CARDIAC AND GLYCEMIC BIOMARKERS FOR EARLY DECISION MAKING

THE COMBINATION OF CARDIOVASCULAR AND GLYCEMIC BIOMARKERS FOR EARLY DECISION MAKING IN PATIENTS PRESENTING TO THE EMERGENCY DEPARTMENT WITH SYMPTOMS OF ACUTE CORONARY SYNDROME

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TITLE: The combination of cardiovascular and glycemic biomarkers for early decision making in patients presenting to the emergency department with symptoms of acute coronary syndrome

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Lay Abstract

Myocardial ischemia is a reduction in coronary blood flow that is insufficient for heart cell demand, which can lead to myocardial injury and cell death. Acute Coronary Syndrome (ACS) encompasses three clinical presentations of myocardial ischemia: ST-elevation MI (STEMI), non-STEMI (NSTEMI) and unstable angina (UA). Current guidelines recommend using electrocardiogram (ECG) findings and multiple cardiac troponin (cTn) measurements over several hours to diagnose (rule-in) or rule-out ACS in the emergency department (ED). However, given these recommendations patients may spend several hours in the ED, consuming valuable time and resources.

This project explores the use of glycemic biomarkers [e.g., glucose and haemoglobin A1c] in combination with cTn to rule-in/rule-out MI and other major cardiovascular events (MACE) to facilitate early decision-making in the ED. This thesis demonstrates that a combination of cTn and glucose at presentation is both an efficient and cost-effective tool for early decision-making in the ED.

Abstract

Chest pain is a common presenting complaint to emergency departments (EDs) and is a symptom of serious cardiovascular events such as myocardial infarction (MI) and possibly cardiovascular death. Early decision-making regarding patient disposition is crucial for early intervention and to avoid ED congestion. The Third Universal Definition of MI states that MI diagnosis be made using electrocardiogram (ECG) findings and/or a rise and/or fall in cardiac troponin (cTn) concentrations. However, patients with ECG abnormalities represent less than 1/3 of all ACS patients, leaving the remaining to be diagnosed using multiple measurements of cTn over several hours. I therefore aimed to develop a strategy to identify patients at low-risk for major adverse cardiovascular events (early rule-out), as well as those at greatest short-term cardiac risk (early rule-in).

In this thesis I present published work on the clinical utility of glycogen phosphorylase Isoenzyme BB (metabolic marker) in combination with high-sensitivity cTn (hs-cTn) to rule-out adverse cardiac events within 72hrs for patients presenting to the ED within 6hrs of ACS symptom onset. I further assessed the utility of metabolic markers using glucose in this setting. Preliminary results show that using a “healthy” hs-cTn concentration with a normal glucose measurement at presentation can be

used to rule-out patients who present to the ED with clinical suspicion of ischemia.

Further expansion of this hypothesis demonstrated that an algorithm incorporating both glucose and cTn can effectively rule-in/rule-out MI or MI/cardiovascular death in patients who present to the ED with symptoms of ACS. In addition, presentation hemoglobin A1c identified previously unknown diabetes; which may have overall health implications for these patients. I also demonstrate that using glucose in combination with cTn is a cost-effective decision-making tool in the ED as compared to cTn alone.

Application of these rule-in/rule-out algorithms can improve morbidity/mortality rates, and alleviate healthcare burdens.

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Preface

This thesis is prepared in the “sandwich” format as outlined in the “Guide for the preparation of Master’s and Doctoral Theses”. Chapter 1 is a general introduction regarding the content of subsequent chapters providing background information, as well as outlines the general theme. The body of this thesis consists of 4 chapters (Chapter 2-5), each one an independent study, all of which are published in peer reviewed scientific journals. There is a small introduction to each chapter, as well as a description of each authors’ contributions following each publication. Finally, Chapter 6 includes the discussion of this thesis aimed to summarize the overall implications of the thesis and discuss possible future directions.

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List of Abbreviations and Symbols

ACS	Acute Coronary Syndrome
ADA	American Diabetes Association
ADAPT	Accelerated Diagnostic Protocol to Assess Patients with Chest Pain Symptoms Using Contemporary Troponins as the Only Biomarker study
ADP	Accelerated Diagnostic Pathway
AG	Admission Glucose
AMI	Acute Myocardial Infarction
APACE	Advantageous Predictors of Acute Coronary syndrome Evaluation study
AUC	Area Under the Curve
CABG	Coronary Artery Bypass Grafting
CAD	Coronary Artery Disease
CDA	Canadian Diabetes Association
CHD	Coronary Heart Disease
CIHI	Canadian Institute for Health Information
CI	Confidence Interval
CK	Creatine Kinase
CP	Chest Pain
cTn	Cardiac Troponin
CTAS	Canadian Triage and Acuity Scale
CV	Coefficient of Variation
CVD	Cardiovascular Disease
CVE	Cardiovascular Events
DIGAMI	Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction study
DM	Diabetes Mellitus
ECG	Electrocardiogram
ED	Emergency Department
EDTA	Ethylenediaminetetraacetic acid
EDACS	Emergency Department Assessment of Chest pain score randomised controlled trial
ESC	European Society of Cardiology
FAST	Fast Assessment of Thoracic Pain
FASTER	Fast Assessment of Thoracic Pain by nEuRal networks
FG	Fasting Glucose
FPG	Fasting Plasma Glucose
FFA	Free Fatty Acids
GDP	Gross Domestic Product
GPBB	Glycogen Phosphorylase Isoenzyme BB

GRACE	Global Registry of Acute Coronary Events
HbA1c	Hemoglobin A1c
HEART	History, ECG, Age, Risk factors and Troponin
hFABP	Heart Type Fatty Acid Binding Protein
hs-cTn	High-sensitivity cardiac troponin
HOMA-IR	Homeostatic Model Assessment and Insulin Resistance
HPLC	High Performance Liquid Chromatography
ICU	Intensive Care Unit
IHC	Integrative Health Coaching
IQR	Interquartile Range
JAMA	Journal of the American Medical Association
LoB	Limit of Blank
LoD	Limit of Detection
LR	Likelihood Ratio
MACE	Major Adverse Cardiovascular Events
MI	Myocardial Infarction
mmol/L	Millimole per Litre
N/A	Not Applicable
ng/L	Nanogram per Litre
NO	Nitric Oxide
NPV	Negative Predictive Value
NSTEMI	Non-ST Elevation Myocardial Infarction
NT-pro BNP	N-Terminal pro B-Type Natriuretic Peptide
NYD	Not Yet Diagnosed
NYHA	New York Heart Association
OCCI	Ontario Case Costing Initiative
OGTT	Oral Glucose Tolerance Test
p	p-value
PCI	Percutaneous Coronary Intervention
PG	Plasma Glucose
PPV	Positive Predictive Value
QC	Quality Control
RBC	Red Blood Cells
ROC	Receiver-Operating Characteristic
RING	Reducing the time Interval for identifying New Guideline defined MI in patients with suspected ACS in the ED
SIRS	Systemic Inflammatory Response Syndrome
SOB	Shortness of breath
STEMI	ST elevation Myocardial Infarction
TIMI	Thrombolysis in Myocardial Infarction
TIS	Total Ischemic Score
UA	Unstable Angina

USPSTF	United States Preventive Services Task Force
ug/L	Microgram per Litre
URL	Upper Reference Limit
WHO	World Health Organization
7d	Seven Day

Declaration of Academic Achievement

All studies within this thesis are original work. The individual contributions of each co-author are outlined in each chapter following the references.

Chapter 1: Introduction

1.1 Background

Over the past several decades the life expectancy of adults has increased significantly (Statistics Canada 2009), and with that, the prevalence of chronic diseases has also risen (Statistics Canada 2013). According to Stats Canada, heart disease remains the second leading cause of death in Canada as of January 2014 (Statistics Canada 2016), despite the overall decreasing trend in heart disease related deaths (Statistics Canada 2016). Furthermore, heart disease and stroke continue to be the leading causes of hospitalization in Canada, accounting for approximately 20% of all hospitalizations to acute care hospitals in 2009 (Heart and Stroke Foundation 2015). Heart disease encompasses a wide range of heart conditions; this thesis focuses on acute coronary syndrome (ACS).

Early detection (rule-in) and exclusion (rule-out) of acute cardiovascular events and complications has many implications, including the potential to decrease mortality and morbidity and lessen the economic burden of ACS on the Canadian healthcare system. Early diagnostic assessment of patients with suspected cardiac injury is necessary to maximize the benefits of treatments and reduce the burden on the healthcare system (Dekker et al. 2010).

This thesis provides some insight into these issues and some solutions.

1.2 Myocardial Ischemia and Acute Coronary Syndrome

Myocardial ischemia occurs due to inadequate blood flow to the myocardial tissue. When ischemia is prolonged, cellular necrosis begins, and in this setting, clinically the condition may be defined as myocardial infarction (MI) (Thygesen et al. 2012; Amsterdam et al. 2014). Myocardial ischemia can be detected using electrocardiograms (Thygesen et al. 2012; Channer and Morris 2002) and myocardial injury by measurement of biomarkers such as cardiac troponin (cTn) (Thygesen et al. 2012). There are several pathological processes that can increase cTn and are considered myocardial injury, MI is just one subset of myocardial injury that can occur (see Fig.1.2.1) (Thygesen et al. 2012).

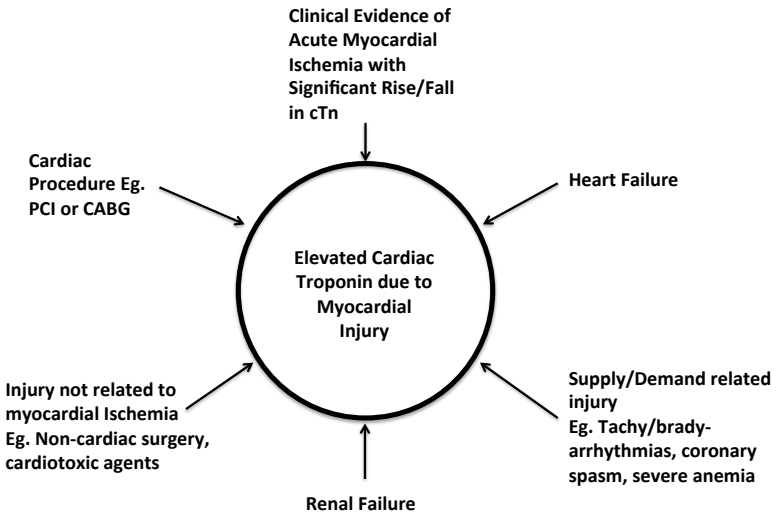

Figure 1.2.1. Various clinical scenarios resulting in cardiac troponin elevations (Adapted from The Third Universal Definition of Myocardial Infarction) (Thygesen et al. 2012)

Given the complexity of many of these clinical entities, expert clinical and laboratory groups have highlighted the importance of distinguishing between acute and chronic elevations of cTn, with the former thought to require a rise and/or fall pattern of cTn concentrations over several hours (Thygesen et al. 2012; Amsterdam et al. 2014). However, even when using these criteria, there are a variety of pathophysiological conditions that may cause elevations (some examples include: aortic dissection, severe anemia,

myocarditis, renal failure and sepsis), it is therefore important to incorporate clinical features into the diagnostic process (Thygesen et al. 2012).

Acute coronary syndrome (ACS) encompasses a spectrum of clinical manifestations of myocardial ischemia including ST-elevation MI (STEMI), non-ST elevation MI (NSTEMI), and unstable angina (UA). Currently, electrocardiograms (ECG) are used to distinguish STEMIs from NSTEMI and UA, as the dynamic changes (i.e., ST segment elevation) on the ECGs are indicative of MI (Thygesen et al. 2012). However, challenges do exist with regards to distinguishing NSTEMIs, UA and non-cardiac chest pain, due to similar presentations, differing severity in myocardial ischemia often accompanied by non-specific ECG findings at presentation and varying cTn concentrations over several hours (Thygesen et al. 2012; Amsterdam et al. 2014). ACS symptoms include, but are not limited to chest pain, arm pain, epigastric pain, jaw pain, and shortness of breath (SOB) – symptoms often associated with many other disorders (e.g., musculoskeletal disorders, pulmonary issues or gastrointestinal discomfort). Furthermore, atypical presentation is common in the elderly and in women (Thygesen et al. 2012). Thus, differentiation between NSTEMI, UA and non-cardiac disorders can be difficult and often time consuming (Thygesen et al. 2012).

1.3 Cardiac Troponin and Cardiac Troponin Assays

Biomarkers are quantifiable biological entities used to indicate a physiological state within a subject; and accordingly their measurement may allow for improved diagnostic accuracy and provide important prognostic information on diseases (Chan and Ng 2010). Troponin is a regulatory protein of striated muscle (Wu et al. 1998). It is 3-unit protein complex (troponin I, T, and C) that is involved in the calcium-mediated regulation of skeletal and cardiac muscle contraction. Cardiac troponins I (cTnI) and T (cTnT) are cardiac specific isoforms of troponin (Thygesen et al. 2012; Wu et al. 1998), located primarily in the contractile apparatus of the muscle (Wu et al. 1998), however, a small percentage of cTn is found unbound in the cytosol and is thought to be released first upon myocardial injury (White 2011). Both cTnI and cTnT are released into the bloodstream following myocardial injury (Wu et al. 1998; Amsterdam et al. 2014) thus making cardiac troponin an ideal biomarker for diagnosing MI.

Cardiac troponin measured in blood is the reference standard by which MI is diagnosed and myocardial injury is identified (Thygesen et al. 2012; Amsterdam et al. 2014). Cardiac troponin is most often measured using an immunochemical technique, an example being a chemiluminescent sandwich immunoassay. Briefly, this method typically employs the use of at least two antibodies and a label (i.e.,

chemiluminescent label). Cardiac troponin in the patient's blood sample is bound to anti-troponin antibodies; in the sandwich format, one is usually the capture antibody and the other is usually labeled with an enzyme or molecule (i.e., a conjugate) that can be activated to produce a signal (e.g., emitted light from a chemiluminescent immunoassay that uses alkaline phosphatase as the enzyme conjugated to the second antibody) (Cox et al. 2012).

Although cTn is the reference standard for the diagnosis of MI along with an electrocardiogram (ECG), studies have shown that not all patients experiencing ACS or a serious cardiac outcome are identified using these methods (Dong et al. 2013; Eggers et al. 2012). Furthermore, with the introduction of high-sensitivity cardiac troponin assays (hs-cTn) there is growing concern that the diagnosis of NSTEMI may increase, while UA and other diagnoses decrease (Amsterdam et al. 2014) due to the increased analytical sensitivity and precision of these assays – meaning that lower concentrations are able to be detected with higher precision (Korley and Jaffe 2013). Given the need for timely and accurate identification of those with and without MI, early rule-out and rule-in options must be examined.

