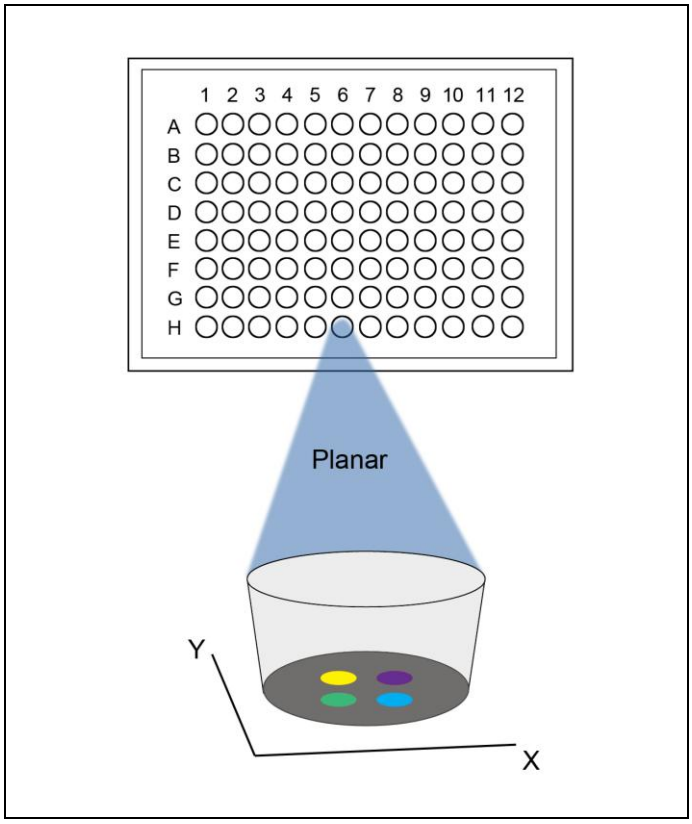



**Figure 1.3** – **Planar multiplex formats.** With the planar format, capture antibodies are affixed in discrete spots to the surface with different biomarkers distinguished by their geographical location (adapted from Ellington et al., 2010)(54).

biomarkers can be simultaneously measured in the ACS setting. Such an approach would provide several benefits over singleplex testing (56). Multiplex testing is cost-efficient as a lower volume of reagents are consumed. Furthermore, since many markers are evaluated in parallel, a decreased sample volume is required and testing also becomes time-efficient. However, multiplex testing is not without its limitations. Most assays are developed for research and nonclinical purposes with only a handful currently approved for clinical testing (57). Furthermore, the analytical performance of the various assays depends on a variety of factors including proprietary antibody pair information and the

analysis software. Finally, there is not a single kit on which all analytes can be optimally measured. Thus, kit selection should take into consideration the assay sensitivity for a particular analyte.

## 1.4 RATIONALE AND OBJECTIVES

Although the hospitalization and mortality rates for cardiovascular disease (CVD) have been decreasing over the past 20 years, ischemic heart disease remains the main cause of these occurrences (58). Furthermore, there is an increasing number of Canadians with high blood pressure, diabetes, and obesity. These conditions, along with the aging population, are all significant risk factors for CVD. Therefore, it is predicted that the actual number of hospitalizations and deaths due to CVD will rise dramatically (59). Chest pain is one of the most common ED presenting complaints (60). As it stands, between 300,000 and 500,000 Canadians present to the ED with chest pain every year (61). The majority of these individuals are not immediately diagnosed and require blood tests. While current clinical guidelines recommend serial cTn measurements for assessing risk in patients with suspected ACS, the process requires a time delay of at least six hours from presentation (62). Therefore, for patients with chest pain in the ED, the key is the early identification of individuals at high risk for MI. To this end, markers upstream from those reflecting myocardial necrosis (i.e., cTn) may provide an earlier indication of risk. Moreover, the use of multiple biomarkers may be superior by providing a more complete assessment.

Thus, the overall objective of this thesis was to investigate whether an early biomarker panel, measured at presentation to the ED, could be useful in predicting those who are at risk for short term cardiovascular events. More specifically:

1) Evaluate the merits of using multiplex testing to measure a biomarker panel.
2) Identify those who are at short term risk (within the first 3 days after presentation) for a MI or another adverse cardiac event (including PCI, CABG, refractory ischemic symptoms, ventricular dysrhythmia, non-fatal cardiac arrest, heart failure, stroke, and death).

The utility of the panel was assessed as a tool used in conjunction with the next generation of high-sensitivity cardiac troponin tests.

# CHAPTER 2: METHOD COMPARISON

## 2.1 Materials and methods

### 2.1.1 MSD quality control

Commercially, Meso Scale Discovery (MSD) does not provide any control material to assess their multiplex platform. Therefore, a set of 20 different controls (consisting of pooled patient samples, controls provided with Randox cytokine biomarker kits, and spiked samples) were collected and analysed to assess precision (i.e., CV for coefficient of variation). Following three days of assessment, 13 of the 20 controls were selected based on precision and run for another two days thereby completing quality control testing over five days. Five MSD arrays encompassing 13 biomarkers were analysed on the SECTOR® Imager 2400: hEGF (human epidermal growth factor), NT-proBNP, vascular injury panel (VEGF, PlGF, sFlt-1, bFGF), growth factor panel (E-selectin, P-selectin, sICAM-3 (soluble intercellular adhesion molecule-3), thrombomodulin), and the cytokine panel (IL-6, IL-10, and MCP-1). Based on CVs and concentration levels across the 13 biomarkers assessed (an effort was made to ensure there was a 'low' and 'high' control for each biomarker), two controls were selected to be run on each MSD kit during the analytical testing phase. Saline was used to determine the limit of blank (LoB) of each array (n=20). All assays were run according the manufacturer's protocol.

| Control material # | Source |
|---|---|
| 1 | Randox cytokine control level 1 |
| 2 | Randox cytokine control level 2 |
| 3 | Randox cytokine control level 3 |
| 4 | CTL Plasma #18 |
| 5 | CTL Plasma #19 |
| 6 | CTL Plasma #20 |
| 7 | CTL Serum Pool 1 |
| 8 | CTL Serum Pool 3 |
| 9 | CTL Serum #24 |
| 10 | EDTA cTN |
| 11 | Cancer patient serum pool |
| 12 | Serum #4 |
| 13 | Serum #5 |
| 14 | Serum #6 |
| 15 | EDTA Neat |
| 16 | EDTA Spike 1 |
| 17 | EDTA Spike 2 |
| 18 | Serum Neat |
| 19 | Serum Spike 1 |
| 20 | Serum Spike 2 |

**Table 2.1** – List of 20 control materials assessed during quality control phase.

### 2.1.2 Population and sample collection

To compare singleplex and multiplex testing methods, blood samples from patients enrolled in the RING study (Reducing the time Interval for identifying New Guideline defined MI in patients with suspected ACS in the ED study) were used. The population consisted of 154 patients that presented to the Hamilton General Hospital's ED within six hours of chest pain onset between December 2008 – December 2010. Enrolled participants were ≥18 years with a-non-diagnostic echocardiogram (ECG), whereas

ineligible participants included those with a STEMI, those sent directly to surgery, or considered trauma patients. Informed consent was obtained and the study was approved by the research ethics board (REB). Blood samples were drawn by research nursing staff at presentation and then 90 minutes and 3 hours afterwards into ethylenediaminetetraacetic (EDTA) vacutainer tubes. Following processing, the EDTA plasma samples were aliquoted into three cryovials and stored below -70°C for subsequent testing. Patients were contacted three times after blood was collected for a follow-up 72 hours after presentation, and again at 30 days and one year. The patients' location (discharged, ward bed, etc.), any medication given at the follow-up and whether any cardiovascular events had occurred since registration in the trial had begun were recorded.

### 2.1.3 Biomarker analysis

During biomarker testing, the samples remained thawed at room temperature for no longer than two hours and were stored at 4°C until testing was finished. The specimens were refrigerated for a maximum period of four days. The 13 biomarkers were analysed using the above mentioned method (see Section 2.1.1.) on the following five arrays on the SECTOR® Imager 2400: i) hEGF, ii) NT-proBNP, iii) vascular injury panel (VEGF, PlGF, sFlt-1, bFGF), iv) growth factor panel (E-selectin, P-selectin, sICAM-3, thrombomodulin), and v) the cytokine panel (IL-6, IL-10, MCP-1). The same EDTA plasma samples were also analysed using a Randox cytokine multiplex array on the Evidence Investigator™ biochip

platform to measure IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, interferon-γ (IFN-γ), TNF-α, IL-1α, IL-1β, MCP-1, and hEGF. At a separate location (Hamilton General Clinical Research and Clinical Trials Laboratory), IL-6 was measured on the Access II platform (Beckman Coulter) while PlGF, sFlt-1, and NT-proBNP were measured on the Elecsys 2010 platform (Roche Diagnostics). All assays were run according to the protocol specified by the manufacturer. The patient samples underwent a single freeze-thaw cycle prior to all of the testing described above.

### 2.1.4 Statistical analyses

Comparisons between methods were performed only when there were detectable concentrations as determined by the manufacturer (Randox, Beckman, Roche) or CLSI guidelines (MSD). The between-day CV for the MSD multiplex panel (n=15 days) was determined. A greater number of quality control materials were used for both the Beckman (5 controls, 4 days) and Roche (3 controls, 4 days) platforms. The correlation between singleplex (clinical assays) and multiplex assays was evaluated using Spearman's r and Passing-Bablok regression. Bland-Altman difference plots were used to assess bias. The LoB for the MSD platforms was determined using the upper 95$^{th}$ percentile of saline measurements. Analyses were performed according to CLSI EP15 A2 guidelines with Analyse-It software (Analyse-It, UK) (63).

## 2.2 RESULTS

Of the 20 samples analyzed in the quality control phase, an EDTA plasma and a serum sample (control material 7 and 10, respectively) were selected as control material to be run with subsequent plates on MSD arrays. Between-day coefficients of variation (CVs) for these controls ranged between 8% (s-Flt-1) and 90% (E-selectin) over the five arrays assessed (Table 2.2, 2.3). The LoB for the markers ranged from 0.01 (thrombomodulin) to 9.99 (MCP-1) ng/L (Table 2.4).

| Analyte | CM 7 - CTL Serum Pool 1 | | |
|---|---|---|---|
| | Mean (ng/L) | Between-day | |
| | | SD | CV, % |
| hEGF | 153.85 | 28.93 | 18.81 |
| NT-proBNP | 579.82 | 253.86 | 43.78 |
| IL-6 | 168.18 | 30.34 | 18.04 |
| IL-10 | 2.81 | 0.52 | 18.62 |
| MCP-1 | 744.99 | 76.75 | 10.30 |
| bFGF | 9.85 | 2.90 | 29.49 |
| PlGF | 23.60 | 5.38 | 22.80 |
| sFlt-1 | 231.01 | 18.87 | 8.17 |
| VEGF | 600.15 | 56.82 | 9.47 |
| E-selectin | 26.12 | 20.63 | 78.96 |
| P-selectin | 89.12 | 38.45 | 43.14 |
| sICAM-3 | 1.64 | 1.11 | 67.60 |
| Thrombomodulin | 4.34 | 1.87 | 43.13 |

**Table 2.2** – Mean concentration and between-day CVs obtained during quality control phase with control material #7 on the MSD SECTOR® Imager 2400

| Analyte | CM 10 - EDTA cTN | | |
|---|---|---|---|
| | Mean (ng/L) | Between-day | |
| | | SD | CV, % |
| hEGF | 32.77 | 7.27 | 22.19 |
| NT-proBNP | 8917.66 | 3154.72 | 35.38 |
| IL-6 | 370.23 | 58.28 | 15.74 |
| IL-10 | 6.13 | 1.30 | 21.18 |
| MCP-1 | 621.19 | 109.39 | 17.61 |
| bFGF | 27.09 | 4.54 | 16.75 |
| PlGF | 29.76 | 4.16 | 13.98 |
| sFlt-1 | 305.96 | 37.23 | 12.17 |
| VEGF | 160.18 | 24.70 | 15.42 |
| E-selectin | 8.25 | 7.40 | 89.71 |
| P-selectin | 49.62 | 7.24 | 14.60 |
| sICAM-3 | 1.14 | 0.67 | 58.50 |
| Thrombomodulin | 2.74 | 0.54 | 19.70 |

**Table 2.3** - Mean concentration and between-day CVs obtained during quality control phase with control material #10 on the MSD SECTOR® Imager 2400

Following analytical testing with the RING patient samples, MSD (2 controls, 15 days) and Randox (3 controls, 4 days) between-day CVs ranged from 4.1-27% and 1.8-21%, respectively. Duplicate measurements on the MSD platform produced average CVs ranging from 4.2-21%. Between-day CVs for IL-6 measured on the Access II platform (5 controls, 4 days) were between 2-5%. The Elecsys 2010 (3 controls, 4 days) produced the following CVs: PlGF = 2.8-3.4%, sFlt-1 = 3.7-5.8%, and NT-proBNP = 1.6-4.4%.

| Analyte | LoB |
|---|---|
| IL-6 | 0.12 |
| IL-10 | 0.47 |
| MCP-1 | 9.99 |
| bFGF | 0.67 |
| PlGF | 2.32 |
| sFlt-1 | 3.38 |
| VEGF | 6.61 |
| hEGF | 0.12 |
| NT-proBNP | 0.62 |
| E-selectin | 0.28 |
| P-selectin | 0.16 |
| sICAM-3 | 0.02 |
| Thrombomodulin | 0.01 |

**Table 2.4** – Limit of blank, calculated as the upper 95$^{th}$ percentile of saline measurements of the 13 biomarkers on the MSD SECTOR® Imager 2400

| Analyte | Control #7 | Control #10 |
|---|---|---|
| hEGF | 27 % | 14 % |
| NT-proBNP | 16 % | 8.0 % |
| IL-6 | 20 % | 12 % |
| IL-10 | 20 % | 20 % |
| MCP-1 | 6.5 % | 15 % |
| E-selectin | 16 % | 23 % |
| P-selectin | 12 % | 11 % |
| sICAM-3 | 16 % | 17 % |
| Thrombomodulin | 6.5 % | 11 % |
| bFGF | 12 % | 12 % |
| PlGF | 15 % | 16 % |
| sFlt-1 | 4.1 % | 6.7 % |
| VEGF | 4.2 % | 15 % |

**Table 2.5** – Precision, as measured by coefficients of variation, of control material used during biomarker measurement of RING patient samples on the MSD SECTOR® Imager 2400

Assay performance comparisons between the two multiplex platforms (MSD and Randox) were possible for VEGF, MCP-1, and hEGF. Furthermore, evaluation of multiplex testing (MSD) against singleplex testing (Roche) was carried out between PlGF, sFlT-1, and NT-proBNP. Finally, the performance of IL-6 was compared across MSD, Randox, and Beckman. Comparison of IL-10 between MSD versus Randox was not possible because 93% of biomarker concentrations fell below the LoB of the MSD and/or limit of detection of Randox platforms. In assessing MCP-1, hEGF, and VEGF between the multiplex platforms, Passing-Bablok analysis revealed regressions of [MCP-1 (MSD)] = 1.43[Randox] + 40; [hEGF (MSD)] = 2.91[Randox] + 0.38; and [VEGF (MSD)] = 6.69[Randox] – 14.57. Bland-Altman difference plots revealed significant bias among all three markers ranging from 29.5-133.23 (Figures 2.1-2.6). Only IL-6 on the MSD platform correlated with that of Randox having a regression of [MSD] = 1.05[Randox]+0.46 (Figure 2.7). There was also agreement of IL-6 between the multiplex (MSD) and singleplex platforms (Beckman) with a regression of [MSD] = 1.08[Beckman]+0.25 (Figures 2.8, 2.10). Passing-Bablok analysis for PlGF, sFlt-1, and NT-proBNP between multiplex and singleplex platforms yielded regressions of [MSD PlGF] = 1.36[Roche]-0.64, [MSD sFlt-1] = 2.68[Roche]-53.11, [MSD NT-proBNP] = 3.50[Roche]-11.87, respectively. Significant bias was also shown via difference plots with biases of 4.91, 57.17, 546.47, respectively (Figures 2.11-2.16).