1.3.1 High-Sensitivity Cardiac Troponin and the 99th Percentile upper reference limit concentration from a healthy population

With the introduction of hs-cTn assays into clinical use, it is important to understand what "high-sensitivity" refers to in this context. Briefly, there are two defining characteristics that distinguish high-sensitivity assays from the contemporary cardiac troponin assay. First, the total imprecision [coefficient of variation or CV: measure of variability of repeated measures, ratio of standard deviation and the mean (Shechtman 2013)] at the 99th percentile value should be ≤10% and second, a measurable concentration of cTn can be quantified in the majority of healthy people (Apple and Collinson 2012). The 99th percentile upper reference limit (URL) is typically determined by the manufacturer of each assay and is based on the measurement of cTn in a healthy reference population (Apple and Collinson 2012; Hickman et al. 2014; Ungerer et al 2016) as cardiac troponin can be found in the circulation of healthy individuals due to a variety of reasons including apoptosis and normal myocyte turnover (White 2011). However, there is not currently a consensus of what constitutes a healthy reference population as most manufacturers and investigators have different definitions (Apple and Collinson 2012; Hickman et al. 2014; Ungerer et al. 2016).

Given the variability in determination of the 99th percentile URL, it is important to evaluate the different 99th percentile cutoffs, especially with respect to how these cutoffs perform clinically to diagnose MI. One study compared the 99th percentile cutoffs between the Roche hs-cTnT assay and the Abbott ARCHITECT hs-cTnI assay [population size (n)=1540] using the same healthy reference population. They report numerically similar 99th percentile cutoffs for hs-cTnT [15ng/L, 95% confidence interval (CI)[1]: 13-16] and hs-cTnI (13ng/L, 95%CI: 5-15), which are different then the manufacturers' reported 99th percentile cutoffs (14ng/L for hs-cTnT and 26ng/L for hs-cTnI) (Kimenai et al. 2016). Another study also sought to determine the 99th percentile cutoffs using a presumably healthy reference population. They report 99th percentile concentrations similar to the previously mentioned study for hs-cTnT [15ng/L (95%CI: 13-28) for the Roche hs-cTnT assay] but rather different for hs-cTnI [23ng/L (95%CI: 16-63) for the Abbott ARCHITECT hs-cTnI assay] (Apple et al. 2012).

Mueller and colleagues sought to determine if hs-cTn values below the manufacturers 99th percentile could be used to rule-out MI at presentation (MI adjudicated based on 2007 Universal Definition of MI). They report a sensitivity of 89.6% (95%CI: 86.4-92.3%) and NPV

[1] Confidence interval: range of reasonable values that contain the parameter of interest (eg. 99th percentile) with a certain degree of confidence (ie. 95% confidence interval)(Pagano and Gaureau 2013)

of 96.5% (95%CI: 95.4-97.4%) for hs-cTnT when using the 99th percentile cut-off of 14ng/L and a sensitivity of 77.2 (95%CI: 72.1-81.7%) and NPV of 94.3% (95%CI: 92.8-95.5%) for the Abbott hs-cTnI when using the 99th percentile cut-off of 26.2ng/L with the highest sensitivity and specificity in patients who presented more than 6 hours after symptom presentation. They conclude that using a single presentation cTn below the manufacturers' 99th percentile should not be used to rule-out MI (Hoeller et al. 2013).

Pickering and colleagues sought to determine if using a threshold of the 99th percentile for serial sampling (0-4hrs) of hs-cTn could effectively rule-out patients who present to the ED with symptoms of ACS. They also evaluated sex-specific cutoffs for the 99th percentile URL. Acute MI (AMI) was defined using the 2007 task force definition of MI using the contemporary assay and was independently adjudicated by local cardiologists. They found that using the overall 99th percentile or sex-specific cutoffs, even when combined with ECG findings, was not a safe rule-out method in this population (Pickering et al. 2015).

Another paper examined four different study populations from three different countries (i.e., ADAPT, ADP, EDACS, and RING studies) of patients presenting with chest pain to the ED. Myocardial infarction was defined using the 2007 global task force recommendations for all

four studies and was independently adjudicated by clinicians. Using serial sample of hs-cTnI and hs-cTnT (0-3-hour testing) they determine that using the manufacturers' 99th percentile cut-off has too low of a sensitivity to rule-out MI within this timeframe (Pickering et al. 2016).

1.4 Other Biomarkers of Cardiac Ischemia

There are many different biomarkers of cardiac ischemia under investigation, three non-cTn ACS biomarker are of particular interest in this thesis: Heart-type Fatty Acid Binding protein (hFABP), Glycogen Phosphorylase Isoenzyme BB (GPBB) and glucose. An in-depth review of each marker can be found in sections 1.4.1, 1.4.2 and 1.5.

1.4.1 Heart-type Fatty Acid Binding protein (hFABP)

Heart-type Fatty Acid Binding protein is a small hydrophilic protein involved in fatty-acid metabolism (Chan and Ng 2010) that is found in large quantities in cardiomyocytes (Ozdemir et al 2011; Suzuki et al. 1998) and in much lower quantities in the kidneys, skeletal muscle and brain tissue (Rick Body 2009). Injury to myocardial cells allows this small protein to quickly leak into the bloodstream in proportion to the extent of damage and as a result, has been suggested as an alternative to other biomarkers, such as myoglobin, in a multi-marker approach for diagnosing MI (Jaffe et al 2006). Evidence suggests that

hFABP may be a viable biomarker for a variety of high-risk cardiac populations (Ozdemir et al. 2011; Suzuki et al. 1998; Jaffe et al. 2006; Hasegawa et al. 2004) and may be useful in the ED setting. Specifically, in a study (n=181) evaluating hFABP alone and in combination with hs-cTnT for the diagnosis of MI (NSTEMI) (Defined using the 2007 Universal definition of MI and American College of Cardiology Foundation/ American Heart Association Task Force guidelines) in ED patients presenting with symptoms of ACS found that the addition of hFABP to hs-cTnT increased the area under the curve (AUC[2]) of hs-cTnT alone from 0.893 (95%CI: 0.812-0.974) to 0.908 (95%CI: 0.839-0.977), although this difference was not statistically significant (p=0.07). The addition of hFABP at 5.8ng/mL to hs-cTnT (14ng/L) increased both sensitivity and NPV for NSTEMI diagnosis as well. The authors note that hFABP is not an alternative to CK-MB or cTn but rather should be considered an additive marker for the rule-out of MI (Dupuy et al. 2014). Conversely, patients enrolled in the APACE (the Advantageous Predictors of Acute Coronary syndrome Evaluation study) were evaluated to determine the

[2] AUC: Area under the receiver-operating characteristic (ROC) curve shows how good a test is a distinguishing those with and without the disease in question. The closer an AUC is to 1, the better the test (Pagano and Gaureau 2013)

ROC curve: Graphical representation of sensitivity vs. 1-specificity. The probability if a true positive result vs. the probability if a false positive result for a range of different cutoffs (Pagano and Gaureau 2013)

incremental diagnostic value of hFABP to hs-cTnT in early presenting (within 1 hour) (n=105) ED patients with symptoms of ACS (AMI adjudicated using the Third Universal Definition of MI) and found that the addition of hFABP increased the AUC of hs-cTnT from 0.88 (95%CI: 0.81-0.94) to 0.90 (95%CI: 0.83-0.98), yielding similar results to the above study. However, in repeating the analysis of patients presenting within two hours of symptom onset (n=398) found that hFABP no longer increased the AUC of hs-cTnT alone (Schoenenberger et al. 2016). Further exploration of hFABP as an early marker of ischemia is necessary to determine its clinical utility.

1.4.2 Glycogen Phosphorylase Isoenzyme BB (GPBB)

Glycogen Phosphorylase Isoenzyme BB (GPBB) is a member of the GP family. It is involved in glycogenolysis and is found in the sarcoplasmic reticulum of brain tissue and in cardiomyocytes. During ischemia it is activated and released from glycogen, where it then enters into circulation within hours of chest pain onset (Lillpopp et al. 2012; Krause et al. 1996). The clinical utility of GPBB is still disputed (Meune et al. 2011). Early studies suggest that GPBB had the highest AUC as compared to CK-MB, myoglobin or cTnT for the detection of MI within the first four hours of symptom onset (Rabitzsch et al. 1995). However, recent studies suggest that GPBB is not an alternative to hs-

cTn assays for the early diagnosis of MI (Adjudicated using the 2007 Universal Definition of MI) (Lillpopp et al. 2012) but rather that the addition of GPBB to the contemporary cTn assays may help to increase the sensitivity for MI diagnosis (Lillpopp et al. 2012; Meune et al. 2011; Apple et al. 2005). Further research is required in order to explore GPBB in the ED setting and determine its utility for ACS patients.

1.5 ACS and Hyperglycemia

Acute and chronic hyperglycemia have been shown to be associated with increased incidents of adverse cardiovascular outcomes (Kawano et al. 1999; Timmer et al. 2005; Deedwania et al. 2008; Lippi, Cervellin, and Targher 2012; Naito et al. 2014; Pai et al. 2013; Cakmak et al. 2008). Therefore, I have reviewed both long-term (Hemoglobin A1c; HbA1c) and acute (glucose) indicators of glycemic control as part of a risk assessment tool for ACS. Sections 1.5.1 to 1.5.4 and 1.6 outline some of the relevant research and pathophysiological mechanisms associated with hyperglycemia and adverse cardiovascular outcomes.

1.5.1 Proposed Pathophysiological Mechanism of Acute Hyperglycemia

Acute hyperglycemia in the presence of MI has been documented (Oswald et al. 1986; Capes et al. 2000; Angeli et al. 2015; Deedwania et al. 2008) and is associated with increased mortality and morbidity in the critically ill (Viana et al. 2014), as well as in patients with ACS (Deedwania et al. 2008) , regardless of diabetic status. The precise underlying pathophysiology for how hyperglycemia affects ACS is not well understood, however some of the proposed mechanisms of actions are summarized in Figure 1.5.1.

The question arises, “is glucose a marker or a mediator of adverse outcomes in patients with ACS?”. Many studies have sought to determine the physiological link between hyperglycemia and adverse outcomes in ACS patients. Studies in animals have shown that acute hyperglycemia decreases coronary collateral blood flow by reducing nitric oxide (NO) availability (Kersten et al. 2001), eliminating ischemic preconditioning and dose-dependently increasing infarct size (Kersten et al. 1998). In addition, hyperglycemia has been shown to induce apoptosis and nitrotyrosine formation in animal hearts (Ceriello et al. 2002), and to increase Q-T interval via ventricular instability (D’Amico et al. 2001). Further, hyperglycemia has been shown to cause increased oxidative stress (Ceriello 1997) causing a reduction in the bioavailability of NO which can lead to endothelial dysfunction (Valgimigli et al. 2003; De Caterina 2000). Hyperglycemia may also

reflect a state of decreased insulin which is associated with increased lipolysis and free fatty acid (FFA) circulation (Capes et al. 2000). Increased FFA concentration in turn increases myocardial oxygen demand (Kumar et al. 2013), reduces myocardial contractility (Kumar et al. 2013), and is generally toxic to the ischemic myocardium (Capes et al. 2000). Hyperglycemia also leads to increased platelet activation (Oswald et al. 1988). Additionally, MI is associated with local and systemic inflammation (Mulvihill and Foley 2002; Marfella et al. 2003), and the resulting stress hyperglycemia has been shown to increase the inflammatory response and worsen cardiac outcomes (Marfella et al. 2003) .

It is also thought that hyperglycemia may be a marker of adverse events in patients with ACS, for example; as stress increases, so do stress hormones, which in turn promote glycogenolysis and hyperglycemia (Capes et al. 2000).

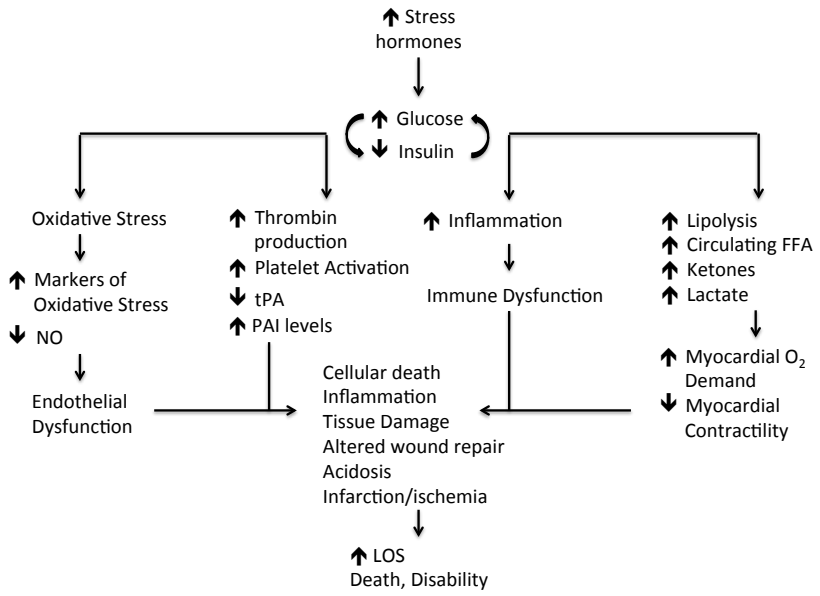

Fig.1.5.1. Physiological link between hyperglycemia and adverse events (Adapted from Deedwania et al. 2008)

Despite the lack of agreed upon physiological mechanisms, evidence suggests hyperglycemia is associated with poor outcomes.

1.5.2 Studies Assessing MI and Hyperglycemia

There is a multitude of research that has examined the association of hyperglycemia at admission to hospital for MI/ACS to adverse outcomes. Terlecki et al have demonstrated that in patients admitted to hospital for STEMI, those who had acute hyperglycemia (defined as >7.8mmol/L) had a significantly higher rate of in-hospital mortality

compared to those defined as normoglycemic (Terlecki et al. 2013). Another study investigating the link between admission glucose and acute MI in elderly patients found that a large proportion of patients with elevated glucose did not have any previously reported diabetes and that higher admission glucose concentrations were associated with increased risk of mortality at both 30 days and one year. Particularly in the patients without known diabetes, there was a steep increase in 30-day and one year mortality as glucose concentrations increased – this trend was not noted in patients with previously documented diabetes. Conversely, the authors found that mortality in patients with diabetes was only associated when glucose concentrations were extremely high (>13.3mmol/L or >240mg/dL) (Kosiborod et al. 2005).

A recent study sought to investigate the importance of hyperglycemia [oral glucose tolerance test (OGTT)] in the prediction of long-term mortality and cardiovascular events (CVE) in ACS patients. The authors report a higher rate of mortality and reinfarction for patients who had a positive OGTT or known diabetes suggesting that disturbed glucose metabolism, even in its early stages negatively affects outcomes. The authors also suggest routine glucose testing in patients with ACS for the detection of diabetes and dysglycemia (Kuhl et al. 2015).

Another study sought to compare fasting blood glucose (normal FG defined as <6.1mmol/L) and admission glucose (AG) (normal AG defined as <7.8mmol/L) in non-diabetic patients with MI for the prediction of 30-day mortality. The authors found that elevated FG was a strong predictor of 30-day mortality and that a ROC derived cutoff of 6.3mmol/L for fasting glucose and 8.4mmol/L for admission glucose maximized sensitivity and specificity. A modest correlation was found between fasting and admission glucose and that both FG and AG were independent predictors of 30-day mortality, when used alone, FG was superior to AG (Suleiman et al. 2005).