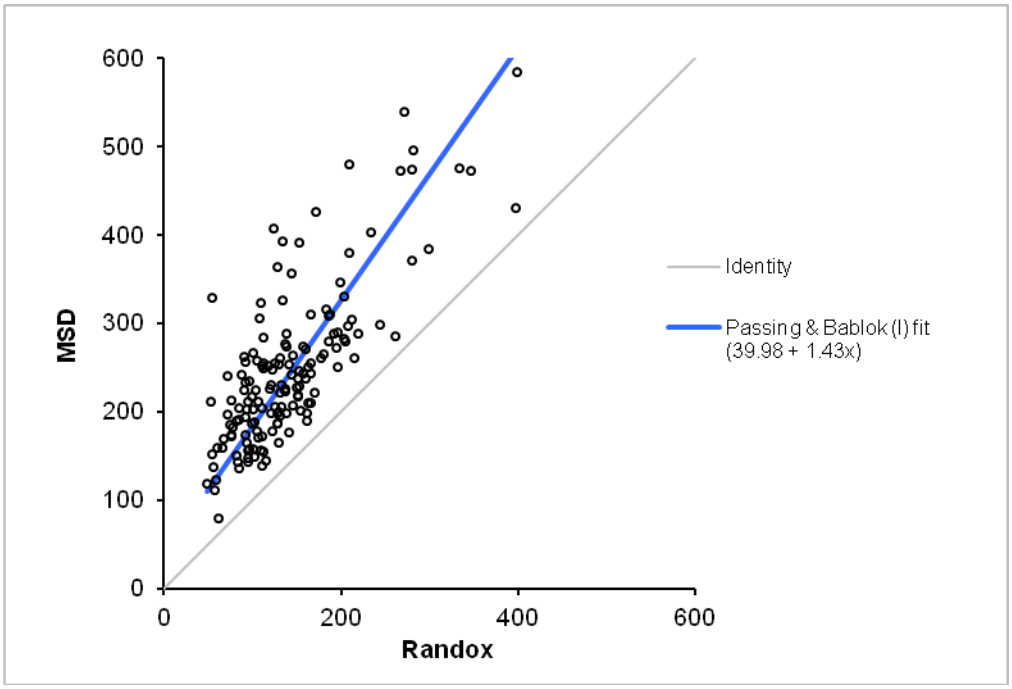



**Figure 2.1** – Passing-Bablok regression of MCP-1 measurements on MSD against Randox

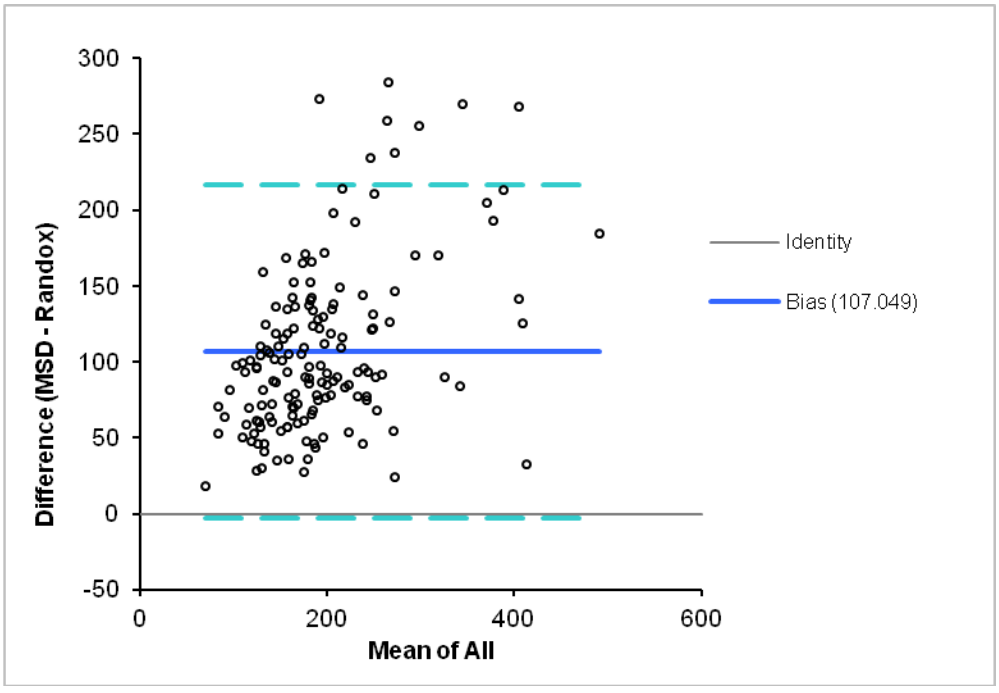



**Figure 2.2** – Bland-Altman difference plot between MCP-1 measurements

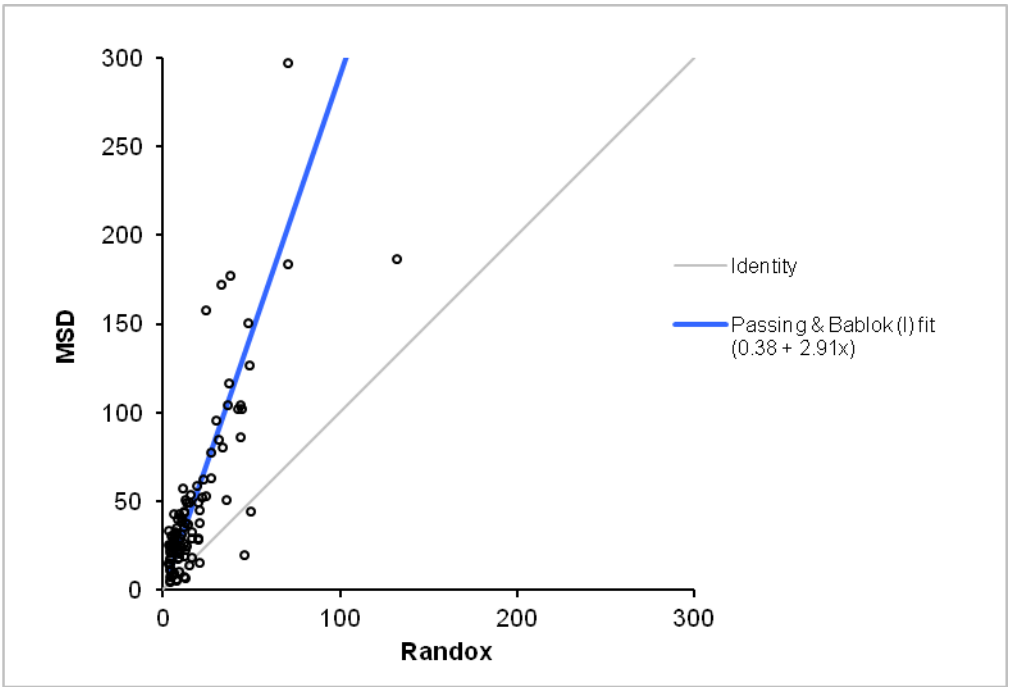



**Figure 2.3** – Passing-Bablok regression of hEGF measurements on MSD against Randox

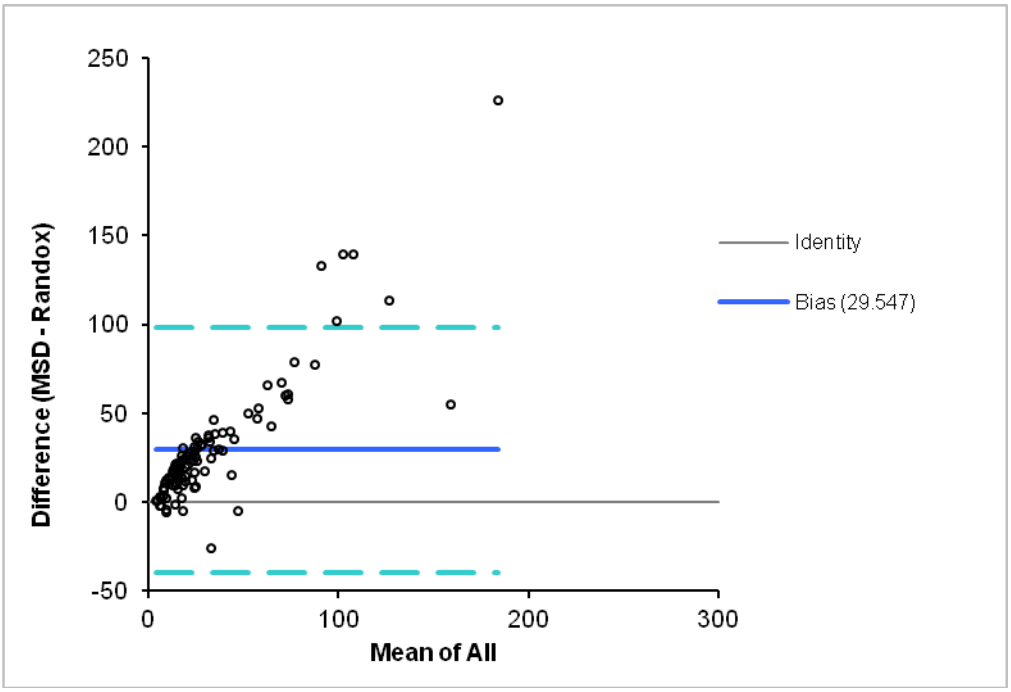



**Figure 2.4** – Bland-Altman difference plot between hEGF measurements

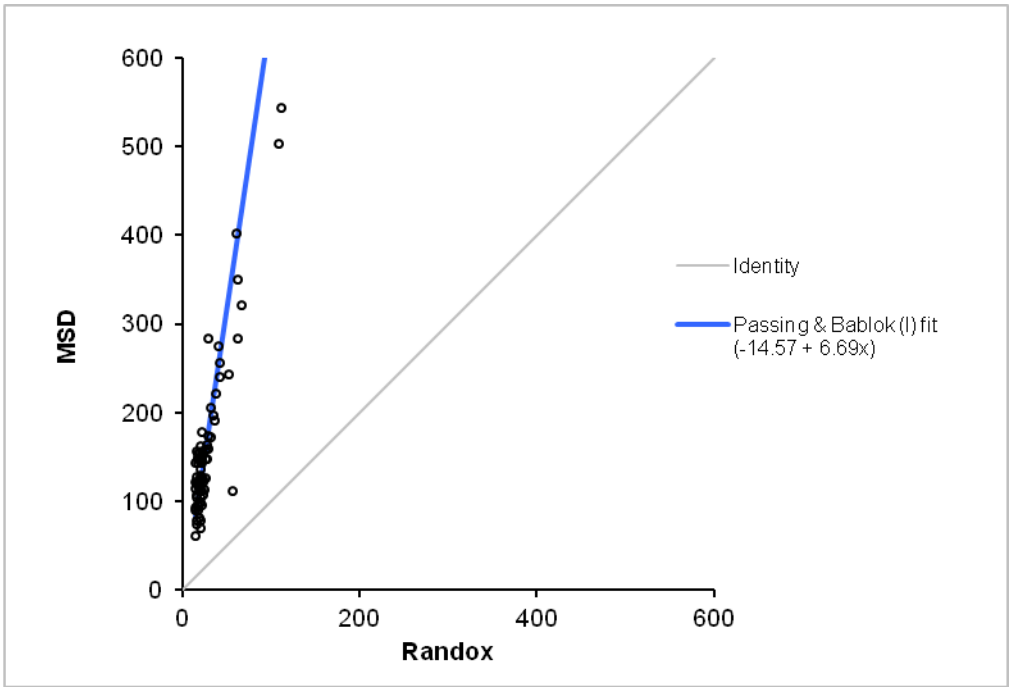



**Figure 2.5** – Passing-Bablok regression of VEGF measurements on MSD against Randox

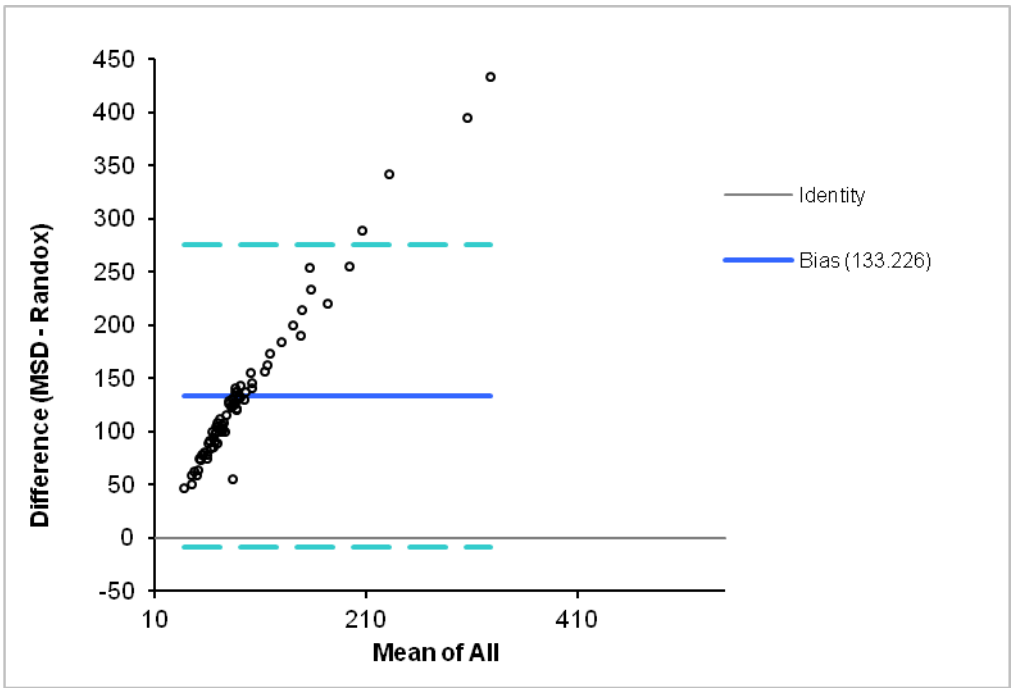



**Figure 2.6** – Bland-Altman difference plot between VEGF measurements

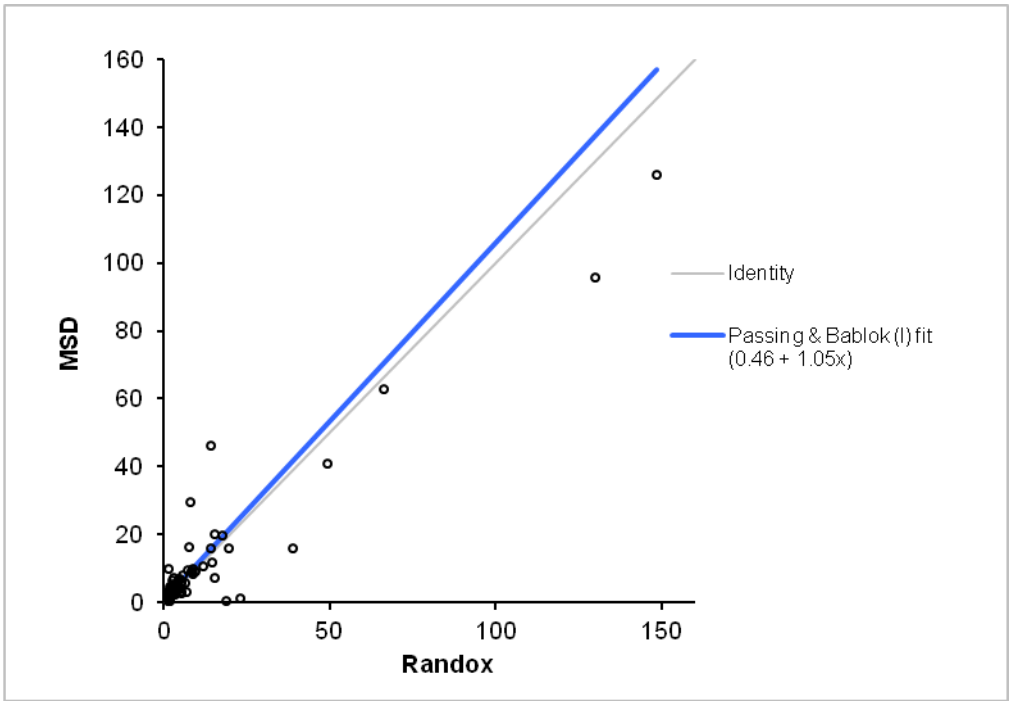



**Figure 2.7 -** Passing-Bablok regression of IL-6 measurements on MSD against Randox

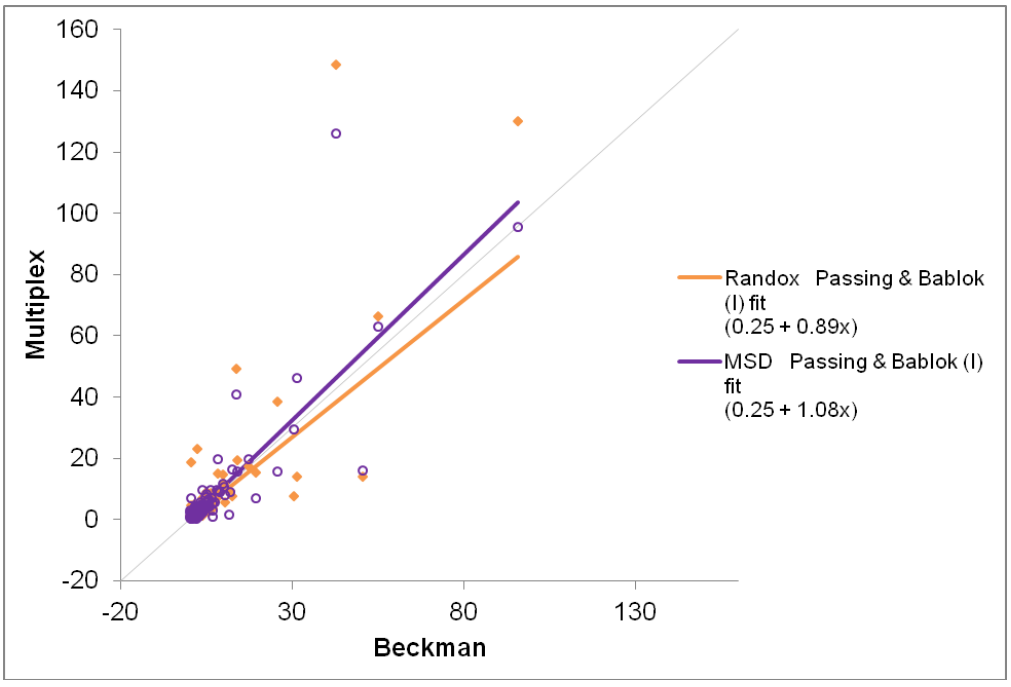



**Figure 2.8** – Passing-Bablok regression of IL-6 measurements on MSD and Randox against Beckman

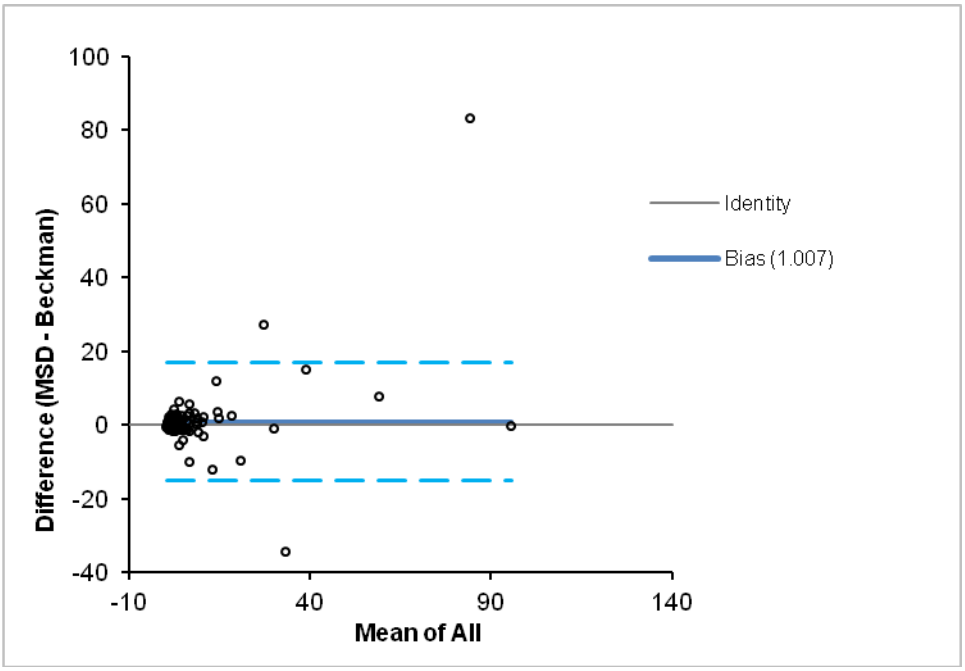



**Figure 2.9** – Bland-Altman difference plot of IL-6 measurements between MSD and Beckman

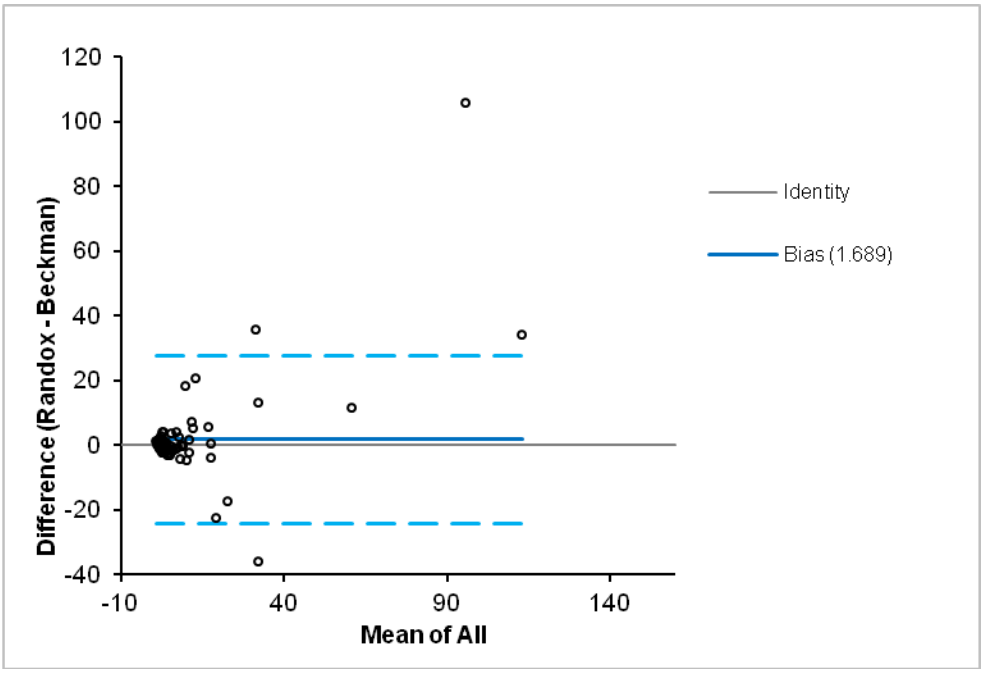



**Figure 2.10** - Bland-Altman difference plot of IL-6 measurements between Randox and Beckman

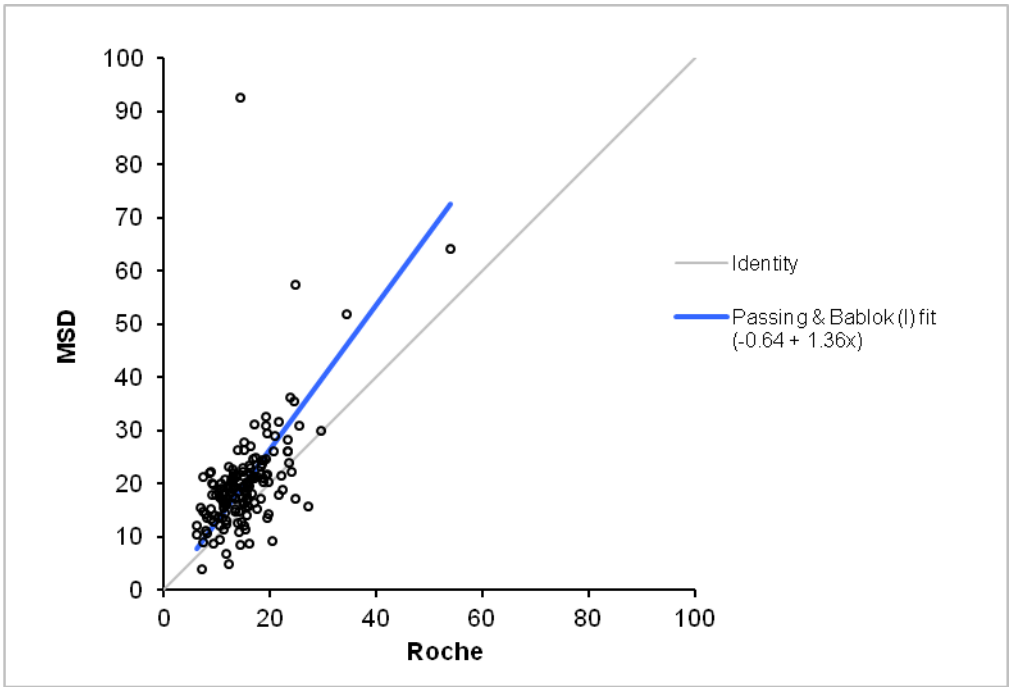



**Figure 2.11** – Passing-Bablok regression of PlGF measurements on MSD against Roche

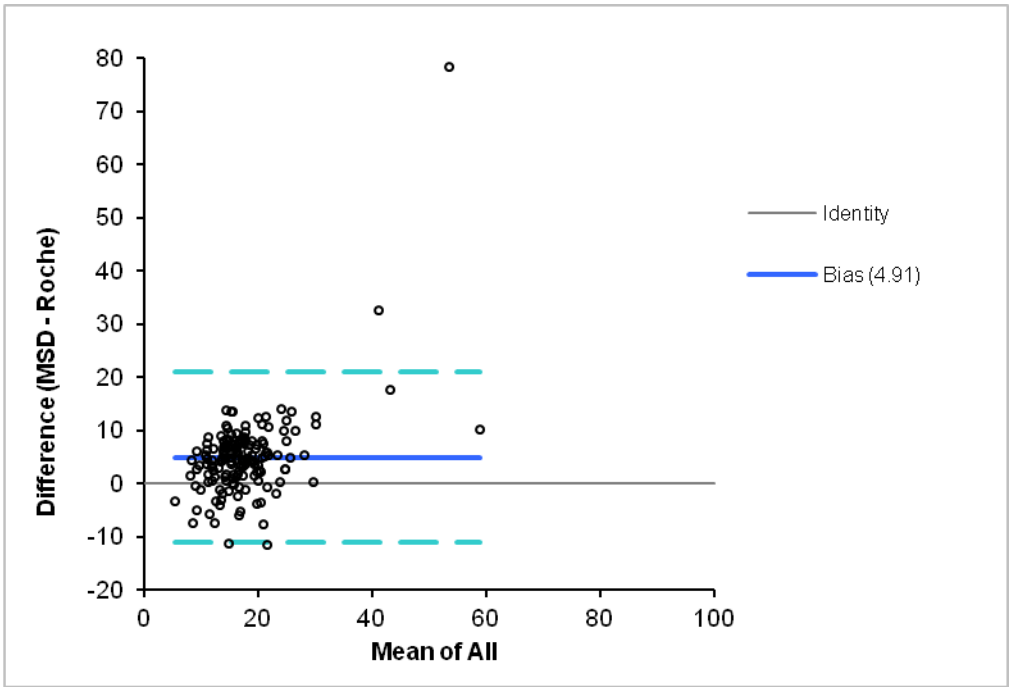



**Figure 2.12** – Bland-Altman difference plot between PlGF measurements

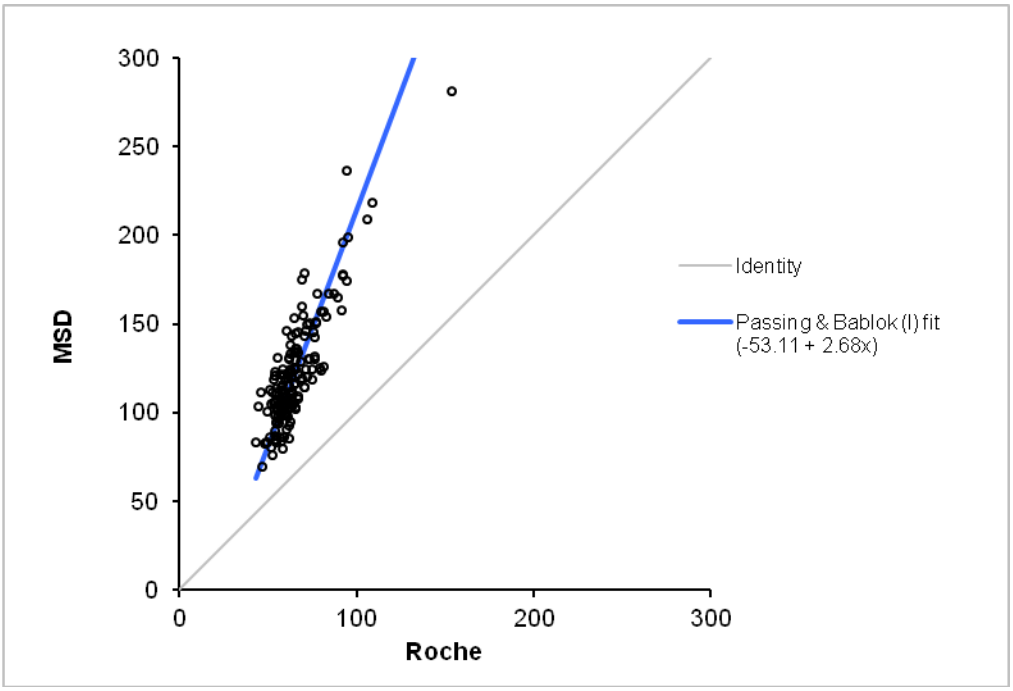



**Figure 2.13** – Passing-Bablok regression of sFlt-1 measurements on MSD against Roche

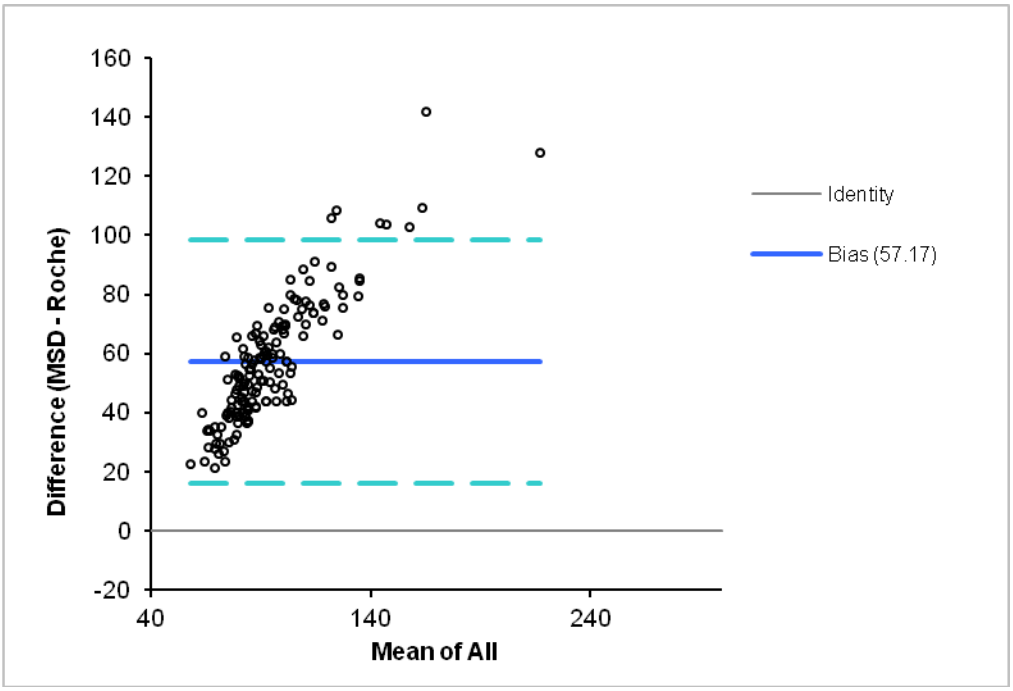



**Figure 2.14** – Bland-Altman difference plot between sFlt-1 measurements

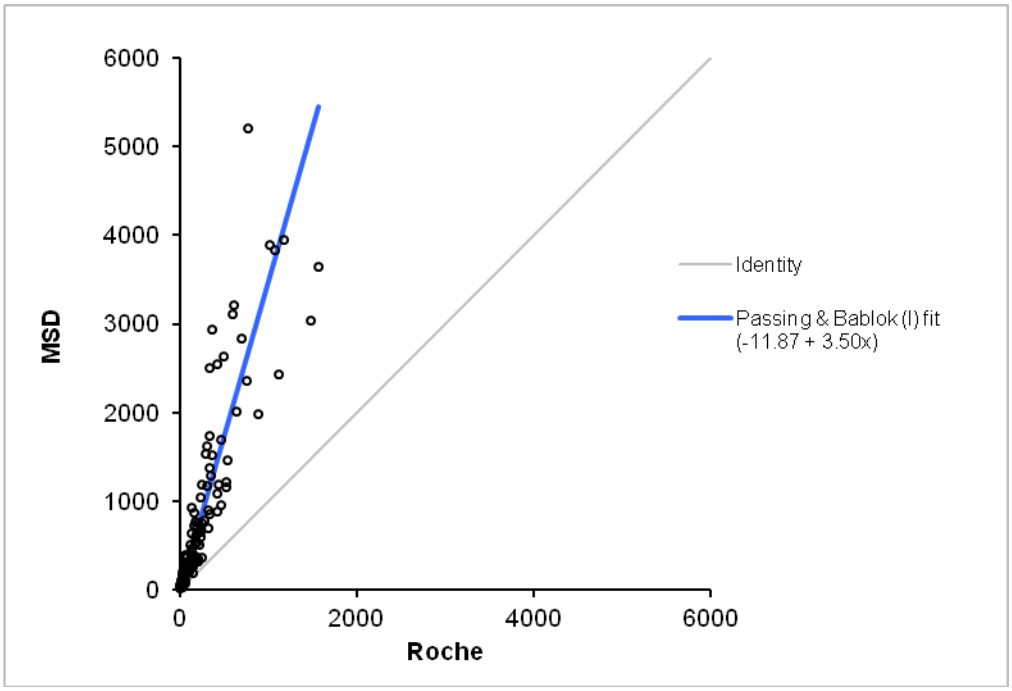



**Figure 2.15** – Passing-Bablok regression of NT-proBNP measurements on MSD against Roche

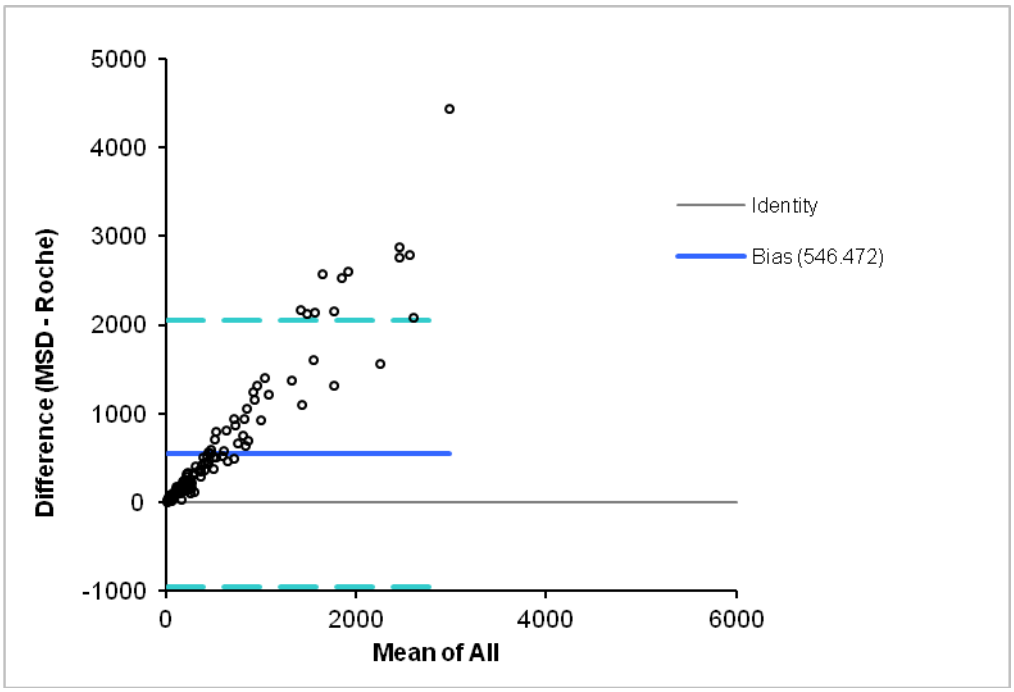



**Figure 2.16** – Bland-Altman difference plot between NT-proBNP measurements

| Analyte | Platform method | Analysed samples | | | Regression analysis | | | Assay parameters Measurable range, ng/L |
|---|---|---|---|---|---|---|---|---|
| | | n | QC material | CV, % (mean conc.) | Slope (95% CI) | Intercept (95% CI) | r | |
| MCP-1 | Multiplex (Randox) | 153 | Low<br>Medium<br>High | 60% (57 ng/L)<br>16% (154 ng/L)<br>25% (796 ng/L) | 1.43 (1.27 to 1.63) | 39.98 (12.92 to 58.88) | 0.784 | 0-1500 |
| | Multiplex (MSD) | | Plasma pool<br>Serum pool | 12% (620 ng/L)<br>7.1% (768 ng/L) | | | | 10-11000 |
| hEGF | Multiplex (Randox) | 102 | Low<br>Medium<br>High | 16% (44 ng/L)<br>9.1% (94 ng/L)<br>57% (679 ng/L) | 2.91 (2.56 to 3.42) | 0.38 (-6.49 to 4.42) | 0.795 | 0-900 |
| | Multiplex (MSD) | | Plasma pool<br>Serum pool | 14% (31 ng/L)<br>25% (136 ng/L) | | | | 0.1-2500 |
| VEGF | Multiplex (Randox) | 74 | Low<br>Medium<br>High | 12% (51 ng/L)<br>14% (238 ng/L)<br>15% (635 ng/L) | 6.69 (5.65 to 8.09) | -14.57 (-49.71 to 9.46) | 0.912 | 0-3000 |
| | Multiplex (MSD) | | Plasma pool<br>Serum pool | 7% (181 ng/L)<br>11% (658 ng/L) | | | | 7-9000 |
| IL-6 | Singleplex (Beckman) | 139 | Plasma pool<br>Serum pool<br>Access IL-6 QC Lvl 1<br>Access IL-6 QC Lvl 2<br>Access IL-6 QC Lvl 3 | 4.8% (26 ng/L)<br>2.5% (194 ng/L)<br>5.9% (8 ng/L)<br>5.1% (277 ng/L)<br>4.6% (750 ng/L) | 1.06 (0.97 to 1.17) | 0.27 (-0.001 to 0.52) | 0.870 | 0.5-1500 |
| | Multiplex (MSD) | | Plasma pool<br>Serum pool | 11% (374 ng/L)<br>16% (172 ng/L) | | | | 0.1-11000 |
| PlGF | Singleplex (Roche) | 153 | Plasma pool<br>PreciControl Lvl 1<br>PreciControl Lvl 2 | 3.4% (25 ng/L)<br>2.8% (91 ng/L)<br>3.4% (1812 ng/L) | 1.36 (1.16 to 1.62) | -0.64 (-4.17 to 2.17) | 0.567 | 2-10000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Multiplex (MSD) | | Plasma pool<br>Serum pool | 24% (35 ng/L)<br>19% (25 ng/L) | | | | 2.7-9000 |
| sFLt-1 | Singleplex (Roche) | 153 | Plasma pool<br>PreciControl Lvl 1<br>PreciControl Lvl 2 | 5.8% (184 ng/L)<br>3.7% (951 ng/L)<br>3.8% (9501 ng/L) | 2.68<br>(2.39 to 3.05) | -53.11<br>(-74.86 to -35.16) | 0.864 | 6-85000 |
| | Multiplex (MSD) | | Plasma pool<br>Serum pool | 12% (332 ng/L)<br>9% (225 ng/L) | | | | 2.1-9000 |
| NT-proBNP | Singleplex (Roche) | 134 | Plasma pool<br>PreciControl Cardiac II Lvl 1<br>PreciControl Cardiac II Lvl 2 | 4.4% (4176 ng/L)<br>3.4% (130 ng/L)<br>1.6% (4441 ng/L) | 3.50<br>(3.14 to 3.84) | -11.87<br>(-32.96 to 3.58) | 0.881 | 5-35000 |
| | Multiplex (MSD) | | Plasma pool<br>Serum pool | 12% (9249 ng/L)<br>17% (606 ng/L) | | | | 0.3-5000 |

**Table 2.6** - Method comparisons of MCP-1, hEGF, VEGF, IL-6, PlGF, sFlt-1, and NT-proBNP

## 2.3 DISCUSSION

Prior to evaluating the performance of the MSD system, quality control was established and implemented due to a lack of commercially provided controls. This was necessary to ensure the reliability of laboratory performance from plate to plate as well as day to day, thus providing confidence obtained from arrays performed on the SECTOR® Imager 2400. An effort was made to select controls with concentrations of analytes at two different levels with relatively low CVs to better assess assay performance. Inclusion of both controls on every MSD array allowed for the monitoring of analytical quality control as results on assays with control concentrations outside 2 SD of the established mean were rejected. Though the between-day CVs during the quality control evaluation phase fell within a large range of 8-90%, the CVs were acceptable (below 20%) for all biomarkers except NT-proBNP, E-selectin, P-selectin, sICAM-3, and thrombomodulin during the testing phase. Furthermore, it should be noted that, over time, as experience in running the MSD assays was gained, the CVs improved significantly for these five biomarkers in particular. Throughout analytical testing of the RING study samples, the CVs ranged from 4.1-27% with the greatest imprecision observed for NT-proBNP, MCP-1, thrombomodulin, sFlt-1, and VEGF (Table 2.5).

With established control material, evaluation of multiplex biomarker technology was possible. This was done by comparing its performance against the 'gold standard' singleplex assays. MSD and Randox represented the multiplex platforms while Roche and Beckman instruments represented the singleplex methods. Overall, singleplex

immunoassays demonstrated greater precision than multiplex assays for all the analytes compared. Of the multiplex assays, IL-6 had the highest correlation and closest agreement with the Beckman singleplex assay. According to Passing-Bablok regression analysis, this was followed by PlGF and MCP-1, the latter having a significant constant bias however. Bland-Altman plots revealed that IL-6, PlGF, sFlt-1, and NT-proBNP all had a positive bias as compared to the clinical assays. With the multiplex assays, sFlt-1 showed the greatest bias with measurements falling 60% higher than those obtained on the singleplex platform. In the event that a similar bias occurs across the range of all concentrations, however, such constant biases may affect the interpretation of results.