A systematic review of 15 cohorts was published in 2000 and reported that mean glucose concentrations across admissions for MI were significantly higher in patients with an adverse outcome and that diabetic patients who died had a significantly higher glucose concentration than those who survived, the same trend was seen in patients without known diabetes – although the mean glucose concentrations were lower. Admission or fasting glucose was used in 11 of the studies rather than the mean glucose concentration. The definition of hyperglycemia ranged from 6.7mmol/L to 11.0mmol/L on admission or 6.1mmol/L to 8.0mmol/L for fasting. The range of patients who had stress hyperglycemia was from 3-71% in patients without known diabetes and 46-84% in those with diabetes. They found that

the pooled unadjusted relative-risk[3] of in-hospital death after an MI in patients without diabetes but who had stress hyperglycemia was 3.9 (95%CI: 2.9-5.4) compared to those without diabetes or stress hyperglycemia. The pooled unadjusted relative risk for diabetic patients with hyperglycemia was 1.7 (95%CI:1.2-2.4) compared to diabetic patients without hyperglycemia (Capes et al. 2000).

A key theme among major studies of acute hyperglycemia in ACS patients is the lack of a defined and accepted definition of hyperglycemia in these populations. There is also no agreed upon time to measure glucose or the number of measurements. Of note, many of the studies examined the hospital admission glucose, rather than the ED presentation glucose. This may increase the likelihood that significant results were found, as patients would have been deemed ill enough to require hospital admission.

1.5.3 Diabetes, Hyperglycemia and ACS

A key question arises: “why not simply use diabetic status to indicate a dysglycemic state and therefore cardiac health?”. Several studies have attempted to evaluate this approach. One study sought to determine if type two diabetes mellitus (DM) and hyperglycemia were

[3] Relative risk: chance that someone with exposure (i.e., Hyperglycemia) will develop the disease (e.g., MI) divided by the chance that someone without the exposure (i.e., Normal glucose) will develop the disease(Pagano and Gaureau 2013).

independent predictors of mortality in elderly patients with ACS. They found that diabetic status is not useful for prognostication but that hyperglycemia is associated with worse outcomes in the elderly. However, the authors were unable to determine if hyperglycemia was simply a marker of worse baseline characteristics rather than a causative factor (Savonitto et al. 2014).

Another study examining the effect of diabetic status, and new-onset hyperglycemia on short and long-term cardiovascular outcomes in ACS patients was undertaken in the middle-eastern gulf countries. New-onset hyperglycemia was defined as glucose ≥7.0mmol/L and based off a fasting glucose within 24 hours of admission. Almost half (49.2%) had a previous diagnosis of diabetes and 8.8% had new-onset hyperglycemia – these patients had similar baseline characteristics as patients with no known diabetes and no new-onset hyperglycemia. They report that patients with new-onset hyperglycemia had higher rates of 30-day mortality and one-year mortality compared to those without new-onset hyperglycemia or known DM and that new-onset hyperglycemia was a strong predictor of in-hospital mortality, cardiogenic shock, and major bleeding even after adjustment for confounders (Alfaleh et al. 2014).

Further to these studies, an Australian group highlighted that the extent of undiagnosed diabetes can be up to half of all patients with

ACS who have abnormal glucose concentrations (Chih et al. 2008). It has also been suggested that 30% of people in the US who have diabetes are unaware of their medical condition (Dungan et al. 2009) and, according to the Canadian Diabetes Association (CDA), as many as 15% of ACS patients have undiagnosed diabetes. Furthermore, the CDA states that hyperglycemia is closely related to in-hospital mortality in ACS patients, more-so then diabetes status (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee 2013).

Given these data, at this time it is unlikely that simply using diabetic status as a surrogate marker for hyperglycemia would be adequate to capture the extent of hyperglycemia in ACS, for use as a rule-in/out tool, or use as a prognostic marker.

1.5.4 Clinical Decision Making Tools

A variety of clinical decision making tools exist in order to aid physicians in diagnosis and risk stratification for patients with possible ACS; examples include: TIMI (Thrombolysis in Myocardial Infarction), GRACE (Global Registry of Acute Coronary Events) and HEART (History, ECG, Age, Risk factors and Troponin) (Gardner et al. 2015). These commonly used tools use a variety of clinical components in the risk score, including diabetes status, (Gardner et al. 2015) however

they do not include blood glucose concentration, despite the evidence of elevated glucose concentrations in non-diabetic patients (as mentioned above). Therefore, several studies have investigated clinical decision making tools and glucose.

A recent study sought to determine the incremental value of admission glucose when added to the GRACE score (Global Registry of Acute Coronary Events) for the prediction of one-year mortality in ACS patients. This cohort included all patients who had admission glucose, including those with STEMIs (there was no exclusion criteria). They compared GRACE score alone and then GRACE score with admission glucose. They report that glucose was an independent predictor of one-year mortality, independent of GRACE score and the presence of diabetes and that the AUC increased significantly after the inclusion of glucose in the GRACE score. They note as well that inclusion of glucose was associated with a net reclassification index[4] of 37%, and was mainly in the non-event group. The authors also noted that 48% of patients who did not die had a substantial and significant reduction in overall risk when glucose was added to their model – suggesting the new model better identified patients without an

[4] Net reclassification index: Used to assess diagnostic improvements with the addition of a new marker. NRI classifies patients based on event status, then re-classifies patients using the new marker and determines if the re-classification was correct (Pencina et al. 2008)

event. Their analysis also determined that a cut-point of 8.9 mmol/L provided the highest sensitivity and specificity. The authors also reported that patients with hyperglycemia on admission had higher rates of mortality as well as highlighted the potential for using glucose for identifying patients at low-risk (Timóteo et al. 2014).

In 2015, another study investigated the prognostic role of admission glucose and insulin resistance [using Homeostatic Model Assessment and insulin resistance (HOMA-IR)] in patients with ACS complicated by heart failure for death during their hospital admission (Lazzeri et al. 2015). They included patients with NSTEMIs and STEMIs and measured fasting blood glucose, cTnI and NT-proBNP (N-Terminal pro B-Type Natriuretic Peptide) at the intensive care unit (ICU) admission. Hyperglycemia was defined as ≥7mmol/L for non-diabetic patients and ≥10mmol/L for diabetic patients. They found that hyperglycemia in non-diabetic patients had the highest odds ratio for death in the ICU, followed by hyperglycemia in diabetic patients. They also found fasting admission glucose concentrations were independent predictors of ICU mortality, while acute insulin resistance was not and that it was independently associated with ICU mortality in non-diabetic patients (Lazzeri et al. 2015).

Other clinical decision making tools have also incorporated other markers or diagnostic tools such as ECGs (Richard Body et al. 2015),

age , smoking and hypertension (Backus et al. 2013; Cohen et al. 2013; Carlton, Khattab, and Greaves 2015). Although these approaches appear to be useful it is important to consider the limitations of such tools. First, this researcher's observations suggest that not all physicians interpret ECGs exactly the same, that patients may not always be aware of their medical conditions such as hypertension, and that smoking status may be subject to recall bias and may in fact be associated with lower cardiac troponin levels (Lyngbakken et al. 2016). Further, evidence suggests that biological age versus chronological age likely has an effect on cardiovascular risk and risk factors (Liang et al. 2016).

1.6. HbA1c and Acute Coronary Syndrome

1.6.1 What is HbA1c?

Glycated hemoglobin (HbA1c) is a marker of long-term glycemic control (Naito et al. 2014) and is used for screening, diagnosis and management of diabetes mellitus (DM) (Pai et al. 2013). HbA1c is a molecule in which glucose is bound to the N-terminal valine of hemoglobin and is produced following the glycation of circulating glucose to hemoglobin, hence glycated hemoglobin or HbA1c (Hare et al. 2012). HbA1c is most commonly expressed as the percentage or fraction of total hemoglobin. Given the average lifespan of red blood cells (RBCs), HbA1c concentrations correlate to the average glucose

concentrations over the two to three months preceding HbA1c measurement (Hare et al. 2012). There are many different ways to measure HbA1c (immunoassay, ion-exchange high-performance liquid chromatography (HPLC), boronate affinity HPLC and enzymatic assays) but in each method the goal is to separate HbA1c from other hemoglobins in order to accurately measure this analyte (Little and Roberts 2009).

HbA1c concentrations have been reported to vary depending on a variety of factors such as genetics, oxidative stress, iron and vitamin B12 deficiency, renal failure and a high rate of RBC turnover (Hare et al. 2012).

Among the many recommendations for HbA1c testing and utilization, the United States Preventive Services Task Force (USPSTF) in 2015 updated their recommendations from 2008. They now recommend screening for abnormal blood glucose as part of a cardiovascular risk assessment in adults aged 40-70 years who are overweight or obese using HbA1c, fasting glucose or an oral glucose tolerance test for screening. They note that there is inadequate evidence that directly shows that measuring blood glucose leads to improved outcomes but that the risks/harm of measuring it are limited (Siu 2015).

HbA1c is an ideal biomarker for determining long-term hyperglycemia, as it is not subject to day-to-day changes (Pai et al. 2013) or affected by acute changes in glucose concentrations due to stress of illness (Siu 2015). It also does not require a fasting state, unlike the OGTT which most often is performed in the morning in a fasting state (Siu 2015) thus making HbA1c an ideal marker for use in acute care when steady state or long term glycemic status is desired. Recent studies have begun to evaluate the use of HbA1c as a prognostic marker for cardiovascular disease.

1.6.2 HbA1c and ACS: A Literature Review

In 2015, a study was published on the predictive value of HbA1c versus the GRACE score for the prediction of MACE in ACS patients undergoing PCI with no known history of diabetes, as well as the incremental value of adding HbA1c to the GRACE score. Diagnostic criteria for diabetes were: HbA1c ≥ 6.5%, FPG ≥ 7.0mmol/L, 2-h PG (plasma glucose) ≥ 11mmol/L [2010 American Diabetes Association (ADA) guidelines]. Sampling was done in the fasting state. They found that HbA1c was positively correlated with GRACE scores and that HbA1c was an independent predictor of MACE and the cumulative risk of MACE increased as HbA1c increased. Combining HbA1c with the GRACE score increased the AUC from 0.75 (95% CI 0.69-0.82) to 0.80 (95% CI 0.75-0.85) (p=0.012). They also found that the net

reclassification index for HbA1c reclassified patients appropriately and significantly in both directions, particularly, 28% of patients without an event were reclassified as lower risk when using HbA1c and 42% of patients with events were reclassified as high-risk (Liu et al. 2015).

Another study reviewed the relationship between HbA1c and MACE in patients who underwent elective PCI. They found that HbA1c concentrations between 6% to 7% were significantly associated with increased risk of MACE and cardiovascular mortality in non-diabetic patients and that their event rate was similar to those who had diabetes. These data suggest that an abnormal HbA1c may have prognostic significance in non-diabetics who undergo PCI (Corpus et al. 2003).

A 2004 study reported on the relationship between HbA1c, cardiovascular disease and death. The average follow-up was six years. They reported that patients with previously undiagnosed diabetes and an HbA1c >7.0% had a greater risk for all-cause mortality and CVD than those without diabetes. The risk of CVD or all-cause mortality increased as HbA1c concentrations increased. In both men and women HbA1c concentrations predicted an increased risk of CVD and mortality, independently of other known risk factors, including diabetic status. An increase in HbA1c by 1% was associated

with a 20-30% increase in event rate. They also reported a less linear relationship in HbA1c concentrations and adverse events in women – in order for risk to be increased, a higher HbA1c percentage was needed (Khaw 2004).

Another study found similar results when they evaluated the relationship between glycemic exposure over time and the development of microvascular complications. They reported that in patients with type 2 DM there was a significant association between diabetic complications, including death with a range of glycemic exposures. They also noted that risk for microvascular complications decreased by 37% with every 1% reduction in HbA1c (Stratton et al. 2000).

Furthermore, a 2013 study examined the association of HbA1c and coronary heart disease (CHD) in two prospective case-control studies of health professionals without a previous history of diabetes or HbA1c above 6.5%. Among these patients, greater HbA1c concentrations were found to be associated with increased risk of CHD in both men and women. They found that patients with an HbA1c of 6.0 to <6.5% had an 85% higher risk of CHD than those at lower concentrations. This association persisted when adjusted for traditional risk factors (Pai et al. 2013).

Despite the evidence and recommendations supporting the measurement and monitoring of the diabetic status in ACS patients, measurement and use of HbA1c to evaluate ACS patients varies greatly (Siu 2015). An Australian study reported on 1743 diabetic ACS patients from 33 different hospitals to determine the frequency and predictors of HbA1c measurement in-hospital. They found that only 41% of ACS patients had HbA1c measured in hospital and that the frequency varied greatly across hospitals. Measurement was more likely to be done in hospitals that had catheterization capabilities. Those patients who had an HbA1c measurement were more often younger, male, indigenous to Australia, English-speaking, with a history of smoking. Interestingly, patients with chronic renal failure, a history of CVD and history of cardiac interventions were less likely to have an HbA1c measurement. HbA1c measurement was associated with catheterization, revascularization, measurement of cholesterol and lipids and greater use of evidence-based practices, as well as referrals for cardiac rehabilitation. They also noted that patients who received an HbA1c measurement in-hospital had significantly lower mortality rates in-hospital – perhaps due to the inherently lower risk nature of these patients (i.e., age and history of CVD) (Snir et al. 2016).

Furthermore, in acute care where time is limited, there does not

always seem to be agreement/knowledge of previously diagnosed diabetes. Personal observations from chart reviews and data collection from the acute care setting indicate that patients are not always aware of their health conditions and that physicians may not have the time for an in-depth history in a fast-paced environment such as in the ED.

1.7 Glucose and HbA1c Studies

Given the interest in the metabolic status and adverse outcomes in patients with ACS, Norhammer et al. sought to determine the prevalence of glucose abnormalities in patients without diabetes who had a diagnosis of MI (Adjudicated using the European Society of Cardiology and the American College of Cardiology). Glucose was measured at admission, HbA1c was measured the first morning of admission and concentrations of capillary overnight fasting glucose each morning until discharge. At discharge, an OGTT was taken. Three months following discharge, another HbA1c test, fasting glucose concentrations and a new OGTT was conducted. Diabetes mellitus was defined according to the 1998 WHO definition and the ADA definition for fasting blood glucose from 1997. Criteria were as follows: a fasting glucose >6.0mmol/L (1997 ADA definition) or a 2-h post-load glucose concentration >11.0 mmol/L, or both; impaired glucose tolerance defined as a fasting glucose >6.1 mmol/L and 2-h

glucose between 7.8–11.0 mmol/L. Normal glucose tolerance was defined as fasting glucose <6.1 mmol/L and 2-h glucose <7.8 mmol/L. They found a high prevalence of abnormal glucose metabolism in patients with MI, that these changes can be detected before hospital discharge and suggest that the true prevalence of diabetes in patients with MI may be as high as 45%. They concluded that HbA1c at admission, a single fasting glucose 4 or 5 days into the admission or an oral glucose tolerance test can predict risk (Norhammar et al. 2002).

A 2008 study evaluated the relationship between admission HbA1c and myocardial perfusion abnormalities in patients with acute MI (Defined using the European Society of Cardiology and American College of Cardiology criteria). Blood glucose and HbA1c were measured within three hours of admission - fasting status was not considered. They found a significant relationship between glucose concentrations at admission and death after MI, as well as glucose concentrations and a positive exercise test and total ischemic score (TIS). They also report a significant relationship between HbA1c and mortality after MI, a positive exercise test and TIS. HbA1c also correlated with the number of diseased vessels and that patients with an HbA1c concentration >6.5% had significantly higher ischemic scores and had a higher risk for death (Cakmak et al. 2008). They

concluded that both HbA1c and glucose on admission were associated with higher ischemic scores and rates of mortality (Cakmak et al. 2008).