This data underlines the differences in precision and concentrations between multiplex and singleplex assays. While the employment of multiplex testing is accompanied with the benefits of smaller sample volume, shorter testing periods, and more efficient costs, the precision of singleplex assays may be sacrificed. Sources of imprecision and variability include differences in the degree of automation, the quality of the calibration curve, detection methods, manufacturing process, matrix type, cross-reactivity and nonspecific binding, and antibody selection. While the singleplex platforms analysed in this thesis were on the automated clinical instruments, the multiplex arrays were performed on semi-automated platforms – requiring the manual addition of reagents, samples, and washing of plates. Secondly, with curve fitting accomplished via a mathematic approach, different platforms may vary in the quality of the calibration curve. A series of calibrator samples of known analyte concentration is

used to produce a curve against which sample light intensity plotted. The number of calibrators used and the mathematical model applied can affect the accuracy of the assay results. Concentrations of samples with a light signal outside the standard curve were determined by extrapolating the curve or by diluting and reanalyzing samples. This also introduces avenues through which the true value might be missed. Thus assays with a wider measurable range and that incorporate analytes with similar physiological ranges would limit the need for extrapolations and dilutions (64,65). Moreover, the detection methods of the MSD assays, electrochemiluminesence, may be more specific than chemiluminescence and may be associated with a lower amount of background noise as the electrical field used to induce the release of light is narrow (66,67). Spotting irregularities between each well/plate due to the assay manufacturing process would also introduce variability. The printing of microarrays can be accomplished with the use of solid pins or via piezoelectric printers. The former uses a needle that deposits a set amount of antibodies by capillary action in a pre-defined pattern. The latter employs an electric pulse to place a specific amount of antibodies on a particular spot (55,68). Inconsistent spots between wells and plates could explain the intra- and inter-assay imprecision and the duplicate measurements with relatively little or no analyte concentration observed during the testing of RING samples.

The matrix, whether it is serum or plasma, can also affect the results obtained from immunoassays. For example, it has been reported that VEGF concentrations are higher when measured in serum as opposed to plasma (69). Furthermore, intra-well

interference issues such as the occurrence of cross-reactivity and non-specific binding could also explain the quantitative differences between platforms. This could not be assessed with the MSD arrays as the arrays are pre-coated with capture antibodies already affixed to the planar surface of each well. However, pre-incubation periods and thorough washing between additions of reagents are effective in preventing such interferences (70). Differences in analyte concentrations across platforms may be explained by the use of different antibodies by the respective assays. These antibodies may target different epitopes of the analyte under investigation. Characteristics such as antigen specificity and binding affinity may differ between these antibodies thus affecting the reported analyte concentration. Since information on the particular antibodies used in the evaluated assays is proprietary and cannot be accessed, this effect on the correlations obtained here could not be evaluated.

The small sample size of the RING study represents a limitation of the method comparison performed here. The strength of the correlation would be greater with a much larger population. Furthermore, since the samples were obtained from early presenters to the ED, the concentrations of the majority of the analytes were on the lower end of the assays' measurable range. Therefore, the agreement between the assays could only be assessed within a small concentration range.

Overall, without proper validation and optimization of a multiplex immunoassay platform, various factors can affect the repeatability, reproducibility, precision, and accuracy of the analytical methods. Although quantitative differences were observed

between the MSD, Randox, Beckman, and Roche platforms, the relative differences between the platforms were often comparable. With the consideration of analyte performance relative to clinical assays, multiplex assays may be useful as investigational tools, but validation of results generated by these platforms is of the utmost importance.

# CHAPTER 3: CTS STUDY

## 3.1 MATERIALS AND METHODS

### 3.1.1 Population and sample processing

The Cardiac Troponin testing in the emergency Setting (CTS) study involved an all-comer population of 110 patients who presented to the ED at the Hamilton General Hospital with chest pain. Blood samples were drawn by nurses at presentation, 90 min, and 3h into serum-separating tubes (SSTs) in May-June 2010. Informed consent was obtained and the study was approved by the research ethics board. Following processing, the samples were separated into three cryogenic vials and stored below -70°C prior to subsequent testing.

### 3.1.2 Biomarker testing

Five MSD arrays were performed on the SECTOR on blinded samples for the following markers on the presentation specimen: NT-proBNP, hEGF, growth factor panel (bFGF, PlGF, sFlt-1, VEGF), vascular injury panel (E-selectin, P-selectin, sICAM-3, thrombomodulin), and the cytokine panel (IL-6, IL-10, and MCP-1). The Randox cardiac multiplex array was performed on the evidence investigator to measure the levels of CK-MB, myoglobin, glycogen phosphorylase isoenzyme BB (GPBB), H-FABP, carbonic anhydrase III (CAIII), and cTnI. At an off-site location (Hamilton General Clinical Research

and Clinical Trials Laboratory), IL-6, regular AccuTnI, enhanced AccuTnI, and hs-cTnI were measured on the Access II platform (Beckman Coulter) while PlGF, sFlt-1, NT-proBNP, hs-cTnT were measured on the Elecsys 2010 platform and cTnT and creatinine (eGFR) measured on the Modular platform (Roche). The markers assessed were selected for their association with various pathophysiological processes involved with ACS. Controls comprised of the two samples selected during the quality control phase (MSD) or those provided by the manufacturer (Randox, Roche, Beckman). For MSD arrays, samples on an array with control measurements beyond 2 SD of the mean were rejected and rerun. All assays were run using the manufacturer's protocol.

### 3.1.3 Panel selection and assessment

Patients were evaluated for major adverse cardiovascular events, defined as MI, percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG), refractory ischemic symptoms, ventricular dysrythmia, heart failure, stroke, non-fatal cardiac arrest, and death, at 72 hours following presentation to the ED. The patients were identified as either 1) outcome-free or 2) having one or more outcomes. Descriptive statistics were used to compare patients with and without an outcome. Biomarker concentrations were assessed using receiver operator characteristics (ROC) curve analyses and comparisons of area under the curve (AUC). Following assessment of various combinations of 3-5 biomarker panels, four biomarkers were selected based on the AUC and physiological function. A positive panel was achieved when at least three of

the four biomarker concentrations fell above the ROC-derived cut-off. Furthermore, both the panel and cardiac troponin levels must have been positive for the test to produce a positive result. Alternatively, the test was also evaluated with either the cardiac troponin or the panel being positive for a positive test result. Various cutoffs for both the early biomarkers and the cardiac troponins were also evaluated. For the cardiac troponins, different cutoffs from the literature were assessed (71,72). The panel was evaluated for its ability to identify ED patients at high risk for the composite outcome.

## 3.2 RESULTS

Of the 110 patients enrolled in the CTS study, eight were omitted from analyses as they were either a direct hospital admission (i.e., not an ED patient; n=2), was a duplicate of another patient entry (n=1), could not be associated with any patient records (n=2), or their sample volumes did not allow for measurement of the entire biomarker set (n=3). The rate of patients with one or more MACE among the remaining 102 patients was 13% (13 patients) with the following outcome occurrences: MI (n=4), PCI (n=1), HF (n=2), ventricular dysrythmia (n=3), and CABG (n=3), refractory ischemic symptoms (n=2), and non-fatal cardiac arrest (n=1).

The biomarkers were assessed using ROC analyses and ranked by their AUCs. Glucose had the largest AUC of 0.86 (95% CI: 76-96) followed by hs-cTnI, AccuTnI (enhanced) and AccuTnI (regular) with AUCs of 0.82 (95%CI: 0.68-0.95), 0.79 (95%CI: 0.64-0.95), and 0.78 (95%CI: 0.63-0.93), respectively. After all biomarkers were ranked

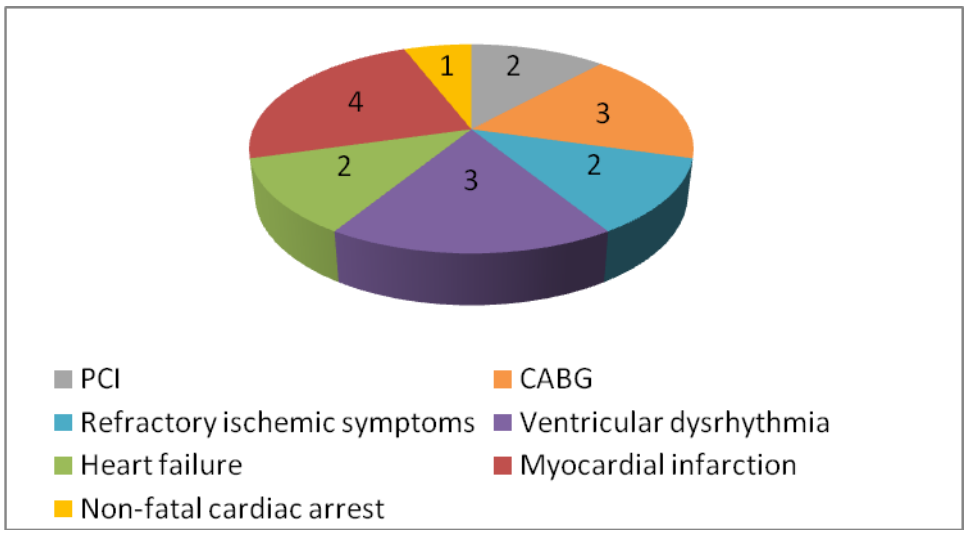



**Figure 3.1** – The distributions of all outcomes reported by CTS study patients

according to AUC and various panels were analysed, the combination of sFlt-1, Creatinine, MCP-1, and NT-proBNP identified patients at risk for adverse cardiac outcomes with the highest accuracy.

ROC-derived cutoffs for sFlt-1, Creatinine, MCP-1, and NT-proBNP were 157.52 ng/L, 90 µmol/L, 484.85 ng/L, and 1835.78 ng/L, respectively. The panel, used in combination with either hs-cTnI or hs-cTnT, had a sensitivity of 100% (95% CI: 75-100%) and specificity of 54% (95% CI: 43-65%) and 52% (95% CI: 41-62%), respectively. The sensitivity/specificity for hs-cTnI or hs-cTnT alone were 92% (95% CI: 64-100%)/57% (95% CI: 46-68%) and 85% (95% CI: 55-98%)/55% (95% CI: 44-66%), respectively (Table 3.6).

| Marker | Age | | Gender | | Outcomes | |
|---|---|---|---|---|---|---|
| | *<65* | *≥65* | *F* | *M* | *-* | *+* |
| Glucose | 6.0 (5.4-7.0) | 6.5 (5.5-8.2) | 6.1 (5.4-7.6) | 6.5 (5.7-7.7) | 6.1 (5.4-20) | 9.9 (8.0-13) |
| hs-cTnI | 7.6 (4.4-17) | 10 (6.8-23) | 7.8 (4.7-13) | 12 (6.7-30) | 8.3 (4.9-16) | 39 (13-105) |
| AccuTnI (enhanced) | 0.01 (0.01-0.02) | 0.02 (0.00-0.03) | 0.01 (0.01-0.02) | 0.02 (0.01-0.04) | 0.01 (0.01-0.02) | 0.05 (0.02-0.13) |
| AccutTnI (regular) | 0.01 (0.00-0.02) | 0.01 (0.00-0.03) | 0.01 (0.00-0.02) | 0.02 (0.01-0.04) | 0.01 (0.00-0.02) | 0.04 (0.02-0.13) |
| hs-cTnT | 3.8 (2.9-17) | 18 (8.9-39) | 7.6 (2.9-20) | 18 (5.5-37) | 8.9 (2.9-21) | 29 (15-75) |
| cTnT | 0.01 (0.01-0.01) | 0.01 (0.01-0.02) | 0.01 (0.01-0.01) | 0.01 (0.01-0.02) | 0.01 (0.01-0.01) | 0.02 (0.01-0.05) |
| sFlt-1 | 147 (135-201) | 157 (129-185) | 145 (129-183) | 158 (135-208) | 146 (130-186) | 181 (158-289) |
| VEGF | 446 (281-824) | 469 (283-864) | 480 (283-862) | 443 (260-797) | 433 (274-736) | 861 (544-1063) |
| Creatinine | 75 (66-89) | 91 (77-117) | 78 (65-94) | 89 (76-113) | 83 (68-103) | 92 (80-127) |
| MCP-1 | 464 (391-583) | 504 (395-638) | 506 (391-618) | 469 (394-598) | 463 (388-600) | 576(487-774) |
| NT-proBNP | 182 (57-658) | 1150 (491-4640) | 560 (173-1922) | 601 (162-3023) | 497 (151-1748) | 2237 (357-9673) |
| hEGF | 65 (38-196) | 93 (40-186) | 69 (35-189) | 87 (45-187) | 74 (37-173) | 186 (68-307) |
| bFGF | 6.6 (5.0-9.5) | 8.0 (5.7-24) | 7.6 (5.7-13) | 6.9 (4.9-19) | 7.0 (5.1-13) | 22 (5.9-29) |
| eGFR | 88 (77-104) | 60 (45-78) | 68 (55-88) | 79 (57-97) | 78 (57-92) | 59 (47-85) |
| P-selectin | 100 (72-125) | 90 (73-121) | 91 (71-119) | 96 (74-125) | 90 (72-122) | 118 (85-134) |
| IL-10 | 2.6 (1.1-4.8) | 2.4 (1.4-4.4) | 2.4 (1.1-4.1) | 2.2 (1.4-6.0) | 2.4 (1.3-4.3) | 3.9 (1.2-132) |
| IL-6 | 4.6 (0.37-7.3) | 6.8 (3.8-10) | 5.2 (2.9-9.1) | 5.7 (3.3-13) | 5.3 (3.1-9.0) | 7.0 (3.1-15) |
| PlGF | 19 (17-23) | 21 (18-26) | 20 (17-24) | 21 (17-27) | 20 (17-25) | 22 (18-26) |
| E-selectin | 18 (13-23) | 17 (9.8-20) | 19 (13-24) | 16 (10-19) | 17 (11-21) | 18 (14-25) |
| Thrombomodulin | 3.2 (2.6-4.3) | 4.3 (3.1-5.4) | 3.8 (2.9-5.3) | 3.5 (2.7-3.5) | 3.7 (2.9-4.8) | 3.5 (2.4-5.8) |
| sICAM-3 | 1.1 (0.93-1.5) | 1.0 (0.76-1.7) | 1.1 (0.81-1.8) | 1.1 (0.80-1.5) | 1.1 (0.84-1.6) | 1.0 (0.65-1.7) |

**Table 3.1**–Median (and interquartile range) of biomarker concentrations between 1) patients aged under 65 and aged 65 years and above, 2) males and females, 3) patients with and without reported outcome

| Marker | Mann-Whitney test p-value | | |
|---|---|---|---|
| | **Age** *<65 vs. ≥65* | **Gender** | **Outcomes** |
| Glucose | 0.31 | 0.32 | <0.01 |
| hs-cTnI | 0.03 | <0.01 | <0.01 |
| AccuTnI (enhanced) | 0.09 | <0.01 | <0.01 |
| AccutTnI (regular) | 0.38 | 0.01 | <0.01 |
| hs-cTnT | <0.01 | <0.01 | <0.01 |
| cTnT | 0.03 | <0.01 | <0.01 |
| sFlt-1 | 0.71 | 0.24 | 0.01 |
| VEGF | 0.71 | 0.69 | 0.03 |
| Creatinine | <0.01 | <0.01 | 0.03 |
| MCP-1 | 0.45 | 0.87 | 0.04 |
| NT-proBNP | <0.01 | 0.91 | 0.04 |
| hEGF | 0.52 | 0.58 | 0.05 |
| bFGF | 0.08 | 0.69 | 0.08 |
| eGFR | <0.01 | 0.21 | 0.11 |
| P-selectin | 0.74 | 0.64 | 0.11 |
| IL-10 | 0.93 | 0.46 | 0.14 |
| IL-6 | 0.11 | 0.45 | 0.40 |
| PlGF | 0.18 | 0.46 | 0.41 |
| E-selectin | 0.34 | 0.07 | 0.74 |
| Thrombomodulin | <0.01 | 0.24 | 0.70 |
| sICAM-3 | 0.58 | 0.59 | 0.57 |

**Table 3.2 –** Mann-Whitney test p-values for all biomarkers by age, gender, and outcome

| Marker | ROC analysis | | |
|---|---|---|---|
| | AUC | 95% CI | p-value |
| Glucose | 0.86 | 0.76-0.96 | <0.01 |
| hs-cTnI | 0.82 | 0.68-0.95 | <0.01 |
| AccuTnI (enhanced) | 0.79 | 0.64-0.95 | <0.01 |
| AccutTnI (regular) | 0.78 | 0.63-0.93 | <0.01 |
| hs-cTnT | 0.76 | 0.61-0.91 | <0.01 |
| cTnT | 0.74 | 0.58-0.90 | <0.01 |
| H-FABP | 0.74 | 0.57-0.92 | <0.01 |
| sFlt-1 | 0.72 | 0.58-0.85 | <0.01 |
| VEGF | 0.69 | 0.52-0.86 | 0.02 |
| Creatinine | 0.68 | 0.54-0.82 | <0.01 |
| MCP-1 | 0.68 | 0.53-0.83 | 0.01 |
| NT-proBNP | 0.68 | 0.51-0.85 | 0.02 |
| hEGF | 0.67 | 0.51-0.83 | 0.02 |
| bFGF | 0.65 | 0.47-0.84 | 0.06 |
| eGFR | 0.64 | 0.47-0.80 | 0.05 |
| P-selectin | 0.64 | 0.47-0.80 | 0.05 |
| IL-10 | 0.63 | 0.43-0.83 | 0.11 |
| IL-6 | 0.57 | 0.39-0.75 | 0.21 |
| PlGF | 0.57 | 0.42-0.72 | 0.18 |
| E-selectin | 0.53 | 0.36-0.70 | 0.37 |
| Thrombomodulin | 0.47 | 0.24-0.69 | 0.61 |
| sICAM-3 | 0.45 | 0.26-0.65 | 0.69 |