A population-based study out of Reykjavik, Iceland evaluated citizens without a known history of MI on the association of markers of dysglycemia with CHD incidence. They also performed a systematic review and meta-analysis of Western cohorts. In 300 000 participants, they reported that people without diabetes, fasting and post-load glucose concentrations were moderately associated with CHD in both studies. Conversely, they found that the meta-analysis indicated a relative risk for CHD of 1.20 per 1% increase in HbA1c in those without diabetes (Sarwar et al. 2010).

Naito and colleagues reported on the effect of both admission glucose and HbA1c concentrations in ACS patients and their relation to adverse outcomes. They showed that elevated admission glucose combined with HbA1c concentrations were associated with increased risk of cardiovascular disease in patients with and without diabetes who underwent PCI (Naito et al. 2014).

Given the variability of results and lack of definitive evidence for cutoffs; further investigation is required to elucidate the utility of the

use of HbA1c and glucose in ACS patients, especially for early decision making in the ED.

1.8 Economic implications of ACS in Canada

Cardiovascular diseases are not only a large health concern but also present a large financial burden on the Canadian healthcare system. Direct costs include physicians, nurses, laboratory costs, bed usage, prescriptions, specialized diets etc., whereas indirect costs can include long or short-term disability, lost time at work due to hospitalization or death, loss of leisure activities by both patients and family members and more (Rivers Charles Associates 2010). In 2000, it was estimated that the cost of CVD in Canada was $22.2 billion (included both direct and indirect costs), the second highest total after musculoskeletal disease ($22.3 billion). Many of the direct costs associated with CVD can be attributed to those over 65 years of age, an age category that is on the rise. Furthermore, ischemic heart disease (including MI) accounts for the largest portion of in-hospital expenditures, and premature death. In 2008/9 in Canada there were almost 110 000 hospitalizations for heart attacks and chest pain alone (Rivers Charles Associates 2010). Prescriptions for CVD also accounted for approximately 15% of all prescriptions in Canada in 2007, a significant increase from previous years (Public Health Agency of Canada 2009). Data from the 2009 Canadian Institute for Health

Information (CIHI) database estimated that 1.3 million days of work or 3500 years were lost in 2009 due to morbidity associated with ACS – a cost of almost $1.8 billion or 0.12% of the 2009 GDP (Gross Domestic Product) of Canada (Rivers Charles Associates 2010). Given these figures, the estimated direct and indirect costs for ACS is $3.4 billion per year. If considering quality and length of life, ACS contributes another estimated $15.3 billion in losses (Rivers Charles Associates 2010).

Given the large economic burden ACS places on our healthcare system appropriate strategies for early decision-making are necessary to reduce ED wait times and overcrowding, triage those in need of immediate care as quickly as possible and discharge those considered low risk. The cost and workflow advantages of such a strategy are explored in chapter 5 of this thesis.

1.9 Summary

The key purpose of this thesis is to demonstrate the use of glycemic biomarkers in combination with currently used hs-cTn assays in ruling-out and ruling-in MI and other major cardiovascular events (MACE). This thesis describes the process of exploring different combinations of hs-cTn with other proposed biomarkers, to the selection of the optimal biomarker combinations, as well as the algorithms from an economic standpoint.

This thesis is a sandwich thesis and contains co-authored original work where I was the first/lead author. Specifically, Colleen Shortt's contributions to each paper are outlined below:

Chapter 2: Contribution Statement:
Colleen Shortt developed the idea for this paper, alongside Dr. Peter Kavsak. She participated in the laboratory analysis, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Chapter 3: Contribution Statement:
Colleen Shortt developed the idea for this paper, alongside Dr. Peter Kavsak. She participated in data abstraction from charts, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Chapter 4: Contribution Statement:
Colleen Shortt developed the idea for this paper, alongside Dr. Peter Kavsak. She participated in data collection in the ED, outcome preparation/adjudication, patient follow-up, data abstraction from charts, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions

Chapter 5: Contribution Statement:

Colleen Shortt developed the idea for this paper, alongside Dr. Peter Kavsak, Dr. Richard Whitlock and Dr. Feng Xie. She participated in data collection in the ED, outcome preparation/adjudication, patient follow-up, data abstraction from charts, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions

Chapter 2

Comparison of hs-cTnI, hs-cTnT, hFABP and GPBB for identifying early adverse cardiac events in patients presenting within six hours of chest pain-onset

Publication:

Shortt CR, Worster A, HA Hill, Kavsak PA. Comparison of hs-cTnI, hs-cTnT, hFABP and GPBB for predicting early adverse cardiac events in patients presenting within six hours of chest pain-onset. Clinica Chimica Acta 2013;4.19: 39–41

Introduction

There is growing evidence that a multi-marker approach could be beneficial when evaluating patients with chest pain (Dupuy et al. 2014). Two biomarkers of interest are hFABP and GPBB. Heart-type Fatty Acid Binding Protein is thought to be a useful early marker of myocardial ischemia (Dupuy et al. 2014) as it is released from injured myocardium into the blood stream quickly following myocardial injury (Liebetrau et al. 2015). During myocardial ischemia GPBB, a key enzyme involved in

energy metabolism in the heart, is free to move into extra-cellular fluid following the breakdown of glycogen. It is thought that GPBB may be a key biomarker in indicating ischemia (Krause et al. 1996).

This study highlights two key results. First, that neither hs-cTnI, nor hs-cTnT are capable of identifying all MACE at 72 hours when using the 99th percentile cut-off. Second, using a combination of hs-cTn and GPBB for the identification of patients at risk for a short-term major cardiovascular event is superior to hs-cTn alone or when combined with hFABP. One of the concerns highlighted in the literature with regards to hFABP is that it is cleared by the kidneys and therefore greatly affected by renal status (Glatz et al. 1998). Furthermore, a review of 6 studies concluded that using hFABP to diagnose MI would provide predictive values similar to that of using only an ECG (Rick Body 2009).

This study demonstrated the potential for the use of a metabolic biomarker (GPBB) in clinical decision-making, and the need for more data concerning assessment of low-risk patients presenting early after pain onset.

Letter to the Editor

Comparison of hs-cTnI, hs-cTnT, hFABP and GPBB for predicting early adverse cardiac events in patients presenting within six hours of chest pain-onset.

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Key words: high-sensitivity cardiac troponin I; high-sensitivity cardiac troponin T, heart-type fatty-acid binding protein; glycogen phosphorylase BB, chest pain population

To the Editor:

A recent publication comparing and assessing high-sensitivity cardiac troponin T (hs-cTnT) with heart-type fatty-acid binding protein (hFABP) at presentation in a pooled population of patients included in the FAST II (Fast Assessment of Thoracic Pain) and FASTER I (Fast Assessment of Thoracic Pain by nEuRal networks) studies indicated no incremental value for the diagnosis of myocardial infarction (MI) with the addition of hFABP to hs-cTnT [1]. An additional study in 3 centers in Germany on patients admitted with suspected acute coronary syndrome (ACS) to chest pain units also suggested limited utility of combining another early biomarker (glycogen phosphorylase BB; GPBB) with a sensitive cardiac troponin I (cTnI) assay for the diagnosis of acute MI [2]. However, neither of these studies assessed peak concentrations on early serial measurements (i.e., at presentation and 3 h, and 6 h later) for the diagnosis of MI as recommended by recent guidelines [3] or for predicting other short-term serious cardiac adverse events. Further work is needed here, as the pathophysiological role of GPBB, an enzyme of cellular metabolism, is believed to be released from its binding to glycogen during conditions of ischemia, which is then rapidly moved into circulation within the first hours of chest pain onset [2]. Moreover, hFABP, which is highly concentrated in the myocardium, is also rapidly released into circulation following myocardial injury [4]. Accordingly, to further delineate the role of

early biomarkers (i.e., hFABP, GPBB) for identifying short-term serious adverse cardiac events in the presence of either hs-cTnT or hs-cTnI assays, we measured these early biomarkers in samples collected at presentation and 3 h and 6 h later in the emergency department (ED) in an early chest pain population.

The study population has been previously described [5] as well as the performance by the area under the curve (AUC) of hs-cTnI (AUC = 0.86) and hs-cTnT (AUC = 0.82) assays using the presentation sample for predicting a short-term adverse cardiac event [6]. Briefly, the inclusion criteria in the study were as follows: i) ≥18 years of age, ii) patients with possible ACS symptoms within 6 h before presentation, and iii) a cTnI ordered by an ED physician. On the other hand, patients were excluded if: i) they refused to participate, ii) were referred directly to trauma/surgery, or iii) had an outcome before the initial cTnI result. The study outcomes (composite adverse cardiac events within 72 h after presentation) were: MI, heart failure, serious arrhythmia, refractory ischemic cardiac pain, or death [5,6]. After obtaining research ethics board approval, available serum samples collected at presentation and 3 h and 6 h later in the ED were thawed and analyzed for hs-cTnT (Roche Elecsys 2010) and hs-cTnI (investigational-use only assay; Beckman Coulter Access II) (first thaw) and levels of hFABP and GPBB (second thaw) were measured (Randox Evidence Investigator). Analyses were then performed (with the Analyse-it

and GraphPad Prism software) on the peak concentration for each biomarker (n = 163 patients) to obtain the AUC (ROC curve analysis with the Delong–Clarke–Pearson method used for comparison), the diagnostic sensitivity, specificity, likelihood ratios (LR), positive predictive value (PPV), and negative predictive value (NPV) calculated using the reported 99th percentile cutoffs for each biomarker (hs-cTnT = 14 ng/L [1,6]; hs-cTnI = 10 ng/L [6]; hFABP = 5.2 μg/L [4]; GPBB = 7.88 μg/L [manufacturer]).

The average (standard deviation) age of the study cohort was 62 (15) years with 59% being males. Those patients with an adverse cardiac event (n = 21) within 72 h after presentation had significantly higher peak biomarker concentrations as compared to those without an event (n = 142) (median hs-cTnI = 45 ng/L vs. 7 ng/L, p b 0.0001; hs-cTnT = 75 ng/L vs. 6 ng/L, p b 0.0001; hFABP = 4.6 ng/L vs. 2.6 ng/ L, p = 0.0007; GPBB = 16.6 μg/L vs. 13.4 μg/L, p = 0.0059; via Mann– Whitney test). ROC curve analyses indicated that hs-cTnI, but not hs-TnT, had a significantly higher AUC as compared to either hFABP or GPBB (Fig. 1). When the 99th percentile cutoffs for the peak concentra- tion were applied, only the dual combination of hs-cTnI > 99th or GPBB > 99th was able to identify all patients with an adverse cardiac event (sensitivity = 100%; 95% CI: 82–100), albeit at a lower specificity of 32% (95% CI: 25–41) as compared to either biomarker alone (Table 1). By comparison, the dual combination of

hs-cTnI > 99th or hFABP > 99th did not improve performance above hs-cTnI. Restricting the analysis to only those with a diagnosis of ACS (n = 16; MI = 11 and refractory ischemic cardiac pain = 5) the dual combination of hs-cTnT >99th or GPBB > 99th produced a sensitivity of 100% (95% CI: 77–100) with a specificity of 38% (95% CI: 31–47), similar to the performance of hs-cTnI combined with GPBB. On the other hand, for the combination of hFABP > 99th with either hs-cTnI > 99th or hs-cTnT >99th, the sensitivity for ACS diagnosis was only 81% (95% CI: 56–94).

The present analyses indicate that measuring either hs-cTnI or hs-cTnT at presentation and 3 and 6 h later in the ED and interpreting them based on guideline cutoffs (i.e., 99th percentile) cannot identify all patients that will experience an adverse cardiac outcome over the short-term. These data reinforce the need for clinical judgment in assessing low-risk patients presenting early after pain onset, irrespective of the cTn concentration obtained within the first 6 h in the ED. Moreover, these data also suggest that different hs-cTn assays may have different clinical characteristics. For instance, by ROC curve comparison only the hs-cTnI assay and not the hs-cTnT assay was superior to either hFABP or GPBB for diagnosing a short-term adverse cardiac event. A possible explanation may be the higher analytical sensitivity of the hs-cTnI assay as compared to the hs-cTnT assay as evidenced by more individuals with detectable concentrations in either a healthy reference population [7] or a stable high-

risk popu- lation [8,9]. Additional studies should clarify what, if any, clinical differences exist between hs-cTn assays in this setting.

Finally, the combination of GPBB, but not hFABP, with hs-cTn as- says appears to identify more patients at risk for short-term adverse cardiac events. The ability to rule out any adverse cardiac event by 6 h as demonstrated by this dual combination needs to undergo subse- quent testing in different and larger ED chest pain populations, and in comparison with other emerging biomarkers with possible roles in acute cardiac care [10]. The present data, although pilot in nature, does open the door to multimarker approaches for identifying ED patients at high-risk for a short-term cardiac outcome, even in the era of hs-cTn assays.

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Contribution Statement

Colleen Shortt developed the idea for this paper, alongside Dr. Peter Kavsak. She participated in laboratory analyses, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Andrew Worster contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

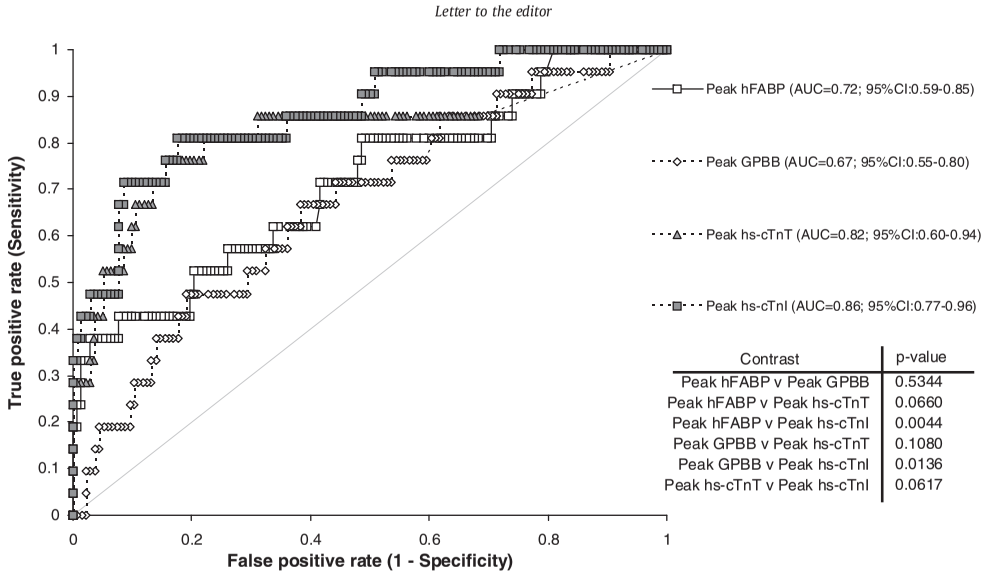
Stephen Hill contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

Peter Kavsak contributed to the original study design as well as participating in developing the idea for the study, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Table 1. Clinical Characteristics of the biomarkers using the 99th percentile cutoffs for predicting an adverse cardiac event at 72 hours

	hs-cTnI	hs-cTnT	hFABP	GPBB	hs-cTnI OR hFABP	hs-cTnT OR hFABP	hs-cTnI OR GPBB	hs-cTnT OR GPBB
Sensitivity (95%CI)	86% (65-96)	81% (59- 92)	43% (24- 64)	67% (45-83)	86% (65- 96)	81% (59- 93)	100% (82-100)	95% (76->99)
Specificity (95%CI)	63% (55-70)	73% (65- 79)	80% (73- 86)	58% (50-66)	54% (45- 62)	65% (57-73)	32% (25-41)	39% (31-47)
PPV (95%CI)	25% (17-37)	30% (20- 43)	24% (13- 40)	20% (12-31)	21% (14- 31)	26% (17- 38)	19% (12-27)	19% (13-28)
NPV (95%CI)	97% (91-99)	96% (91- 99)	90% (84- 95)	92% (84-96)	96% (89- 99)	96% (90- 99)	100% (90-100)	98% (89- >99)
LR+	2.30	2.95	2.17	1.59	1.84	2.35	1.48	1.56
LR-	0.23	0.26	0.71	0.57	0.27	0.29	0.00	0.12

Figure 1. ROC curve analysis for the peak concentration of hs-cTnI, hs-cTnT, hFABP, and GPBB for predicting an adverse cardiac event at 72 hours.