**Table 3.3** – Ranking of the ROC AUC on the measured biomarkers

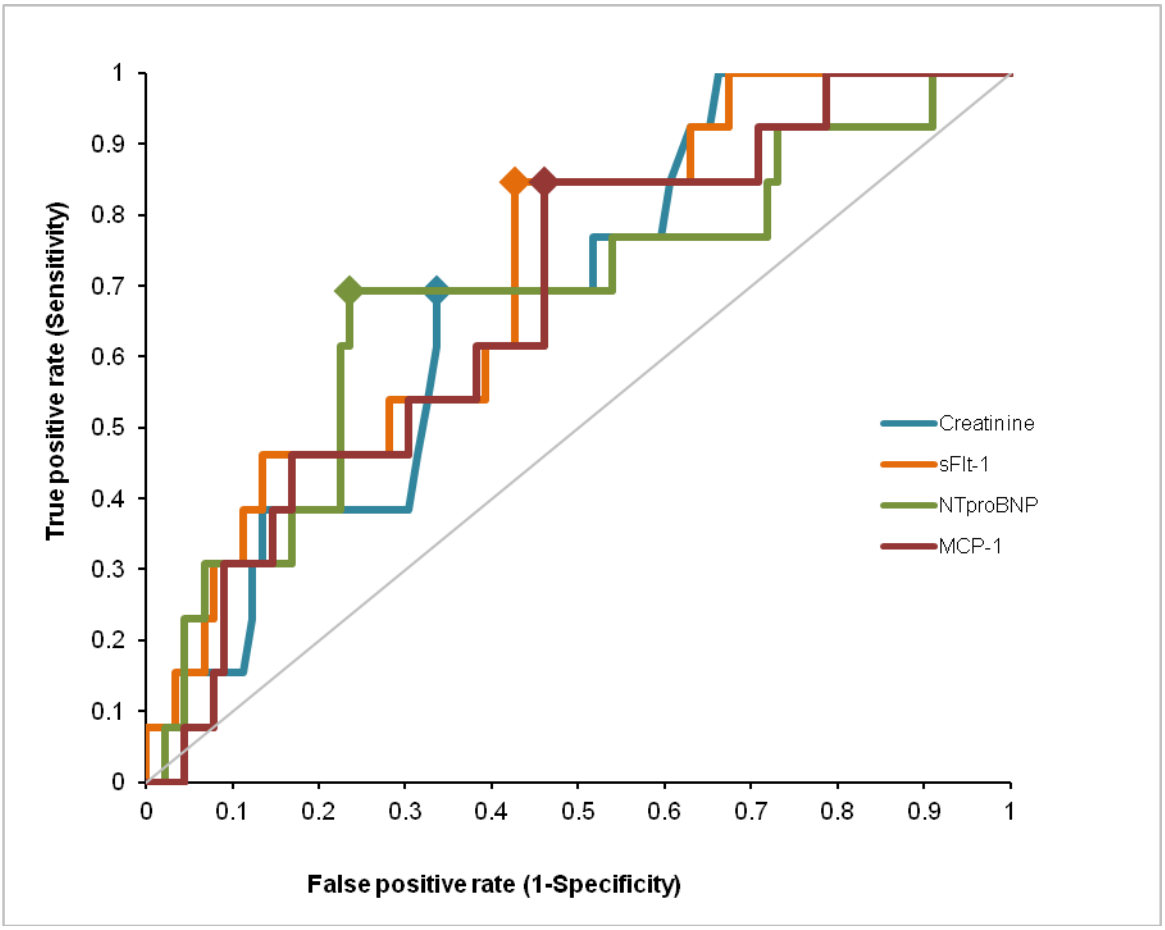



**Figure 3.2 –** ROC plot of panel biomarkers with relative cutoffs identified by diamond markers

| | hs-cTnI | AccuTnI (enhanced) | AccuTnI (regular) | hs-cTnT | cTnT | sFlt-1 | VEGF | NT-proBNP | MCP-1 | Creatinine | hEGF | bFGF | P-selectin | eGFR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **hs-cTnI** | | 0.89* | 0.81* | 0.75* | 0.57* | 0.13 | -0.09 | 0.49* | 0.1 | 0.33* | 0.03 | 0.02 | 0.06 | -0.24* |
| **AccuTnI (enhanced)** | | | 0.81* | 0.75* | 0.53* | 0.17 | <0.01 | 0.40* | 0.05 | 0.23* | 0.09 | <0.01 | 0.01 | -0.13 |
| **AccuTnI (regular)** | | | | 0.65* | 0.50* | 0.16 | <0.01 | 0.29* | 0.11 | 0.15 | 0.11 | 0.11 | 0.12 | -0.08 |
| **TnThs** | | | | | 0.70* | 0.18 | 0.03 | 0.55* | 0.16 | 0.44* | 0.01 | 0.05 | 0.01 | -0.38* |
| **TnT** | | | | | | 0.07 | -0.04 | 0.35* | 0.11 | 0.21* | -0.08 | 0.09 | 0.02 | -0.23* |
| **sFlt-1** | | | | | | | 0.39* | 0.25* | 0.12 | 0.21* | 0.21* | 0.33* | 0.23* | -0.17 |
| **VEGF** | | | | | | | | -0.06 | 0.11 | 0.06 | 0.18 | 0.27* | 0.26* | -0.03 |
| **NT-proBNP** | | | | | | | | | 0.12 | 0.55* | -0.09 | 0.01 | 0.03 | -0.62* |
| **MCP-1** | | | | | | | | | | 0.08 | 0.11 | 0.17 | 0.37* | -0.13 |
| **Creatinine** | | | | | | | | | | | 0.04 | 0.03 | 0.05 | -0.89* |
| **hEGF** | | | | | | | | | | | | 0.25* | 0.26* | 0.01 |
| **bGFG** | | | | | | | | | | | | | 0.39* | -0.08 |
| **P-selectin** | | | | | | | | | | | | | | -0.01 |
| **eGFR** | | | | | | | | | | | | | | |

**Table 3.4** – Spearman's r between all biomarkers assessed. * Denotes p <0.05.

| Test | | | Outcome | | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|---|---|
| | | | Yes | No | | |
| Panel (ROC-derived) | | Yes | 12 | 29 | 0.92 (0.64-1.00) | 0.67 (0.57-0.77) |
| | | No | 1 | 60 | | |
| Panel (Creatinine) | | Yes | 10 | 14 | 0.77 (0.46-0.95) | 0.84 (0.75-0.91) |
| | | No | 3 | 75 | | |
| Panel (FABP-3) | | Yes | 10 | 21 | 0.77 (0.46-0.95) | 0.76 (0.66-0.85) |
| | | No | 3 | 68 | | |
| Panel (bFGF) | | Yes | 9 | 13 | 0.69 (0.39-0.91) | 0.85 (0.76-0.92) |
| | | No | 4 | 76 | | |
| Panel (3 of 3) | | Yes | 6 | 6 | 0.46 (0.19-0.75) | 0.93 (0.86-0.98) |
| | | No | 7 | 83 | | |
| ≥ 0.018 | AccuTnI (enhanced) | Yes | 10 | 32 | 0.77 (0.46-0.95) | 0.64 (0.53-0.74) |
| | | No | 3 | 57 | | |
| | AccuTnI (enhanced) & Panel (Creatinine) | Yes | 8 | 8 | 0.62 (0.32-0.86) | 0.91 (0.83-0.96) |
| | | No | 5 | 81 | | |
| | AccuTnI (enhanced) & Panel (ROC) | Yes | 10 | 12 | 0.77 (0.46-0.95) | 0.87 (0.78-0.93) |
| | | No | 3 | 77 | | |
| | AccuTnI (enhanced) & Panel (3 of 3) | Yes | 5 | 4 | 0.39 (0.14-0.68) | 0.96 (0.89-0.99) |
| | | No | 8 | 85 | | |
| | AccuTnI (enhanced) & Panel (bFGF) | Yes | 8 | 7 | 0.62 (0.32-0.86) | 0.92 (0.85-0.97) |
| | | No | 5 | 82 | | |
| | AccuTnI (enhanced) & FABP-3 | Yes | 9 | 14 | 0.69 (0.39-0.91) | 0.84 (0.75-0.91) |
| | | No | 4 | 75 | | |
| ≥ 0.04 | AccuTnI (enhanced) | Yes | 8 | 13 | 0.62 (0.32-0.86) | 0.85 (0.76-0.92) |
| | | No | 5 | 76 | | |
| | AccuTnI (enhanced) & Panel (Creatinine) | Yes | 6 | 2 | 0.46 (0.19-0.75) | 0.98 (0.92-1.00) |
| | | No | 7 | 87 | | |
| | AccuTnI (enhanced) & Panel (ROC) | Yes | 8 | 5 | 0.62 (0.32-0.86) | 0.94 (0.87-0.98) |
| | | No | 5 | 84 | | |
| | AccuTnI (enhanced) & FABP-3 | Yes | 7 | 5 | 0.54 (0.25-0.81) | 0.94 (0.87-0.98) |
| | | No | 6 | 84 | | |
| | AccuTnI (enhanced) & Panel (bFGF) | Yes | 7 | 2 | 0.54 (0.25-0.81) | 0.98 (0.92-1.00) |
| | | No | 6 | 87 | | |
| | AccuTnI (enhanced) & Panel (3 of 3) | Yes | 5 | 1 | 0.39 (0.14-0.68) | 0.99 (0.94-1.00) |
| | | No | 8 | 88 | | |
| ≥ 0.05 | AccuTnI (enhanced) | Yes | 7 | 9 | 0.54 (0.25-0.81) | 0.90 (0.82-0.95) |
| | | No | 6 | 80 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | AccuTnI (enhanced) & Panel (ROC) | Yes | 7 | 4 | 0.54 (0.25-0.81) | 0.96 (0.89-0.99) |
| | | No | 6 | 85 | | |
| ≥ 0.018 | AccuTnI (regular) | Yes | 10 | 33 | 0.77 (0.46-0.95) | 0.63 (0.52-0.73) |
| | | No | 3 | 56 | | |
| | AccuTnI (regular) & Panel (ROC) | Yes | 10 | 13 | 0.77 (0.46-0.95) | 0.85 (0.76-0.92) |
| | | No | 3 | 76 | | |
| | AccuTnI (regular) & Panel (3 of 3) | Yes | 5 | 4 | 0.38 (0.14-0.68) | 0.96 (0.89-0.99) |
| | | No | 8 | 85 | | |
| | AccuTnI (regular) & Panel (bFGF) | Yes | 8 | 7 | 0.62 (0.32-0.86) | 0.92 (0.85-0.97) |
| | | No | 5 | 82 | | |
| | AccuTnI (regular) & FABP-3 | Yes | 9 | 14 | 0.69 (0.39-0.91) | 0.84 (0.75-0.91) |
| | | No | 4 | 75 | | |
| | AccuTnI (regular) & Panel (Creatinine) | Yes | 8 | 8 | 0.62 (0.32-0.86) | 0.91 (0.83-0.96) |
| | | No | 5 | 81 | | |
| ≥ 0.04 | AccuTnI (regular) | Yes | 8 | 12 | 0.62 (0.32-0.86) | 0.87 (0.78-0.93) |
| | | No | 5 | 77 | | |
| | AccuTnI (regular) & Panel (ROC) | Yes | 8 | 6 | 0.62 (0.32-0.86) | 0.93 (0.86-0.98) |
| | | No | 5 | 83 | | |
| | AccuTnI (regular) & Panel (Creatinine) | Yes | 6 | 3 | 0.46 (0.19-0.75) | 0.97 (0.91-0.99) |
| | | No | 7 | 86 | | |
| | AccuTnI (regular) & FABP-3 | Yes | 7 | 5 | 0.54 (0.25-0.81) | 0.94 (0.87-0.98) |
| | | No | 6 | 84 | | |
| | AccuTnI (regular) & Panel (bFGF) | Yes | 7 | 3 | 0.54 (0.25-0.81) | 0.97 (0.91-0.99) |
| | | No | 6 | 86 | | |
| | AccuTnI (regular) & Panel (3 of 3) | Yes | 5 | 2 | 0.38 (0.14-0.68) | 0.98 (0.92-1.00) |
| | | No | 8 | 87 | | |
| ≥ 0.05 | AccuTnI (regular) | Yes | 6 | 8 | 0.46 (0.19-0.75) | 0.91 (0.83-0.96) |
| | | No | 7 | 81 | | |
| | AccuTnI (regular) & Panel (ROC) | Yes | 6 | 5 | 0.46 (0.19-0.75) | 0.94 (0.87-0.98) |
| | | No | 7 | 84 | | |
| ≥ 10 | hs-cTnI | Yes | 12 | 38 | 0.92 (0.64-1.00) | 0.57 (0.46-0.68) |
| | | No | 1 | 51 | | |
| | hs-cTnI & Panel (Creatinine) | Yes | 9 | 11 | 0.69 (0.39-0.91) | 0.88 (0.79-0.94) |
| | | No | 4 | 78 | | |
| | hs-cTnI & Panel (ROC) | Yes | 11 | 17 | 0.85 (0.55-0.98) | 0.81 (0.71-0.89) |
| | | No | 2 | 72 | | |
| | hs-cTnI & Panel (3 of 3) | Yes | 6 | 6 | 0.46 (0.19-0.75) | 0.93 (0.86-0.98) |
| | | No | 7 | 83 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | hs-cTnI & Panel (bFGF) | Yes | 9 | 10 | 0.69 (0.39-0.91) | 0.89 (0.80-0.95) |
| | | No | 4 | 79 | | |
| | hs-cTnI & FABP-3 | Yes | 10 | 18 | 0.77 (0.46-0.95) | 0.80 (0.70-0.88) |
| | | No | 3 | 71 | | |
| ≥ 19 | hs-cTnI | Yes | 8 | 18 | 0.62 (0.32-0.86) | 0.80 (0.70-0.88) |
| | | No | 5 | 71 | | |
| | hs-cTnI & Panel (Creatinine) | Yes | 6 | 4 | 0.46 (0.19-0.75) | 0.96 (0.89-0.99) |
| | | No | 7 | 85 | | |
| | hs-cTnI & Panel (ROC) | Yes | 8 | 7 | 0.62 (0.32-0.86) | 0.92 (0.85-0.97) |
| | | No | 5 | 82 | | |
| | hs-cTnI & FABP-3 | Yes | 7 | 8 | 0.54 (0.25-0.81) | 0.91 (0.83-0.96) |
| | | No | 6 | 81 | | |
| | hs-cTnI & Panel (bFGF) | Yes | 7 | 3 | 0.54 (0.25-0.81) | 0.97 (0.91-0.99) |
| | | No | 6 | 86 | | |
| | hs-cTnI & Panel (3 of 3) | Yes | 5 | 2 | 0.39 (0.14-0.68) | 0.98 (0.92-1.00) |
| | | No | 8 | 87 | | |
| ≥ 38 | hs-cTnI | Yes | 7 | 7 | 0.54 (0.25-0.81) | 0.92 (0.85-0.97) |
| | | No | 6 | 82 | | |
| | hs-cTnI & Panel (ROC) | Yes | 7 | 3 | 0.54 (0.25-0.81) | 0.97 (0.91-0.99) |
| | | No | 6 | 86 | | |
| ≥ 14 | hs-cTnT | Yes | 11 | 40 | 0.85 (0.55-0.98) | 0.55 (0.44-0.66) |
| | | No | 2 | 49 | | |
| | hs-cTnT & Panel (Creatinine) | Yes | 8 | 11 | 0.62 (0.32-0.86) | 0.88 (0.79-0.94) |
| | | No | 5 | 78 | | |
| | hs-cTnT & Panel (ROC) | Yes | 10 | 16 | 0.77 (0.46-0.95) | 0.82 (0.72-0.89) |
| | | No | 3 | 73 | | |
| | hs-cTnT & Panel (3 of 3) | Yes | 5 | 5 | 0.39 (0.14-0.68) | 0.94 (0.87-0.98) |
| | | No | 8 | 84 | | |
| | hs-cTnT & FABP-3 | Yes | 9 | 18 | 0.69 (0.39-0.91) | 0.80 (0.70-0.88) |
| | | No | 4 | 71 | | |
| | hs-cTnT & Panel (bFGF) | Yes | 8 | 9 | 0.62 (0.32-0.86) | 0.90 (0.82-0.95) |
| | | No | 5 | 80 | | |
| ≥ 32 | hs-cTnT | Yes | 6 | 15 | 0.46 (0.19-0.75) | 0.83 (0.74-0.90) |
| | | No | 7 | 74 | | |
| | hs-cTnT & Panel (ROC) | Yes | 6 | 5 | 0.46 (0.19-0.75) | 0.94 (0.87-0.98) |
| | | No | 7 | 84 | | |
| | hs-cTnT & Panel (Creatinine) | Yes | 6 | 4 | 0.46 (0.19-0.75) | 0.96 (0.89-0.99) |
| | | No | 7 | 85 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | hs-cTnT & FABP-3 | Yes | 6 | 9 | 0.46 (0.19-0.75) | 0.90 (0.82-0.95) |
| | | No | 7 | 80 | | |
| | hs-cTnT & Panel (bFGF) | Yes | 6 | 4 | 0.46 (0.19-0.75) | 0.96 (0.89-0.99) |
| | | No | 7 | 85 | | |
| | hs-cTnT & Panel (3 of 3) | Yes | 5 | 3 | 0.39 (0.14-0.68) | 0.97 (0.91-0.99) |
| | | No | 8 | 86 | | |
| ≥ 35 | hs-cTnT | Yes | 6 | 14 | 0.46 (0.19-0.75) | 0.84 (0.75-0.91) |
| | | No | 7 | 75 | | |
| | hs-cTnT & Panel (ROC) | Yes | 6 | 4 | 0.46 (0.19-0.75) | 0.96 (0.89-0.99) |
| | | No | 7 | 85 | | |
| ≥ 0.01 | cTnT | Yes | 8 | 18 | 0.62 (0.32-0.86) | 0.80 (0.70-0.88) |
| | | No | 5 | 71 | | |
| | cTnT & Panel (Creatinine) | Yes | 6 | 4 | 0.46 (0.19-0.75) | 0.96 (0.89-0.99) |
| | | No | 7 | 85 | | |
| | cTnT & Panel (ROC) | Yes | 8 | 6 | 0.62 (0.32-0.86) | 0.93 (0.86-0.98) |
| | | No | 5 | 83 | | |
| | cTnT & FABP-3 | Yes | 7 | 9 | 0.54 (0.25-0.81) | 0.90 (0.82-0.95) |
| | | No | 6 | 80 | | |
| | cTnT & Panel (bFGF) | Yes | 7 | 4 | 0.54 (0.25-0.81) | 0.96 (0.89-0.99) |
| | | No | 6 | 85 | | |
| | cTnT & Panel (3 of 3) | Yes | 5 | 2 | 0.39 (0.14-0.68) | 0.98 (0.92-1.00) |
| | | No | 8 | 87 | | |
| > 0.03 | cTnT | Yes | 6 | 3 | 0.46 (0.19-0.75) | 0.97 (0.91-0.99) |
| | | No | 7 | 86 | | |
| | cTnT & Panel (ROC) | Yes | 6 | 0 | 0.46 (0.19-0.75) | 1.00 (0.96-1.00) |
| | | No | 7 | 89 | | |
| | cTnT & Panel (Creatinine) | Yes | 5 | 0 | 0.39 (0.14-0.68) | 1.00 (0.96-1.00) |
| | | No | 8 | 89 | | |
| | cTnT & Panel (bFGF) | Yes | 5 | 0 | 0.39 (0.14-0.68) | 1.00 (0.96-1.00) |
| | | No | 8 | 89 | | |
| | cTnT & FABP-3 | Yes | 6 | 2 | 0.46 (0.19-0.75) | 0.98 (0.92-1.00) |
| | | No | 7 | 87 | | |
| | cTnT & Panel (3 of 3) | Yes | 4 | 0 | 0.31 (0.09-0.61) | 1.00 (0.96-1.00) |
| | | No | 9 | 89 | | |
| > 0.10 | cTnT | Yes | 0 | 2 | <0.01 (<0.01-0.25) | 0.98 (0.92-1.00) |
| | | No | 13 | 87 | | |
| | cTnT & Panel (ROC) | Yes | 0 | 0 | <0.01 | <0.01 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | No | 13 | 89 | (<0.01-0.25) | (<0.01-0.25) |