Contrast	p-value
Peak hFABP v Peak GPBB	0.5344
Peak hFABP v Peak hs-cTnT	0.0660
Peak hFABP v Peak hs-cTnI	0.0044
Peak GPBB v Peak hs-cTnT	0.1080
Peak GPBB v Peak hs-cTnI	0.0136
Peak hs-cTnT v Peak hs-cTnI	0.0617

Chapter 3

An approach to rule-out an acute cardiovascular event or death in emergency department patients using outcome-based cutoffs for high-sensitivity cardiac troponin assays and glucose

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Introduction

Elevations of cardiac troponin (cTn) in the bloodstream are indicative of myocardial injury. The Third Universal Definition of MI recommends serial measurements of cTn to determine if a significant change (rise or fall) in cTn concentrations exist, with one measurement

exceeding the 99th percentile, in order to aid in identifying evolving myocardial injury. These measurements are recommended to be drawn between 3-6 hours apart (Thygesen et al. 2012). Given the timeframe in which measurements should be taken, patients have the potential to spend numerous hours waiting in the ED. Therefore, the ability to safely and quickly rule-out patients with chest pain could have major implications for ED congestion.

Results from our investigation of hFABP and GPBB indicated that GPBB combined with hs-cTn had potential for predicting MACE, whereas hFABP is not likely an appropriate marker for this population (Shortt et al. 2013). Available research presents conflicting results on the utility of GPBB in the ED setting (Lillpopp et al. 2012; Meune et al. 2011; Mair 1998) suggesting that more information is required and that exploration of another metabolic biomarker, such as glucose, could be of great value. Exploratory analysis of available data from chapter 2 revealed that glucose out-performed GPBB when combined with hs-cTnI or hs-cTnT for the prediction of MACE.

In this study, we demonstrate the potential of a combination of a "healthy" hs-cTn concentration coupled with a normal glucose concentration at presentation for the ruling-out of patients who present to the ED with a clinical suspicion of cardiac ischemia. These results have

been substantiated and replicated in an Australian and New Zealand ED population of 1412 patients presenting with symptoms of ACS (Greenslade et al. 2015).

Article

An approach to rule-out an acute cardiovascular event or death in emergency department patients using outcome-based cutoffs for high-sensitivity cardiac troponin assays and glucose.

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Key words: high-sensitivity cardiac troponin; glucose; acute coronary syndrome; emergency department; rule-out; health outcomes

Objectives: The application of “undetectable” high-sensitivity cardiac troponin (hs-cTn) concentrations to “rule-out” myocardial infarction is appealing, but there are analytical concerns and a lack of consensus on what concentration should be used to define the lower reportable limit; i.e., limit of detection (LoD) or limit of blank. An alternative approach is to utilize a measurable hs-cTn concentration that identifies patients at low-risk for a future cardiovascular event combined with another prognostic test, such as glucose. We assessed both of these approaches in different emergency department (ED) cohorts to rule-out an event.

Design and methods: We used cohort 1 (all-comer ED population, n = 4773; derivation cohort) to determine the most appropriate approach at presentation (i.e., Dual Panel test: hs-cTn/glucose vs. LoD vs. LoD/glucose) for an early rule-out of hospital death using the Abbott ARCHITECT hs-cTnI assay. We used cohort 2 (n = 144) and cohort 3 (n = 127), both early chest pain onset ED populations as the verification datasets (outcome: composite cardiovascular event at 72 h) with three hs-

cTn assays assessed (Abbott Laboratories, Beckman Coulter, Roche Diagnostics).

Results: In cohort 1, the sensitivity was N99% for all three approaches; however the specificity (11%; 95% CI: 10–12%) was significantly higher for the Dual Panel as compared to the LoD approach (specificity = 5%; 95% CI: 4–6%). Verification of the Dual Panel in cohort 2 and cohort 3 revealed 100% sensitivity and negative predictive values for all three hs-cTn assays.

Conclusions: The combination of a “healthy” hs-cTn concentration with glucose might effectively rule-out patients for an acute cardiovascular event at ED presentation.

Introduction

The measurement of cardiac troponin I and T at low concentrations with the high-sensitivity assays (i.e., hs-cTnI and hs-cTnT) has demonstrated utility in long-term risk stratification in both healthy individuals and patients with stable cardiovascular disease [1–8] and in the early decision making process within the emergency setting [9–11]. Specifically within the emergency department (ED), studies have focused on the improved analytical sensitivity of these high-sensitivity assays and have taken the approach of assessing “undetectable” hs-cTn concentrations to effectively “rule-out” patients at presentation for acute myocardial infarction (MI) [9,11]. This approach has merit, in that the lower the cardiac troponin concentration the lower the risk in the ED setting [12]. However, there are analytical concerns when assessing hs-cTn at the limit of detection (LoD), as analytical interferences, appropriate quality control monitoring, calibration effects and calibration/reagent lot-to-lot variation may have significant impacts on the interpretation when using the LoD [13–15].

Another approach taking advantage of the improved analytical sensitivity of the hs-cTn assays for early rule-out would be to use measureable concentrations of cardiac troponin that have identified patients at low risk for future cardiovascular events and couple this

information to a non-cardiac biomarker that provides additive information in this setting, in this case glucose [16]. Specifically, in patients with stable coronary artery disease, low measurable concentrations for hs-cTnI and hs-cTnT that identify patients at low risk can be readily obtained from the literature [1,4–6] as well as a cutoff for normal glycemia (b5.6 mmol/L) [17].

Significant research exists that suggests a link between hyperglycemia and poor outcomes in patients with acute coronary syndrome (ACS). Endothelium dependent vasodilation has been shown to be rap- idly suppressed by hyperglycemia in both diabetic and non-diabetic patients, a process thought to be mediated by increased production of oxygen-derived free-radicals [18]. Timmer and colleagues have also shown that hyperglycemia is an important predictor of impaired coronary flow in patients before percutaneous coronary intervention (PCI) [19]. Other data have also indicated that hyperglycemia is associated with a prothrombotic state, to be associated with increased markers of vascular inflammation, increased generation of reactive oxygen species, higher free fatty acid concentrations, insulin resistance and impaired myocardial glucose utilization [20]. Recently, Lippi and colleagues have demonstrated in a pilot study (n = 46) that the addition of a random plasma glucose measurement to hs-cTnI markedly increased specificity and positive predictive value without affecting sensitivity and negative predictive value

when predicting acute MI in patients in the ED [21].

Given these data on low hs-cTn concentrations and normoglycemia possibly identifying patients at low cardiovascular risk, the goal of the present study was to assess if the combination of hs-cTn and glucose at presentation could be used to effectively rule-out hospital death and an acute cardiovascular event in ED patients. Specifically, we assessed patients using a population-based cut-off for stable coronary artery disease [6] and the American Diabetes Association cut-off for impaired fasting glucose [17]. Using these cut-offs, we compared this approach to using the LoD alone and a combination of the LoD and glucose to determine the optimal rule-out strategy in a derivation cohort. Following this, 2 verification cohorts were used to validate the best approach using three different hs-cTn assays.

Design and methods

hs-cTn assays and study populations

This analysis included three ED study populations, all of which received research ethics board approval with the analytical performance of the hs-cTn assays all previously reported. Briefly, the LoD for these assays is as follows: Abbott hs-cTnI; LoD = 1 ng/L, Beckman hs-cTnI LoD = 3 ng/L, Roche hs-cTnT LoD = 5 ng/L with the precision for these assays during this study listed as follows: cohort 1, CV = 4.8% for the Abbott hs-

cTnI patient QC pool (n = 147; mean = 43.2 ng/L) at the Juravinski Hospital ci8200 analyzer; 5.4% (n = 103; mean = 40.8 ng/L) at the Hamilton General Hospital ci16200#1 analyzer; and 4.7% (n = 117; mean = 41.0 ng/L) at the Hamilton General Hospital ci16200#2 analyzer; with cohort 2 and 3 analyses being performed on research designated analyzers also using low concentration patient pool material to determine the imprecision (CV = 15% at 5.8 ng/L for the Abbott hs-cTnI assay (i1000; n = 43)), CV = 9.6% at 13.7 ng/L for the Beckman Coulter research hs-cTnI assay (Access 2; n = 17) and CV = 14% at 12.5 ng/L for the Roche hs-cTnT assay (Elecsys 2010; n = 19) [22–24].

Briefly, cohort 1 (an all-comer population) consisted of all consecutive ED patients from two EDs over a period of 3 months [24] who had both glucose and cTnI (including Abbott ARCHITECT hs-cTnI measurements on the clinical analyzers; blinded to the treating physicians) available at presentation. The outcome for this prospective observational study was hospital death.

Cohort 2 (from the RING study for Reducing the time Interval for identifying New Guideline defined MI in patients with suspected ACS in the ED) [23] consisted of adult patients who presented with chest pain within 6 hours of pain onset who were clinically managed with the 4th-generation cTnT assay and in whom hs-cTnI (Abbott ARCHITECT i1000 and Beckman Access 2), and hs-cTnT (Roche Elecsys 2010) were

retrospectively measured in the presentation EDTA samples from these patients. Samples underwent one freeze thaw (storage at −70 °C) for the measurement of hs-cTnI (Beckman) and hs-cTnT (Roche) and a subsequent freeze–thaw cycle for the measurement of hs-cTnI (Abbott). The stability of cardiac troponin measured with these assays under these conditions has previously been demonstrated [25–27]. Patients were adjudicated for the composite outcome (PCI, coronary artery bypass graft surgery, and hospital admissions for arrhythmia, refractory ischemic cardiac pain, heart failure, MI, stroke, non-fatal cardiac arrest, or death) at 72 h from presentation.

Cohort 3 (an early chest pain onset ED population in 2003) [28] consisted of adult patients who presented with chest pain also within 6 h of onset who were clinically managed with a standard cTnI assay and in which hs-cTnI (Abbott ARCHITECT i1000 and Beckman Access 2) and hs-cTnT (Roche Elecsys 2010) were retrospectively measured in the presentation serum samples. Samples for the Beckman hs-cTnI and Roche hs-cTnT measurements underwent one freeze–thaw cycle (storage at −70 °C) with the Abbott hs-cTnI measurements performed on samples that underwent a second freeze–thaw cycle. All patients were adjudicated for the composite outcome (death, MI, heart failure, serious arrhythmia, refractory ischemic cardiac pain) at 72 h [22,28] (see Fig. 1).

Biomarker cutoff selection

Cutoffs for the analytes were selected based on several different literature sources. The American Diabetes Association (ADA) impaired glucose cutoff (<5.6 mmol/L) was used to define a normal glucose concentration [17]. For hs-cTn concentrations, the cutoffs were chosen based on the lowest risk group for future cardiovascular events in patients with stable coronary artery disease as these patients are at high-risk for future myocardial infarction [29]. Specifically, the PEACE study was used to select the lowest cutoff for the Abbott ARCHITECT hs-cTnI assay (<4ng/L) [6] while the HOPE study was the source for both the Beckman Access hs-cTnI research assay cutoff (<6 ng/L) [4] and for the Roche hs-cTnT assay cutoff (<8 ng/L) [5]. The reported LoDs for the hs-cTn assays (Abbott hs-cTnI LoD <1 ng/L; Beckman hs- cTnI LoD <3 ng/L; Roche hs-cTnT LoD <5 ng/L) were also used as cutoffs [30].

Algorithm and statistical analysis

Cohort 1 was defined as the derivation cohort and was used to deter- mine the most appropriate approach for early rule-out (i.e. Dual Panel [hs-cTn and glucose] vs. hs-cTn LoD vs. LoD Dual [LoD and glucose] testing). The best combination in cohort 1 was chosen based on the highest reported sensitivity and specificity for the algorithm (differences be- tween algorithms were assessed via McNemar's test).

Specifically, Dual Panel testing was defined as Abbott hs-cTnI <4 ng/L and glucose <5.6 mmol/L to yield a negative panel result, with either hs-cTnI or glucose concentration above these cutoffs yielding a positive result. Also, the LoD was defined as <1 ng/L for a negative result and LoD Dual testing was defined as hs-cTnI <1 ng/L and glucose b5.6 mmol/L for a negative result. Sensitivity, specificity, positive and negative predictive values and likelihood ratios (with 95% confidence intervals) were calculated for each approach with the best combination being employed in cohorts 2 and 3 (i.e., the verification datasets). Briefly, the Dual Panel testing criteria for other hs-cTn assays were defined as Beckman hs-cTnI <6 ng/L and glucose b 5.6 mmol/L and Roche hs-cTnT < 8 ng/L and glucose b 5.6 mmol/L, yielding negative panel results, respectively. Non-parametric and categorical analyses (e.g., Mann–Whitney, Spearman rank correlation, Kruskal–Wallis, McNemar and chi-squared tests) were performed using Graphpad Prism 6.0 and StatsDirect software (version 2.7.9) with p-values <0.05 considered significant.

Results

Cohort 1

In cohort 1 (all-comer population), 4773 patients were included in the analysis (Fig. 1). The population was spilt fairly even amongst males (51.2%) and females with the median age (interquartile range; IQR) being

70 y (56–82) (Table 1) with 4546 hs-cTnI (95.24%) results above the LoD and 897 hs-cTnI (18.79%) results above the 99th percentile (30 ng/L) [31,32] of the Abbott ARCHITECT assay (Fig. 2). There was a positive correlation between presentation glucose and hs-cTnI (Spearman's Rho = 0.22; p <0.001). Applying either the LoD or Dual Panel test (hs-cTnI <4 ng/L and glucose <5.6 mmol/L) in this group only missed 1 hospital death (sensitivity = 99.57% (95% CI: 97.61–99.99); negative likelihood ratio = 0.09 (95% CI: 0.01–0.62) and 0.04 (95% CI: 0.01–0.28), respectively) however, the specificity was higher with the Dual Panel test (Dual Panel specificity = 10.9% (95% CI: 10–12%) vs. LoD specificity = 4.98% (95% CI: 4.4–6.0%), see Table 2). Combining the LoD with glucose (LoD Dual testing) yielded significantly lower specificity (2.47%) as compared to the LoD alone. Using the Dual Panel test potentially 496 patients (10.4% of cohort) could have been discharged after presentation testing, compared to 113 patient (2.4% of cohort) using the LoD Dual test and 227 patients (4.8% of cohort) using the LoD cutoff alone.

Cohort 2

Of the 144 patients in cohort 2 (Fig. 1), 63.9% were male, with a median (IQR) age = 60 y (49–70) (Table 1). Applying the respective LoDs for the Abbott hs-cTnI, Beckman hs-cTnI, and Roche hs-cTnT assays, the percentage of patients with detectable cardiac troponin concentrations

were 65.28%, 77.08% and 59.03%, respectively. The hs-cTn concentrations were correlated with one another (hs-cTnIs Spearman's Rho = 0.81; p <0.0001) (Abbott hs-cTnI and hs-cTnT Spearman's Rho = 0.74; p <0.0001) (Beckman hs-cTnI and hs-cTnT Spearman's Rho = 0.68; p <0.0001). Glucose was also correlated with the hs-cTn assays (Abbott hs-cTnI and Glucose Spearman's Rho = 0.28 p = 0.0005), (Beckman hs-cTnI and glucose Spearman's Rho = 0.24; p = 0.0034) and (hs-cTnT and glucose Spearman's Rho = 0.22; p = 0.0068).

Applying the Dual Panel test of hs-cTn and glucose, 100% sensitivity was achieved by all three assays (95% CI 84% to 100%) (Table 2). Applying the LoDs only, the sensitivity for the Abbott and Beckman hs-cTnI assays was 100%, however, 2 patients were missed by using Roche's LoD (sensitivity = 91.67% (95% CI: 73.00–98.84)). Using the Dual Panel test, potentially 34 patients (23.6% of cohort) could have been discharged following presentation sampling using the Roche assay, 32 patients (22.2% of cohort) using the Abbott assay and 30 patients (20.8% of cohort) using the Beckman assay.

Cohort 3

In cohort 3 (Fig. 1), of the 127 eligible patients 62.2% were male and the median (IQR) age = 59 y (49–73) (Table 1). In this cohort, 100%, 98.43% and 53.54% of patients had detectable hs-cTn concentrations for

the Abbott hs-cTnI, Beckman hs-cTnI and Roche hs-cTnT assays, respectively. There was a positive correlation between all the hs-cTn assays (Abbott hs-cTnI and hs-cTnT: Spearman's Rho = 0.81; p <0.0001), hs-cTnI and Beckman hs-cTnI: Spearman's Rho = 0.76; p < 0.0001), (Beckman hs-cTnI and hs-cTnT: Spearman's Rho = 0.73; p < 0.0001) however, glucose was not significantly correlated (Abbott hs-cTnI and glucose: Spearman's Rho = 0.16 p = 0.0704), (hs-cTnT and glucose Spearman's Rho = 0.12 p = 0.17) and (Beckman hs-cTnI and glucose Spearman's Rho = 0.13, p = 0.1567).

Applying the Dual Panel test of hs-cTn and glucose, 100% sensitivity was also achieved by all three assays in this cohort (95% CI: 78–100%) (Table 2). Applying the LoDs only, the sensitivity for the Abbott hs-cTnI was 100% (with 0% specificity), the Beckman hs-cTnI was 100% (with 2% specificity); however, 3 patients were missed by using Roche's LoD (sensitivity = 82.35% (95% CI: 58.16–94.62); specificity = 50.91% (95% CI: 41.70–60.06)). Using the Dual Panel test, potentially 26 patients (20.4% of cohort) could have been discharged following presentation sampling using the Roche assay, 12 patients (9.4% of cohort) using the Beckman assay and 11 patients (8.7% of cohort) using the Abbott assay.

Discussion

The present study demonstrates that for hs-cTn assays where the

majority of ED patients have detectable concentrations the benefit for utilizing the LoD alone for early rule-out is diminished. Moreover, depending on the population selection and even the sample type measured (EDTA plasma versus serum), the amount of detectable hs-cTn concentrations will vary, and so too will the clinical performance of the LoD as a test. Utilizing a measurable 'normal' hs-cTn concentration as a cutoff to identify a group at low risk is appealing from a laboratory perspective in that hs-cTn assays can now be monitored at this level with quality control material. However, cardiac troponin alone may not be sufficient to rule-out; our approach of coupling hs-cTn concentrations with glucose concentrations, appears to work equally well across different hs-cTn assays. The Dual Panel test of hs-cTn and glucose was effective in the all-comer ED population and in the early onset chest pain populations.

These results support previous work by our group indicating that metabolic markers may be useful in this setting [33]. Specifically, we have demonstrated the ability of the combination of hs-cTnI N99th percentile or glycogen phosphorylase isoenzyme BB (GPBB) (an enzyme of cellular metabolism that is released from an ischemic myocardium) N99th percentile in identifying all patients with an ad- verse cardiac event (sensitivity = 100%; 95% CI: 82–100) (specificity = 32%; 95% CI: 25–41) [33]. However, the added benefit in the present study is that the use of the ubiquitous test, glucose, along with hs-cTn provides excellent performance

for ruling-out a short-term event.

Further to this, a large, prospective, community based study (n=9331) from the United States followed patients for up to 6 y to determine the relationship between pre-diabetes and diabetes to subclinical myocardial damage (assessed by hs-cTnT) [34]. They report that even after adjustment for cardiovascular risk factors, diabetes and pre-diabetes were significantly associated with a higher incidence of subclinical myocardial damage [34]. They also report that patients who developed diabetes over the follow-up period or remained in the pre-diabetes category were at higher risk for adverse cardiac events, particularly if hs-cTnT was elevated [34]. Their findings support the potential harmful effects of hyperglycemia on the myocardium.

There are however several limitations to the present study; one being that we did not assess MI alone and two that the outcomes obtained are different amongst the cohorts. A third limitation is that we only assessed short-term outcomes; and consequently may have missed later events. A fourth limitation is the lack of clinical data for these cohorts. A fifth limitation is we did not capture the fasting status of the patients; however, this would more likely lead to an overestimation of positives with the Dual Panel test, rather than an underestimation. Further studies assessing MI and longer follow-up with knowledge of fasting status and/or HbA1c values are needed to further delineate the combination of hs-cTn

and glycemia status to rule-out an event. In addition to the above limitations, major drawbacks are the different study populations assessed (refer to Table 1) and in particular the small sample size of the verification cohorts. Despite achieving 100% sensitivity, the wide confidence intervals clearly indicate an underpowered analysis. A 10 fold larger study, with the same prevalence would be needed to achieve a 95% CI similar to that of cohort 1. Although these data are exploratory, the well defined study cohorts and application of glucose to different hs-cTn assays for identifying a short-term adverse event do make these findings noteworthy and perhaps provide a new route to explore for early decision making in patients presenting with chest pain to the ED.

Given the growing acceptance of the definition of a high-sensitivity cardiac troponin assay (N50% of healthy subjects have a measurable concentration) the need for appropriate rule-out tools is increasingly necessary [35]. Our preliminary data illustrates the potential of a Dual Panel approach for ruling-out ACS in the ED. Using this approach we have achieved 100% sensitivity, as well as demonstrated an increased potential for early discharge from the ED. Larger prospective trials are needed to further validate this Dual Panel approach if we are to fully realize the potential of hs-cTn to decrease ED congestion without compromising patient care [36].

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Contribution Statement

Colleen Shortt developed the idea for this paper, alongside Dr. Peter Kavsak. She participated in data abstraction from charts, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Kim Phan participated in laboratory analyses, interpretation of data, as well as manuscript revisions

Andrew Worster contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

Stephen Hill contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

Peter Kavsak contributed to the original study design in which these data were sourced. As well as participating in developing the idea for the study, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Figure 1. Flow diagram of cohorts used in this study.

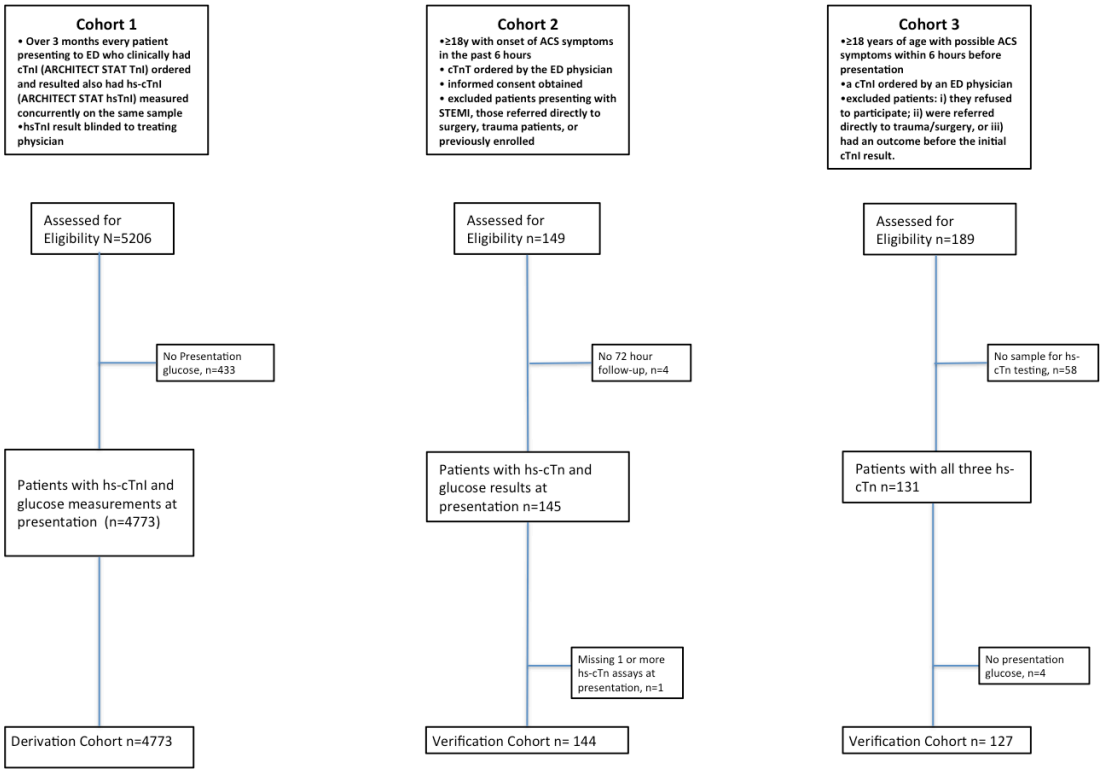

Figure 2. Positivity rate in patients using the overall 99th percentile, the Dual Test, and LoD in Cohort 1 (proportion difference in positivity between LoD and Dual Panel Test = 5.6% (95%CI:4.6 to 6.7), p < 0.0001)

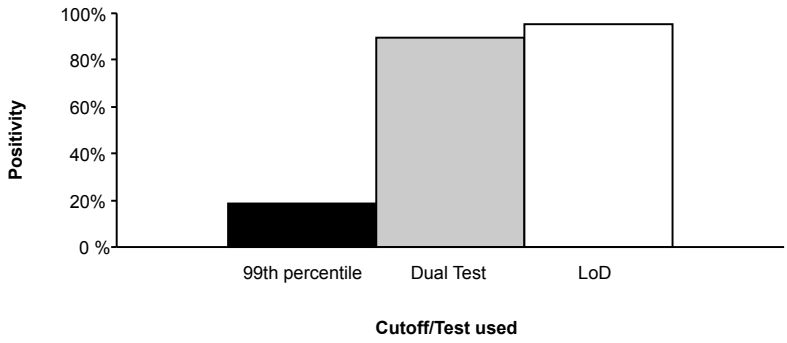

Table 1. Patient characteristics for Cohorts 1, 2 and 3

Variable	Cohort 1 (n=4773)	Cohort 2 (n=144)	Cohort 3 (n=127)	p-value
Demographics				
Age [years; median (IQR)]	70 (56-82)	60 (49-70)	59 (49-73)	< 0.0001
Male Sex (%)	2443 (51%)	92 (64%)	79 (62%)	0.0007
Presenting Symptoms				
Chest Pain	819 (17.6%)	134 (93.1%)	107 (84.3%)	<0.0001
Arm pain (R or L)	40 (0.8%)	37 (25.7)	36 (28.4%)	< 0.0001
SOB	334 (7.0%)	57 (39.6%)	57 (44.9%)	< 0.0001

Table 2. Dual Panel test characteristics in Cohorts 1, 2, 3

Test Characteristics (95% CI)	Cohort 1 (all-comer ED population)					
	Dual Testing		**LoD Dual Test**		**LoD Test**	
	Outcome	No Outcome	Outcome	No Outcome	Outcome	No Outcome
Test Positive	230	4047	230	4430	230	4316
Test Negative	1	495	1	112	1	226
McNemar p-value	Dual vs LoD Dual p<0.0001		LoD Dual vs LoD p<0.0001		Dual vs LoD p<0.0001	
Sensitivity	99.57%	97.34 to 99.99%	99.57%	97.34 to 99.99%	99.6%	97.34 to 99.99%
Specificity	10.9%	10.01 to 11.84%	2.47%	2.05 to 2.96%	4.98%	4.38 to 5.65%
Positive PV	5.38%	4.74 to 6.10%	4.98%	4.35 to 5.60%	5.06%	4.46 to 5.74%
Negative PV	99.80%	98.75 to 99.99%	99.1%	94.67 to 99.99%	99.56%	97.29 to 99.99%
Positive LR	1.12	1.10 to 1.13	1.02	1.01 to 1.03	1.05	1.04 to 1.06
Negative LR	0.04	0.01 to 0.28	0.18	0.03 to 1.25	0.09	0.01 to 0.62

Test Characteristics (95% CI)	Cohort 2 (RING study – early chest pain onset population)					
	Abbott hs-cTnI + Glucose		**Beckman hs-cTnI + Glucose**		**Roche hs-cTnT + Glucose**	
	Dual Testing		**Dual Testing**		**Dual Testing**	
	Outcome	No Outcome	Outcome	No Outcome	Outcome	No Outcome
Test Positive	24	88	24	90	24	86
Test Negative	0	32	0	30	0	34
McNemar p-value	Abbott vs Beckman p=0.48		Beckman vs Roche p 0.21		Roche vs Abbott p=0.32	
Sensitivity	100.0%	87.95 to 100.0%	100.0%	87.95 to 100.0%	100.0%	87.95 to 100.0%
Specificity	26.7%	19.54 to 35.24%	25.0%	18.07 to 33.48%	28.3%	21.01 to 37.00%
Positive PV	21.4%	14.79 to 29.97%	21.1%	14.52 to 29.48%	21.8%	15.07 to 30.47%
Negative PV	100.0%	90.70 to 100.00%	100.0%	90.14 to 100.00%	100.0%	91.20 to 100.00%
Positive LR	1.36	1.22 to 1.52	1.33	1.20 to 1.48	1.39	1.45 to 1.56
Negative LR	0.00	n/a	0.00	n/a	0.00	n/a

Test Characteristics (95% CI)	Cohort 3 (2003 early chest pain onset ED population)					
	Abbott hs-cTnI + Glucose		**Beckman hs-cTnI + Glucose**		**Roche hs-cTnT + Glucose**	
	Dual Testing		**Dual Testing**		**Dual Testing**	
	Outcome	No Outcome	Outcome	No Outcome	Outcome	No Outcome
Test Positive	17	99	17	98	17	84
Test Negative	0	11	0	12	0	26
McNemar p-value	Abbott vs Beckman p=0.71		Beckman vs Roche p 0.0002		Roche vs Abbott p=0.0001	
Sensitivity	100.0%	83.77 to 100.00%	100.0%	83.77 to 100.00%	100.0%	83.77 to 100.00%
Specificity	10.0%	5.52 to 17.18%	11.0%	6.21 to 18.25%	24.0%	16.62 to 32.43%
Positive PV	14.7%	9.26 to 22.32%	14.7%	9.34 to 22.50%	16.8%	10.69 to 25.41%
Negative PV	100.0%	76.88 to 100.00%	100.0%	78.40 to 100.00%	100.0%	88.78 to 100.00%
Positive LR	1.11	1.04 to 1.83	1.12	1.05 to 1.20	1.31	1.18 to 1.45
Negative LR	0.00	n/a	0.00	n/a	0.00	n/a

Chapter 4

Rule-In and Rule-Out of Myocardial Infarction Using Cardiac Troponin and Glycemic Biomarkers in Patients with Symptoms Suggestive of Acute Coronary Syndrome

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Introduction

Our previously reported results indicated that a normal glucose concentration in combination with a "low-risk" measurable concentration of hs-cTn may be useful for early rule-out of ED patients who are investigated for ACS (Shortt et al. 2015). To further expand on these results, I explored different cutoffs for glucose with different combinations of cardiac troponin (cTnI, hs-cTnI, hs-cTnT) at presentation for both the rule-in and rule-out of MI at seven days in patients presenting to the ED with symptoms suggestive ACS. I then tested these algorithms on two different outcomes; MI/cardiovascular death and ACS/cardiovascular

death at seven days. I also explored a longer-term marker of glycemic status, HbA1c. HbA1c provides information on the overall glycemic status of a patient over the prior two to three months and can be used to diagnose diabetes mellitus (DM) (Hare et al. 2012; Canadian Diabetes Association 2013). HbA1c is an ideal marker in the ED setting as it can be measured at any time of the day and is becoming available on automated analyzers that can be operated alongside cTn and glucose in the core laboratory (Hare et al. 2012).