**Table 3.5 -** Sensitivity and specificity of various combinations of biomarker panels when used in conjunction with cardiac troponin (requiring both a positive panel and a positive troponin for a positive test result). Panel (ROC-derived): sFlt-1, NT-proBNP, MCP-1; Panel (Creatinine): sFlt-1, NT-proBNP, MCP-1, Creatinine; Panel (FABP-3): H-FABP, NT-proBNP, sFlt-1; Panel (bFGF): sFlt-1, MCP-1, NT-proBNP, bFGF; Panel (3 of 3): s-Flt-1, NT-proBNP, MCP-1.

| **Test** | | | **Outcome** | | **Sensitivity (95% CI)** | **Specificity (95% CI)** |
|---|---|---|---|---|---|---|
| | | | Yes | No | | |
| Panel (ROC-derived) | | Yes | 12 | 29 | 0.92 (0.64-1.00) | 0.67 (0.57-0.77) |
| | | No | 1 | 60 | | |
| Panel (Creatinine) | | Yes | 10 | 14 | 0.77 (0.46-0.95) | 0.84 (0.75-0.91) |
| | | No | 3 | 75 | | |
| Panel (FABP-3) | | Yes | 10 | 21 | 0.77 (0.46-0.95) | 0.76 (0.66-0.85) |
| | | No | 3 | 68 | | |
| Panel (bFGF) | | Yes | 9 | 13 | 0.69 (0.39-0.91) | 0.85 (0.76-0.92) |
| | | No | 4 | 76 | | |
| Panel (3 of 3) | | Yes | 6 | 6 | 0.46 (0.19-0.75) | 0.93 (0.86-0.98) |
| | | No | 7 | 83 | | |
| ≥ 0.018 | AccuTnI (enhanced) | Yes | 10 | 32 | 0.77 (0.46-0.95) | 0.64 (0.53-0.74) |
| | | No | 3 | 57 | | |
| | AccuTnI (enhanced) OR Panel (Creatinine) | Yes | 12 | 38 | 0.92 (0.64-1.00) | 0.57 (0.46-0.68) |
| | | No | 1 | 51 | | |
| | AccuTnI (enhanced) OR Panel (ROC) | Yes | 12 | 49 | 0.92 (0.64-1.00) | 0.45 (0.34-0.56) |
| | | No | 1 | 40 | | |
| | AccuTnI (enhanced) OR Panel (3 of 3) | Yes | 11 | 34 | 0.86 (0.55-0.98) | 0.62 (0.51-0.72) |
| | | No | 2 | 55 | | |
| | AccuTnI (enhanced) OR Panel (bFGF) | Yes | 11 | 38 | 0.85 (0.55-0.98) | 0.57 (0.46-0.68) |
| | | No | 2 | 51 | | |
| | AccuTnI (enhanced) OR FABP-3 | Yes | 11 | 39 | 0.85 (0.55-0.98) | 0.56 (0.45-0.67) |
| | | No | 2 | 50 | | |
| ≥ 0.04 | AccuTnI (enhanced) | Yes | 8 | 13 | 0.62 (0.32-0.86) | 0.85 (0.76-0.92) |
| | | No | 5 | 76 | | |
| | AccuTnI (enhanced) OR Panel (Creatinine) | Yes | 12 | 25 | 0.92 (0.64-1.00) | 0.72 (0.61-0.81) |
| | | No | 1 | 64 | | |
| | AccuTnI (enhanced) OR Panel | Yes | 12 | 37 | 0.92 | 0.58 |

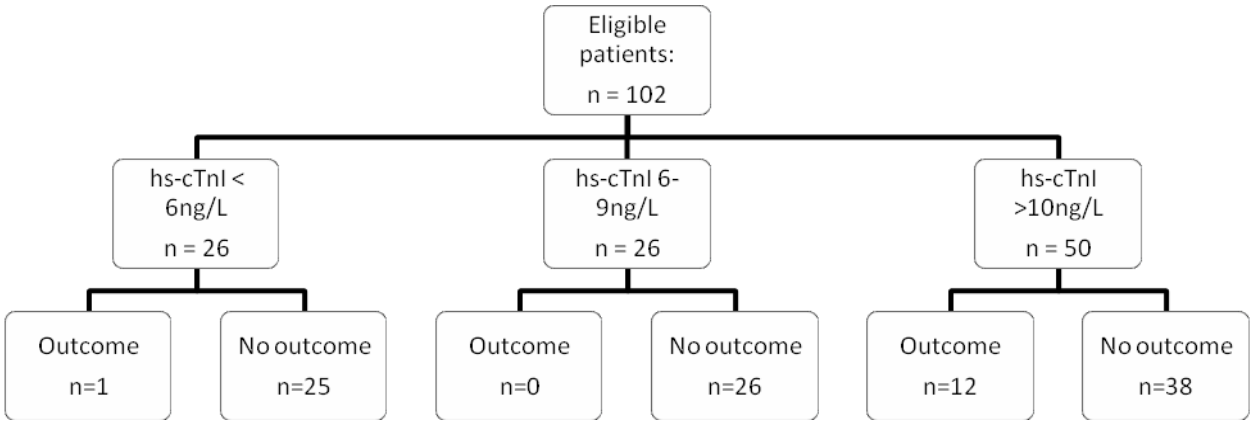
| | | | | | | |
|---|---|---|---|---|---|---|
| | (ROC) | No | 1 | 52 | (0.64-1.00) | (0.48-0.67) |
| | AccuTnI (enhanced) OR FABP-3 | Yes | 11 | 29 | 0.85 (0.55-0.98) | 0.67 (0.57-0.77) |
| | | No | 2 | 60 | | |
| | AccuTnI (enhanced) OR Panel (bFGF) | Yes | 10 | 24 | 0.77 (0.46-0.95) | 0.73 (0.63-0.82) |
| | | No | 3 | 65 | | |
| | AccuTnI (enhanced) OR Panel (3 of 3) | Yes | 9 | 18 | 0.69 (0.39-0.91) | 0.80 (0.70-0.88) |
| | | No | 4 | 71 | | |
| ≥ 0.05 | AccuTnI (enhanced) | Yes | 7 | 9 | 0.54 (0.25-0.81) | 0.90 (0.82-0.95) |
| | | No | 6 | 80 | | |
| | AccuTnI (enhanced) OR Panel (ROC) | Yes | 12 | 34 | 0.92 (0.64-1.00) | 0.62 (0.51-0.72) |
| | | No | 1 | 55 | | |
| ≥ 0.018 | AccuTnI (regular) | Yes | 10 | 33 | 0.77 (0.46-0.95) | 0.63 (0.52-0.73) |
| | | No | 3 | 56 | | |
| | AccuTnI (regular) OR Panel (ROC) | Yes | 12 | 49 | 0.92 (0.64-1.00) | 0.45 (0.34-0.56) |
| | | No | 1 | 40 | | |
| | AccuTnI (regular) OR Panel (3 of 3) | Yes | 11 | 35 | 0.85 (0.55-0.98) | 0.61 (0.50-0.71) |
| | | No | 2 | 54 | | |
| | AccuTnI (regular) OR Panel (bFGF) | Yes | 11 | 39 | 0.85 (0.55-0.98) | 0.56 (0.45-0.67) |
| | | No | 2 | 50 | | |
| | AccuTnI (regular) OR FABP-3 | Yes | 11 | 40 | 0.85 (0.55-0.98) | 0.55 (0.44-0.66) |
| | | No | 2 | 49 | | |
| | AccuTnI (regular) OR Panel (Creatinine) | Yes | 8 | 8 | 0.62 (0.32-0.86) | 0.91 (0.83-0.96) |
| | | No | 5 | 81 | | |
| ≥ 0.04 | AccuTnI (regular) | Yes | 8 | 12 | 0.62 (0.32-0.86) | 0.87 (0.78-0.93) |
| | | No | 5 | 77 | | |
| | AccuTnI (regular) OR Panel (ROC) | Yes | 12 | 35 | 0.92 (0.64-1.00) | 0.61 (0.50-0.71) |
| | | No | 1 | 54 | | |
| | AccuTnI (regular) OR Panel (Creatinine) | Yes | 12 | 39 | 0.92 (0.64-1.00) | 0.56 (0.45-0.67) |
| | | No | 1 | 50 | | |
| | AccuTnI (regular) OR FABP-3 | Yes | 11 | 28 | 0.85 (0.55-0.98) | 0.69 (0.58-0.78) |
| | | No | 2 | 61 | | |
| | AccuTnI (regular) OR Panel (bFGF) | Yes | 10 | 22 | 0.77 (0.46-0.95) | 0.75 (0.65-0.84) |
| | | No | 3 | 67 | | |
| | AccuTnI (regular) OR Panel (3 of 3) | Yes | 9 | 16 | 0.69 (0.39-0.91) | 0.82 (0.73-0.89) |
| | | No | 4 | 73 | | |
| ≥ 0.05 | AccuTnI (regular) | Yes | 6 | 8 | 0.46 (0.19-0.75) | 0.91 (0.83-0.96) |
| | | No | 7 | 81 | | |
| | AccuTnI (regular) OR Panel | Yes | 12 | 32 | 0.92 | 0.64 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | (ROC) | No | 1 | 57 | (0.64-1.00) | (0.53-0.74) |
| ≥ 10 | hs-cTnI | Yes | 12 | 38 | 0.92 (0.64-1.00) | 0.57 (0.46-0.68) |
| | | No | 1 | 51 | | |
| | hs-cTnI OR Panel (Creatinine) | Yes | 13 | 41 | 1.00 (0.75-1.00) | 0.54 (0.43-0.65) |
| | | No | 0 | 48 | | |
| | hs-cTnI OR Panel (ROC) | Yes | 13 | 50 | 1.00 (0.76-1.00) | 0.44 (0.33-0.55) |
| | | No | 0 | 39 | | |
| | hs-cTnI OR Panel (3 of 3) | Yes | 12 | 38 | 0.92 (0.64-1.00) | 0.57 (0.46-0.68) |
| | | No | 1 | 51 | | |
| | hs-cTnI OR Panel (bFGF) | Yes | 12 | 41 | 0.92 (0.64-1.00) | 0.54 (0.43-0.65) |
| | | No | 1 | 48 | | |
| | hs-cTnI OR FABP-3 | Yes | 12 | 41 | 0.92 (0.64-1.00) | 0.54 (0.43-0.65) |
| | | No | 1 | 48 | | |
| ≥ 19 | hs-cTnI | Yes | 8 | 18 | 0.62 (0.32-0.86) | 0.80 (0.70-0.88) |
| | | No | 5 | 71 | | |
| | hs-cTnI OR Panel (Creatinine) | Yes | 12 | 28 | 0.92 (0.64-1.00) | 0.69 (0.58-0.78) |
| | | No | 1 | 61 | | |
| | hs-cTnI OR Panel (ROC) | Yes | 12 | 40 | 0.92 (0.64-1.00) | 0.55 (0.44-0.66) |
| | | No | 1 | 49 | | |
| | hs-cTnI OR FABP-3 | Yes | 11 | 31 | 0.85 (0.55-0.98) | 0.65 (0.54-0.75) |
| | | No | 2 | 58 | | |
| | hs-cTnI OR Panel (bFGF) | Yes | 10 | 28 | 0.77 (0.46-0.95) | 0.69 (0.58-0.78) |
| | | No | 3 | 61 | | |
| | hs-cTnI OR Panel (3 of 3) | Yes | 9 | 22 | 0.69 (0.39-0.91) | 0.75 (0.65-0.84) |
| | | No | 4 | 67 | | |
| ≥ 38 | hs-cTnI | Yes | 7 | 7 | 0.54 (0.25-0.81) | 0.92 (0.85-0.97) |
| | | No | 6 | 82 | | |
| | hs-cTnI OR Panel (ROC) | Yes | 12 | 33 | 0.92 (0.64-1.00) | 0.63 (0.52-0.73) |
| | | No | 1 | 56 | | |
| ≥ 14 | hs-cTnT | Yes | 11 | 40 | 0.85 (0.55-0.98) | 0.55 (0.44-0.66) |
| | | No | 2 | 49 | | |
| | hs-cTnT OR Panel (Creatinine) | Yes | 13 | 43 | 1.00 (0.75-1.00) | 0.52 (0.41-0.62) |
| | | No | 0 | 46 | | |
| | hs-cTnT OR Panel (ROC) | Yes | 13 | 53 | 1.00 (0.76-1.00) | 0.40 (0.30-0.51) |
| | | No | 0 | 36 | | |
| | hs-cTnT OR Panel (3 of 3) | Yes | 12 | 41 | 0.92 (0.64-1.00 | 0.54 (0.43-0.65) |
| | | No | 1 | 48 | | |
| | hs-cTnT OR FABP-3 | Yes | 12 | 43 | 0.92 (0.64-1.00) | 0.52 (0.41-0.62) |
| | | No | 1 | 46 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | hs-cTnT OR Panel (bFGF) | Yes | 12 | 44 | 0.92 (0.64-1.00) | 0.51 (0.40-0.61) |
| | | No | 1 | 45 | | |
| ≥ 32 | hs-cTnT | Yes | 6 | 15 | 0.46 (0.19-0.75) | 0.83 (0.74-0.90) |
| | | No | 7 | 74 | | |
| | hs-cTnT OR Panel (ROC) | Yes | 12 | 39 | 0.92 (0.64-1.00) | 0.57 (0.45-0.67) |
| | | No | 1 | 50 | | |
| | hs-cTnT OR Panel (Creatinine) | Yes | 10 | 25 | 0.77 (0.46-0.95) | 0.72 (0.61-0.81) |
| | | No | 3 | 64 | | |
| | hs-cTnT OR FABP-3 | Yes | 10 | 27 | 0.77 (0.46-0.95) | 0.70 (0.59-0.79) |
| | | No | 3 | 62 | | |
| | hs-cTnT OR Panel (bFGF) | Yes | 9 | 24 | 0.69 (0.39-0.91) | 0.73 (0.63-0.82) |
| | | No | 4 | 65 | | |
| | hs-cTnT OR Panel (3 of 3) | Yes | 7 | 18 | 0.54 (0.25-0.81) | 0.80 (0.70-0.88) |
| | | No | 6 | 71 | | |
| ≥ 35 | hs-cTnT | Yes | 6 | 14 | 0.46 (0.19-0.75) | 0.84 (0.75-0.91) |
| | | No | 7 | 75 | | |
| | hs-cTnT OR Panel (ROC) | Yes | 12 | 39 | 0.92 (0.64-1.00) | 0.57 (0.45-0.67) |
| | | No | 1 | 50 | | |
| ≥ 0.010 | cTnT | Yes | 8 | 18 | 0.62 (0.32-0.86) | 0.80 (0.70-0.88) |
| | | No | 5 | 71 | | |
| | cTnT OR Panel (Creatinine) | Yes | 12 | 28 | 0.92 (0.64-1.00) | 0.69 (0.58-0.78) |
| | | No | 1 | 61 | | |
| | cTnT OR Panel (ROC) | Yes | 12 | 41 | 0.92 (0.64-1.00) | 0.54 (0.43-0.65)] |
| | | No | 1 | 48 | | |
| | cTnT OR FABP-3 | Yes | 11 | 30 | 0.85 (0.55-0.98) | 0.66 (0.56-0.76) |
| | | No | 2 | 59 | | |
| | cTnT OR Panel (bFGF) | Yes | 10 | 27 | 0.77 (0.46-0.95) | 0.70 (0.59-0.79) |
| | | No | 3 | 62 | | |
| | cTnT OR Panel (3 of 3) | Yes | 9 | 22 | 0.69 (0.39-0.91) | 0.75 (0.65-0.84) |
| | | No | 4 | 67 | | |
| > 0.03 | cTnT | Yes | 6 | 3 | 0.46 (0.19-0.75) | 0.97 (0.91-0.99) |
| | | No | 7 | 86 | | |
| | cTnT OR Panel (ROC) | Yes | 12 | 32 | 0.92 (0.64-1.00) | 0.64 (0.53-0.74) |
| | | No | 1 | 57 | | |
| | cTnT OR Panel (Creatinine) | Yes | 11 | 17 | 0.85 (0.55-0.98) | 0.81 (0.71-0.89) |
| | | No | 2 | 72 | | |
| | cTnT OR Panel (bFGF) | Yes | 10 | 16 | 0.77 (0.46-0.95) | 0.82 (0.73-0.89) |
| | | No | 3 | 73 | | |
| | cTnT OR FABP-3 | Yes | 10 | 22 | 0.77 | 0.75 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | No | 3 | 67 | (0.46-0.95) | (0.65-0.84) |
| | cTnT OR Panel (3 of 3) | Yes | 8 | 9 | 0.62 (0.32-0.86) | 0.90 (0.82-0.95) |
| | | No | 5 | 80 | | |
| > 0.10 | cTnT | Yes | 0 | 2 | <0.01 (<0.01-0.25) | 0.98 (0.92-1.00) |
| | | No | 13 | 87 | | |
| | cTnT OR Panel (ROC) | Yes | 12 | 31 | 0.92 (0.64-1.00) | 0.65 (0.54-0.75) |
| | | No | 1 | 58 | | |