Our results demonstrated that an algorithm incorporating glucose with cTn at presentation may allow for early rule-in and rule-out of MI and MI or CV death in patients presenting to the ED with symptoms of ACS. Furthermore, incorporating HbA1c into the algorithm allowed for the identification of previously unknown diabetes in large number of patients without significantly affecting rule-in/rule-out capabilities. The results from this study may help with ED congestion as decisions may be made after the presentation blood work and may even offer some cost saving opportunities for hospitals.

Article

Rule-In and Rule-Out of Myocardial Infarction Using Cardiac Troponin and Glycemic Biomarkers in Patients with Symptoms Suggestive of Acute Coronary Syndrome

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Key words: high-sensitivity cardiac troponin; glucose; Hemoglobin A1c; acute coronary syndrome; emergency department; rule-in/out

Abbreviations: ED= Emergency Department, MI= Myocardial Infarction; ACS= Acute Coronary Syndrome; cTn= cardiac troponin; ECG= Electrocardiogram; hs-cTn= High-sensitivity Cardiac Troponin; HbA1c= hemoglobin A1c; ROMI-3= Rule-out MI in 3 hours; STEMI= ST-Elevation MI; NSTEMI= Non ST-elevation MI; SIRS= systemic inflammatory response syndrome; LoB=Limit of Blank; LoD= Limit of Detection; ROC curve= Receiver Operator Characteristic curve; ADA= American Diabetes Association, CDA= Canadian Diabetes Association; WHO= World Health Organization; NPV= Negative predictive Value; PPV= Positive Predictive Value

Abstract

BACKGROUND: Early rule-in/rule-out of myocardial infarction (MI) in patients presenting to the emergency department (ED) is important for patient care and resource allocation. Given that dysglycemia is a strong risk factor for MI, we sought to explore and compare different combinations of cardiac troponin (cTn) cutoffs with glycemic markers for the early rule-in/rule-out of MI.

METHODS: We included ED patients (n=1137) with symptoms suggestive of acute coronary syndrome (ACS) who had cTnI, high-sensitivity cTnI (hs-cTnI), hscTnT, glucose, and hemoglobin A1c (HbA1c) measurements. We derived rule-in/rule-out algorithms using different combinations of ROC-derived and literature cutoffs for rule-in and rule-out of MI within 7 days after presentation. These algorithms were then tested for MI/cardiovascular death and ACS/cardiovascular death at 7 days. ROC curves, sensitivity, specificity, likelihood ratios, positive and negative predictive values (PPV and NPV), and CIs were determined for various biomarker combinations.

RESULTS: MI was diagnosed in 133 patients (11.7%; 95% CI, 9.8 –13.8). The algorithms that included cTn and glucose produced the greatest number of patients ruled out/ruled in for MI and yielded sensitivity 99%, NPV 99.5%, specificity 99%, and PPV 80%. This diagnostic performance was maintained for MI/cardiovascular death but not for

ACS/cardiovascular death. The addition of hemoglobin A1c (Hb A1c) (6.5%) to these algorithms did not change these estimates; however, ever, 50 patients with previously unknown diabetes may have been identified if HbA1c was measured.

CONCLUSIONS: Algorithms incorporating glucose with cTn may lead to an earlier MI diagnosis and rule-out for MI/cardiovascular death. Addition of HbA1c into these algorithms allows for identification of diabetes. Future studies extending these findings are needed for ACS/ cardiovascular death.

ClinicalTrials.gov identifier:NCT01994577

INTRODUCTION

Chest pain is one of the most common presenting complaints to emergency departments (EDs) worldwide. In Canada, the combination of abdominal and chest pain represents the primary reason for ED visits and results in longer ED times than less emergent conditions (1). Part of the reason for these longer wait times may be attributed to guidelines which recommend a formal workup for suspected acute coronary syndrome (ACS), including serial measurements of cardiac troponin (cTn) and electrocardiograms (ECGs) for the diagnosis of myocardial infarction (MI) (2). Identification of low-risk patients is an attractive option to reduce wait times and unburden the ED. The introduction of high-sensitivity cTn (hs-cTn) assays offers the opportunity to not only diagnose MI, but also to rapidly rule out MI in the ED. We have previously demonstrated the utility of a dual-panel approach for determining patients at low risk for in-hospital mortality and a composite cardiovascular (CV) outcome in the ED (3). Briefly, the dual-panel approach uses (a) a measurable concentration of hs-cTn to identify patients at low risk for future cardiac events (i.e., a “low-risk” cutoff) and (b) a normal glycemic cutoff (3). This dual- panel approach has been validated in an external population using acute MI as the primary outcome (4). Previous research also has suggested a link between glucose and MI (5–11). Despite the lack of agreed upon mechanisms, it is clear that dysglycemia is a strong risk factor for MI.

Given the success of incorporating a marker of acute glycemic status, we thought to also assess a marker of longer-term glycemic status, hemoglobin A1c (HbA1c), as it may also be informative in this setting. Hb A1c values reflect the glycemic state of the patient over the previous 2 to 3 months and is not affected by fasting status (12), which may be advantageous for ED patients, most of whom have not fasted. Furthermore, HbA1c is not affected by acute stress and is a screening and monitoring test for type 2 diabetes mellitus (13). Accordingly, our primary objective for our current study was to derive algorithms assessing cTn with glucose and/or HbA1c for an early rule-in/rule-out of MI (primary outcome) for a sensitive cTnI and 2 hs-cTn assays at ED presentation in a large prospective North American ED population with symptoms suggestive of ACS. Our secondary outcome was to assess these derived algorithms for MI/CV death with our tertiary outcome assessing these same algorithms for ACS/CV death 7 days postpresentation.

MATERIALS and METHODS

Study Population

This was an a priori– developed secondary data analysis of a prospective, multicenter observational cohort study conducted across a Canadian city [Optimum Troponin Cutoffs for ACS in the ED (ROMI-3: Rule-out MI 3 h); ClinicalTrials.gov identifier: NCT01994577] approved by

our research ethics board. Briefly, adults (18 years) presenting to the ED with symptoms of and investigated for ACS (i.e., cTn ordered by an ED physician) were screened and enrolled. Symptoms of ACS are listed in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol63/ issue1 and were based on the heart and stroke signs of MI. Patients were excluded if they met any of the following exclusion criteria before cTnI testing: death (all-cause); ST-Elevation MI (STEMI) [AHA criteria (14), adjudicated independently by a cardiologist]; and serious ventricular cardiac dysrhythmia. We also excluded patients who had any of the following health conditions within the previous 30 days: traumatic chest pain, including surgery or cardiac manipulation; STEMI or non-STE Elevation MI (NSTEMI); diagnosis of pulmonary embolus; known active malignancy; sepsis [2 systemic inflammatory response syndrome (SIRS) criteria and an increased lactate concentration]; or who were previously enrolled or transferred from another primary care facility. Patients were included in the analysis if they met the inclusion (and none of the exclusion) criteria, had presentation clinical cTnI and glucose results and research (blinded to treating physician) hs-cTnI, hs-cTnT, and HbA1c results (see Fig. 1).

Data Collection, Laboratory Testing, and MI outcomes

Research staff screened and enrolled eligible patients 24 h daily for four weeks at all 3 adult EDs in a single North American city of 500 000 people. We collected clinical cTnI and glucose results at presentation as part of the standard workup for each patient in the ED, with hscTnI and hs-cTnT measured on EDTA plasma presentation samples for each patient (research tube). From May 17 through August 13, 2013, the CVs for hs-cTnI (Abbott ARCHITECT i2000s) were 4.4%–7.1% at approximately 20 ng/L; 3.6%–5.0% at approximately 200 ng/; and 2.5%– 4.2% at approximately 15 000 ng/L using Abbott QC material (Abbott Laboratories), and for hs-cTnT [or cTnT-hs, per the manufacturer on the Roche Modular E170 platform (Roche)] were 2.3% at approximately 30 ng/L and 2.1% at approximately 2253 ng/L using Roche QC material. The research tube was collected and EDTA plasma and a blood-spotted filter paper were stored at 160 °C. We performed the Hb A1c measurements on blood-spotted filter paper as previously described (15), with CVs ranging from 1.7%–2.2% (conversion of Hb A1c % NGSP to mmol/mol IFCC can be performed via this website: http://www.ngsp.org/ convert1.asp). Since the study was conducted in a publicly funded healthcare system, we were able to complete patient follow-up at 7 days for 100% of patients via telephone and/or a comprehensive healthcare database review that included visits to all hospitals in the healthcare region. The primary

outcome measure for this analysis, MI at 7 days, was defined and interpreted based on the current MI definition using the contemporary cTnI assay (2, 16). Our secondary outcome was MI/CV death at 7 days, with our tertiary outcome being ACS/CV death at 7 days (see online Supplemental Methods for full definitions of outcomes).

Algorithm Development and Statistical analyses

Development of the rule-in/rule-out algorithms was an iterative process. First, we selected the optimal thresholds for each analyte alone based on previously used literature cutoffs, as well as ROC-derived cutoffs aimed specifically at either rule-in or rule-out of MI within 7 days.

We selected rule-out cutoffs for all cTn using ROC curves that yielded a 99.5% negative predictive value (NPV) (1), 99% sensitivity (18), a negative likelihood ratio (-LR) of 0.1 (18), and the cutoff that maximized the sensitivity and specificity, as well as the manufacturers' 99th percentile cutoff. We selected other low-risk cutoffs from the literature for each analyte, including hs-cTnI 4 ng/L (3, 19), limit of detection (LOD) 2 ng/L (4) and limit of the blank (LOB) 1 ng/L (20), and 5 ng/L (from the High-STEACs study) (17). The cutoffs for hs-cTnT included LOD 5 ng/L (3, 21), LOB 3 ng/L (22), and 8 ng/L from previous literature (22). The cTnI cutoffs for rule-out included the manufacturers' 99th percentile (0.03 g/L), and LOD (0.01 g/L). Selection of glucose cutoffs for rule-out included the ROC-

derived cutoff that yielded the highest sensitivity and specificity; the American Diabetes Association (ADA) cutoff for “normal” glucose, which we have previously used <101 mg/dL (<5.6 mmol/L) (4, 23); and the WHO diabetes cutoff for fasting glucose 126 mg/dL (7 mmol/L) (24). The HbA1c literature cutoff is the ADA cutoff for the diagnosis of diabetes 6.5% (23).

We selected rule-in cutoffs again using ROC curves that yielded an 80% positive predictive value (PPV) (18), 99% specificity (to mirror the 99% sensitivity threshold used to rule out), a positive likelihood ratio (+LR) of 10 (18), and the cutoff that maximizes sensitivity and specificity, as well as the manufacturers’ 99th percentile cutoff. Cutoffs from the literature that have been used to rule in MI include hs-cTnI ≥64 ng/L (25), hs-cTnT ≥52 ng/L (26), cTnI ≥0.3 g g/L (WHO ROC cutoff), and glucose ≥11 mmol/L to diagnose diabetes (27). We evaluated cutoffs for each analyte individually to determine their sensitivity, specificity, NPV, PPV, and LRs. Following this, we evaluated combinations of cTn and/or glucose and/or HbA1c for sensitivity and NPV (rule-out), specificity and PPV (rule-in) using a combination of estimates from each arm. We developed dual or tripositive (for rule-in) and dual or trinegative (for rule-out) criteria. Patients not meeting rule-in/rule-out criteria were assigned to the observational group. For an accepted miss MI rate <1% (25, 28, 29), we selected rule-in and rule-out criteria based on the following:

Algorithm 1—The combination with the most patients ruled in and ruled out.

Algorithm 2—The combination that allows for highest rule-in/rule-out while incorporating HbA1c measurement.

Algorithm 3—The combination of analytes with the highest sensitivity and NPV (rule-out arm) and the highest specificity and PPV (rule-in arm).

Algorithm 4—The combination of cutoffs similar to the Advantageous Predictors of Acute Coronary Syndrome Evaluation/Accelerated Diagnostic Protocol to Assess Patients With Chest Pain Symptoms Using Contemporary Troponins as the Only Biomarker (APACE/ADAPT) studies (this criterion evaluated the presentation sample only) (25, 30).

We performed the statistical analyses using Analyse-it, GraphPad Prism, and SAS version 9.4. Continuous variables are presented as median (interquartile range) and categorical variables as percentages (with 95% CI) and numbers. Baseline characteristics of those with MI vs without MI at 7 days were compared using the 2-sample t-test, Wilcoxon rank sum test, and X^2 test as appropriate, with P values <0.05 considered significant. We calculated CIs using the modified Wald method. We constructed ROC curves using the Delong method to determine the

diagnostic accuracy of each analyte alone, as well as the combination of presentation markers.

Our secondary outcome measure of CV death or MI [consistent with previous literature (17, 31)] and tertiary outcome of ACS or CV death (see online Supplemental Methods for full definitions) were also assessed using algorithms 1, 3, and the LOD as described above. We further assessed all 3 outcomes in those with diabetes (n=333) and those without diabetes (n=804).