**Table 3.6**- Sensitivity and specificity of various combinations of biomarker panels when used in conjunction with cardiac troponin (requiring either a positive panel or a positive troponin for a positive test result). Panel (ROC-derived): sFlt-1, NT-proBNP, MCP-1; Panel (Creatinine): sFlt-1, NT-proBNP, MCP-1, Creatinine; Panel (FABP-3): H-FABP, NT-proBNP, sFlt-1; Panel (bFGF): sFlt-1, MCP-1, NT-proBNP, bFGF; Panel (3 of 3): s-Flt-1, NT-proBNP, MCP-1.



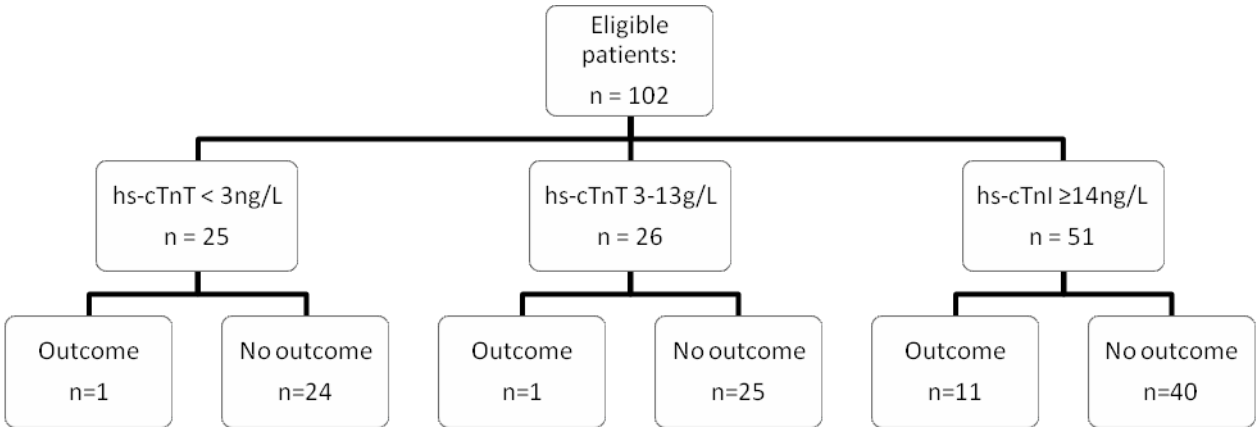

**Panel:** sFlt-1, NT-proBNP, MCP-1, Creatinine (3 out of 4 positive)

| Panel | Outcome: Yes | Outcome: No |
|---|---|---|
| Yes | 1 | 0 |
| No | 0 | 25 |

| Panel | Outcome: Yes | Outcome: No |
|---|---|---|
| Yes | 0 | 3 |
| No | 0 | 23 |

| Panel | Outcome: Yes | Outcome: No |
|---|---|---|
| Yes | 9 | 11 |
| No | 3 | 27 |

**Figure 3.3** – Flow chart depicting biomarker panel performance on patient cohorts separated by high-sensitivity cardiac troponin I values




**Panel B** sFlt-1, NT-proBNP, MCP-1, Creatinine (3 out of 4 markers)

| | | Outcome | |
|---|---|---|---|
| **Panel** | | Yes | No |
| | Yes | 1 | 1 |
| | No | 0 | 23 |

| | Outcome | |
|---|---|---|
| | Yes | No |
| Yes | 1 | 2 |
| No | 0 | 23 |

| | Outcome | |
|---|---|---|
| | Yes | No |
| Yes | 8 | 11 |
| No | 3 | 29 |

**Figure 3.4** - Flow chart depicting biomarker panel performance on patient cohorts separated by high-sensitivity cardiac troponin T values

## 3.3 DISCUSSION

Chest pain is one of chief complaints by patients presenting to the ED. However, a time delay of up to nine hours may be required before a decision is made on their care (10). Here, the examination of serum samples from an all-comer population with suspected ACS revealed that a panel of four biomarkers, in conjunction with cardiac troponin, was more efficient in identifying high-risk patients than cardiac troponin alone.

The combination of sFlt-1, creatinine, MCP-1, and NT-proBNP, when used to compliment hs-cTnI or hs-cTnT at presentation, predicted cardiac outcomes at 72 hours with a sensitivity of 100% (95% CI: 75-100%) and specificity of 54% (95% CI: 43-65%) and 52% (95% CI: 41-62%), respectively. This may address an unmet clinical need by reducing the time required to assess patients in the emergency setting.

The improved prediction of risk may be explained by the roles of each biomarker in the pathophysiology of cardiovascular disease. The biomarkers identified in the panel relay physiologically independent information. By using indicators involved in different processes that lead up to an MI or adverse outcomes, the panel may provide a more comprehensive picture of a patient's risk than with the use of cardiac troponin alone. MCP-1, as an inflammatory cytokine, plays a crucial role in the progression of plaque build-up. Angiogenic factors such as sFlt-1 are early indicators of vascular injury. As a natriuresis marker, high levels of NT-proBNP are indicators of stress on the myocardium. Finally, poor renal function, as reflected by high levels of creatinine or low levels of estimated glomerular filtration rate (eGFR), in the ACS setting has been associated with greater cardiovascular risk. The pressure and volume overload, vascular calcifications, increased sympathetic nervous system activity, oxidative stress, microinflammatory state and the increased levels of homocysteine associated with renal impairment are all potent cardiovascular risk factors (73). While separate reference ranges for creatinine have been established for males (64-104 µmol/L) and females (49-90µmol/L), a single cut-off of 90 µmol/L was used in these analyses (74). Also, it should be noted that eGFR

is often the preferred marker used to identify renal insufficiency since the utility of creatinine can be hindered by covariations in gender, age, race, diet, and muscle mass of each patient (75,76). However, the decision to exclude eGFR in the panel was based on the lower ROC AUC and that no differences have been found between the ability of creatinine and eGFR in identifying adverse cardiac events in patients with ACS (77).

While glucose had the highest AUC among all analytes assessed, it was not incorporated into the final panel since its inclusion did not improve the panel's performance and because glucose is not involved in any physiological processes directly related to ACS. Although H-FABP had the largest AUC among the early ACS biomarkers, its use in conjunction with cardiac troponins did not provide superior incremental benefit to the panel. Other groups have published contradicting reports on this marker in similar populations with some denying its diagnostic utility (78,79) and another supporting the higher performance of H-FABP compared to cardiac troponin (80). Therefore, further investigation will be required to fully assess its clinical role in the ACS setting. VEGF was excluded from panel analyses due to its release in the blood clotting process making serum a suboptimal matrix to measure VEGF. Furthermore, its inclusion would not provide additional physiological information as its receptor is sFlt-1, a marker with a higher AUC in these analyses was used.

This biomarker panel can be incorporated into decision making in various ways. It was first evaluated as a test assessed at admission requiring either a positive cTn test or a positive panel test for the patient to be deemed high-risk. This approach identified

four individuals that were overlooked by cTnT, two by AccuTnI (enhanced), one by hs-cTnI, and two by hs-cTnT. Alternatively, the biomarkers were also assessed for their efficacy when assessing patients in different groups based on cTn levels. In a recent study by Body and colleagues (2011), the lower limit of detection of hs-cTnT was used as an effective early rule-out cutoff for acute MI (81). Using this strategy, one individual in the CTS study who presented with both levels of hs-cTnI and hs-cTnT below the limit of detection would have been ruled-out. With the biomarker panel, however, this patient tested positive and would not have been missed.

The results reported here are limited by the small population evaluated and low incidence of outcomes. With a population of 102, only 13 patients had an outcome. A population with more outcomes will provide strength to these results. Moreover, since the study enrolled an all-comer population it is unknown how this panel would perform in patients presenting early after onset of chest pain to the ED.

# CHAPTER 4: SUMMARY

Here it was determined that while multiplex immunoassays carry greater imprecision than singleplex platforms, with proper validation and optimization, they represent an attractive tool for investigational purposes. Upon comparison several analytes across the platforms it was revealed that the consistency of biomarker detection across platforms is dependent on the biomarker of interest with IL-6 having the best agreement between the MSD, Randox, and Beckman systems.

Furthermore, an early four-biomarker panel (sFlt-1, creatinine, NT-proBNP, and MCP-1) incorporating physiologically independent markers of ACS was more effective in identifying ED patients at risk for cardiac outcomes than cTn alone.

Future work in the validation of multiplex immunoassays should involve more rigorous evaluations using a larger sample size with diverse patient populations. Such a process should also expand on comparisons of intra- and inter-assay precision, recoverability, linearity, and cross-reactivity among more cardiac markers. Finally, optimization of the manufacturing process, antibody selection, and detection method would extend the utility of this technology.

It is also important to assess the efficacy of the biomarker panel on a separate population such as that in the RING study. With the inclusion criteria of presenting to the ED within six hours of chest pain onset, this population strictly includes early presenters and may therefore serve as a suitable validation group. Ultimately, large

multicenter studies would better reveal whether or not early biomarkers could complement cardiac troponin in identifying patients at risk for cardiac outcomes. With consideration for the variability observed in this study between immunoassays, the same platform or platforms with similar performances should be used in comparing such results. Additionally, the MSD assay protocols required at least 3 hours to perform the test. In the clinical setting, a much faster turn-around time is required to provide adequate care for patients. Therefore, an evaluation of this biomarker panel using point-of-care testing may prove to be beneficial in the clinical setting.

Evaluating the inclusion of other novel cardiac biomarkers within a panel is also warranted. Arginine-vasopressin (AVP) is a neurohypophyseal hormone that plays a role in osmotic homeostasis and is released in response to stress. The short half-life, platelet binding, and variation between assays, make the reliable measurement of AVP difficult (82,83). Therefore, the detection of co-peptin, a C-terminal segment of the AVP precursor, is preferred. The early biomarker is more stable and is released in stoichiometric levels relative to AVP. It has been previously shown in two multicenter studies that when used in conjunction with cardiac troponin, co-peptin had a greater AUC than cardiac troponin alone (84,85). With the increased interest in biomarker testing and the ever-evolving multiplex immunoassay technology, other cardiac novel biomarkers will surely surface which may prove useful in assisting cardiac troponin in identifying high-risk cardiac patients.

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