RESULTS

Among the 1137 patients enrolled (Fig. 1), 133 patients (11.7%; 95% CI, 9.8 –13.8) had a final diagnosis of MI [n 65 MI identified from presentation up to 7 h postpresentation and n 69 MI after 7 h and up to 7 days (note: one patient had 2 MIs)]. Those experiencing an MI were older and had a higher prevalence of CV risk factors (see Table 1). The addition of glucose and HbA1c did not significantly change the area under the curve (AUC) compared to hs-cTnI, hs-cTnT, or cTnI alone in the ROC analyses (Fig. 2). Applying the LOD (<2 ng/L) for hs-cTnI, for rule-in/rule-out MI at 7 days yielded a sensitivity 99.2% (95% CI, 95.4 –100), and NPV 99.4 (95% CI, 96.2–100), with only 15.7% (95% CI, 13.6 – 18.1) correctly identified as low risk. Applying the hscTnT LOD (<5 ng/L) yielded a sensitivity of 99.2% (95% CI, 95.4 –100) and NPV of 99.4% (95% CI,

96.2–100), with 17.9% (95% CI, 15.7–20.4) correctly identified as low risk. Applying the cTnI LOD (<0.01 g/L) yielded a lower sensitivity of 82.7% (95% CI, 75.3– 88.3) and NPV of 97.0 (95% CI, 95.6 –98.0). Evaluating the 99th percentile for hs-cTnI, hs-cTnT, and cTnI also failed to meet our prespecified criteria [hs-cTnI sensitivity 72.2% (95% CI, 64.0 –79.1) and NPV 96.0 (95% CI, 94.6 –97.1)], [hs-cTnT sensitivity 92.5% (95% CI, 86.6 –96.0) and NPV 98.3% (95% CI, 96.9 –99.1)], [cTnI sensitivity 67.7% (95% CI, 59.3–75.0) and NPV 95.5% (95% CI, 94.0 –96.6)] (see online Supplemental Table 1).

Hs-cTnI Algorithm (Rule-in and Rule-out) for MI

Diagnostic performance of the four different algorithms can be seen in Table 2. The diagnostic performance of each component of the rule-in/rule-out algorithms can be seen in the online Supplemental Tables 1– 4. The combination of hs-cTnI <5 ng/L and glucose <119 mg/dL (<6.6 mmol/L) (rule-out) and hs-cTnI ≥99 ng/L (rule-in) fulfilled the criteria for Algorithm 1 (highest number of patients ruled in or out) and had a sensitivity of 99.2% (95% CI, 95.4 –100), specificity of 99.0% (95% CI, 98.2–99.5), NPV of 99.7% (95% CI, 98.2–100), and PPV of 85.3% (95% CI, 74.8 –92.0). This method ruled out 29.7% (95% CI, 26.6 –33.1) of

patients, although 1 MI was missed, while 6.0% (95% CI, 4.6 –7.6) or 68 patients were ruled in, of whom 58 had a diagnosis of MI.

The combination of hs-cTnI <5 ng/L, glucose <119 mg/dL (<6.6 mmol/L) and Hb A1c <6.5% (rule-out) and hs-cTnI ≥99 ng/L (rule-in) satisfied our criteria for algorithm 2 (the best combination that included HbA1c) with a sensitivity of 99.2% (95% CI, 95.4 –100), specificity of 99.0% (95% CI, 98.2–99.5), NPV of 99.7% (95% CI, 98.1–100), and PPV of 85.3% (95% CI, 74.8 –92.0). This combination ruled out 29.1% (95% CI, 26.1–32.4) patients, with a miss rate of 1 MI, and 68 patients ruled in (same as algorithm 1 above). Although algorithm 2 ruled out 7 fewer patients at presentation, the addition of Hb A1c identified 50 new cases of increased Hb A1c (≥6.5%) in patients with previously unknown diabetes, 4 of which were ruled out using algorithm 1.

The combination of hs-cTnI <4 ng/L and glucose <119 mg/dL (<6.6 mmol/L) (rule-out) and hs-cTnI ≥82 ng/L and glucose ≥198 mg/dL (≥11 mmol/L) (rule-in) satisfied the criteria for algorithm 3 with a sensitivity 100% (95% CI, 96.6 –100), specificity 99.9% (95% CI, 99.4 –100), NPV 100% (95% CI, 98.4 –100), and PPV 93.8% (95% CI, 69.7–100). Using algorithm 3, 24.5% (95% CI, 21.7–27.6) were ruled out and no MIs were missed; however, only 1.4% (95% CI, 0.8 –2.3) patients were ruled in, of which 15 out of the 16 patients had an MI.

Lastly, the combination most similar to the APACE/ ADAPT study (algorithm 4) used a combination of hscTnI <5 ng/L to rule out and hs-cTnI ≥64 ng/L to rule-in. The sensitivity and specificity were below our prespecified criteria, at 97.0% (95% CI, 92.3–99.1) and 97.5% (95% CI, 96.3–98.3), respectively, with a NPV 99.2% (95% CI, 97.9 –99.8) and PPV 72.8% (95% CI, 62.9 – 80.9). Using this algorithm, 44.3% (95% CI, 40.5–48 – 4) of patients could be sent home; however, 4 MIs would be missed. A total of 8.1% (95% CI, 6.5–9.9) or 92 patients would be ruled in, with 67 MI.

Hs-cTnT Algorithm (Rule-in and Rule-out) for MI

The diagnostic performance of the four different algorithms can be seen in Table 3. The diagnostic performance of each component of the rule-in/rule-out algorithms can be seen in online Supplemental Tables 1, 2, 5, and 6. The combination of hs-cTnT <24 ng/L and glucose <101 mg/dL (<5.6 mmol/L) (rule-out) and hscTnT ≥206 ng/L (rule-in) satisfied the criteria for algorithm 1 (highest number of patients ruled in or out) with a sensitivity 99.2% (95% CI, 95.4 –100), specificity 99.5% (95% CI, 98.8 – 99.8), NPV 99.6% (95% CI, 97.6 –100), and PPV 80.8% (95% CI, 61.7–92.0). This combination ruled out 22.9% (95% CI, 20.3–25.9) of patients and missed only one MI. However, only 2.3% (95% CI, 1.5–3.4) or 26 patients were ruled in, of which 21 had a diagnosis of MI.

The combination of hs-cTnT <24 ng/L, glucose <101 mg/dL (<5.6 mmol/L) and HbA1c <6.5% (ruled-out) and hs-cTnT ≥206 ng/L (ruled-in) satisfied our algorithm 2 criteria, with a sensitivity 99.2% (95% CI, 95.4 – 100), specificity 99.5% (95% CI, 98.8 – 99.8), NPV 99.6% (95% CI, 97.6 – 100), and PPV 80.8% (95% CI, 61.7–92.0). Using algorithm 2, 22.2% (95% CI, 19.5–25.1) of patients would be sent home, with only one MI missed (and the same 26 patients ruled in as algorithm 1). Algorithm 2 ruled out 9 fewer patients at presentation than algorithm 1; however, the addition of Hb A1c still identified those 50 new cases of increased Hb A1c (≥6.5%) in patients with previously unknown diabetes, 5 of which were ruled out using algorithm 1.

The combination of hs-cTnT <14 ng/L and glucose <101 mg/dL (<5.6 mmol/L) (ruled-out) and hs-cTnT ≥206 ng/L and glucose 198 mg/dL (11 mmol/L) (ruled-in) satisfied our algorithm 3 criteria for the highest sensitivity 100% (95% CI, 96.6 –100), specificity 100% (95% CI, 99.5– 100), NPV 100% (95% CI, 97.8 –100), and PPV 100% (95% CI, 62.8 – 100). Using algorithm 3, 18.2% (95% CI, 15.8 –20.9) of patients could be sent home with no missed MIs; however, only 8 patients would be ruled in, all of which had an MI.

The algorithm most similar to the APACE/ADAPT study used a combination of hs-cTnT <14 ng/L (30) (ruled-out) and hs-cTnT ≥52 ng/L (ruled-in). This yielded a sensitivity 92.5% (95% CI, 86.6 –96.0), specificity

92.5% (95% CI, 90.7–94.0), NPV 98.3% (95% CI, 96.9 –99.1), and PPV 46.8% (95% CI, 38.8 –55.0). This algorithm failed to meet our prespecified criteria.

cTnI Algorithm (Rule-in and Rule-out) for 7-Day MI

Results for the optimal rule-in/out criteria combinations can be seen in Table 4 (see online Supplemental Tables 1, 2, 7, and 8 for all combinations).

The combination of cTnI <0.01 g/L and glucose <101 mg/dL (<5.6 mmol/L) (ruled-out) and cTnI ≥0.09 g/L and glucose ≥101 mg/dL (≥5.6 mmol/L) (ruled-in) fit the criteria for algorithm 1 (highest number of patients ruled in or out) with sensitivity 99.2% (95% CI, 95.4 – 100), specificity 99.3% (95% CI, 98.5–99.7), NPV 99.6% (95% CI, 97.6 – 100), and PPV 87.5% (95% CI, 76.1–94.1). This algorithm could send 22.6% (95% CI, 19.9 –25.5) of patients home, missing only one MI, with 4.9% (95% CI, 3.7– 6.4) or 56 patients ruled in, of which 49 had an MI.

The combination of cTnI <0.01 g/L, glucose <101 mg/dL (<5.6 mmol/L), and HbA1c <6.5% (ruled-out) and cTnI ≥0.09 g/L and glucose ≥101 mg/dL (≥5.6 mmol/L) (ruled-in) satisfied our criteria for algorithm 2 (best algorithm incorporating HbA1c). The sensitivity of algorithm 2 was 99.2% (95% CI, 95.4 –100), specificity 99.3% (95% CI, 98.5–99.7), NPV 99.6 (95% CI, 97.5–100), and PPV 87.5% (95% CI, 76.1–94.1) was observed. A total of 21.9% (95% CI, 19.3–24.8) of patients were ruled-out,

while only missing one MI, with the same 56 patients ruled in as algorithm 1. Similar to both the hs-cTn algorithm 2s, the addition of HbA1c identified 50 new cases of increased HbA1c (6.5%) in patients with previously unknown diabetes, of which 5 would be ruled out using algorithm 1.

The combination of cTnI <0.01 g/L and glucose <101 mg/dL (<5.6 mmol/L) (ruled-out) and cTnI ≥0.09 g/L and glucose ≥198 mg/dL (≥11 mmol/L) (ruled-in) satisfied our criteria for the highest sensitivity and specificity (algorithm 3), with a sensitivity 99.2% (95% CI, 95.4 –100), specificity 100% (95% CI, 99.5–100), NPV 99.6% (95% CI, 97.6 –100), and PPV 100% (95% CI, 74.9 –100). This algorithm could send 22.6% (95% CI, 19.9 –25.5) of patients home, missing one MI; however, only 14 patients would be ruled in, of which all had an MI.

Finally, algorithm 4 used a combination of cTnI <0.01 g/L (ruled-out) and cTnI ≥0.3 ng/L (ruled-in) and yielded sensitivity 82.7% (95% CI, 75.3– 88.3), specificity 99.8% (95% CI, 99.2–100), NPV 97.0% (95% CI, 95.6 –98.0), and PPV 93.5% (95% CI, 78.3–99.2). This algorithm failed to meet our prespecified criteria.

Diabetic Status and Secondary & Tertiary Outcomes

For patients with diabetes (n=333), algorithms 1 and 3 maintained their excellent performance; however, for the nondiabetic population (n=804), only algorithm 3 met our prespecified criteria, while algorithm 1

and the LOD did not (see online Supplemental Tables 9 –11). For our secondary outcome of MI or CV death at 7 days in the overall population, both algorithms 1 and 3 maintained their excellent performance; however, for the tertiary outcome of ACS/CV death, neither of the algorithms nor LOD met our prespecified criteria (see online Supplemental Tables 12–17).

DISCUSSION

Using a large, well-characterized cohort of patients presenting to the ED with symptoms suggestive of ACS, we developed several algorithms to rule in/rule out MI at presentation. We highlight 5 major findings.

First, the use of the manufacturers' 99th percentile failed to reach our prespecified acceptable criteria (sensitivity ≥99.0%, NPV ≥99.5%, specificity ≥99.0%, and PPV ≥80.0%) for hs-cTnI, hs-cTnT, and cTnI. This is consistent with other study findings (32–35).

Second, although using an undetectable concentration at presentation achieved a sensitivity above 99%, the NPV was <99.5% and the percentage of patients ruled out was lower than our algorithms 1 and 3. These results are similar to another study that showed a NPV of <99.5% and sensitivity of <99% when only using the LOD for rule-out (35).

Third, the largest and safest reduction in patients required to stay

for observation was achieved using algorithm 1. This highlights the utility of an acute metabolic marker (i.e., glucose) in the diagnostic approach, particularly for rule-out, as well as validating our previous work (3) and that of others (4). These findings are interesting given the recently released recommendations from the US Preventive Services Task Force for screening for abnormal blood glucose concentrations in adults aged 40 – 70 years who are obese (12), as it may provide an opportunity or medium for screening in high-risk patients. The next steps should include a survey of ED physicians on whether the ADA cutoff or another glucose cutoff would be accepted in this setting.

Fourth, the introduction of HbA1c ≥6.5% identified 50 new cases of diabetes. Although HbA1c had no additive value for the diagnosis of MI, there may be added value in the addition of HbA1c to a rule-in/rule-out algorithm for the identification of previously unknown diabetes. Studies out of Australia have reported that measurement of HbA1c in ACS patients with diabetes was associated with better evidence-based care (36), that one in four patients admitted for ACS who had a high admission glucose had undiagnosed diabetes (based on HbA1c values), and that measurement of HbA1c in the ED is both feasible and necessary (37). Together, these findings make a compelling case for the measurement of HbA1c in patients with ACS and hyperglycemia (38). Furthermore, during a service improvement project in a hospital in the UK, universal screening

using HbA1c improved detection rates of diabetes in ACS patients compared to current NICE (National Institute for Health and Care Excellence) guidelines (39).

Fifth, when comparing our algorithms to the algorithm similar to the APACE/ADAPT study, it was clear that presentation-only measurements using these cutoffs are inappropriate, as the missed rate for MI exceeded the clinically acceptable limit for patient safety. Accordingly, a second cTn sample collected after the presentation draw is required to achieve the appropriate diagnostic thresholds, as reported in the APACE/ADAPT studies.

Limitations of this study merit consideration. Our data consist primarily of patients who identify as European descent, which may limit its applicability to non-Caucasian populations; however, the sample is representative of a major North American city. In addition, just over 50% of our population had a presenting complaint of chest pain, which is a lower prevalence than other studies assessing early rule-out protocols and might be explained by the higher prevalence of women (53%) in our study population compared to other studies that had a majority of males in the study population (17, 25). Another limitation is the exclusion of 229 patients for lack of one or more presentation samples for the measurement of all the biomarkers, with the baseline characteristics suggesting that those who were excluded were younger and more racially

diverse. Finally, it is important to note the wide CIs around our measures of diagnostic accuracy; thus, a larger study, with more outcomes, likely will be required to fully determine the applicability of these algorithms.

CONCLUSION

We have shown the utility of both acute (glucose) and chronic (HbA1c) glycemic biomarkers in conjunction with cTn in patients presenting with ACS symptoms for both rule-in/rule-out of MI and for MI/CV death at 7 days, in patients with and without diabetes, as well as for the identification of diabetes. Future directions should include exploration of change criteria for those classified in the observational zone, as well as to prospectively evaluate these algorithms in larger ED chest pain populations. This latter point is especially important for ruling out ACS/CV death. Lastly, the financial implications of any algorithm using multiple biomarkers, as well as the cost-saving opportunities, should be investigated.

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Figure 1. Flow diagram of study cohort

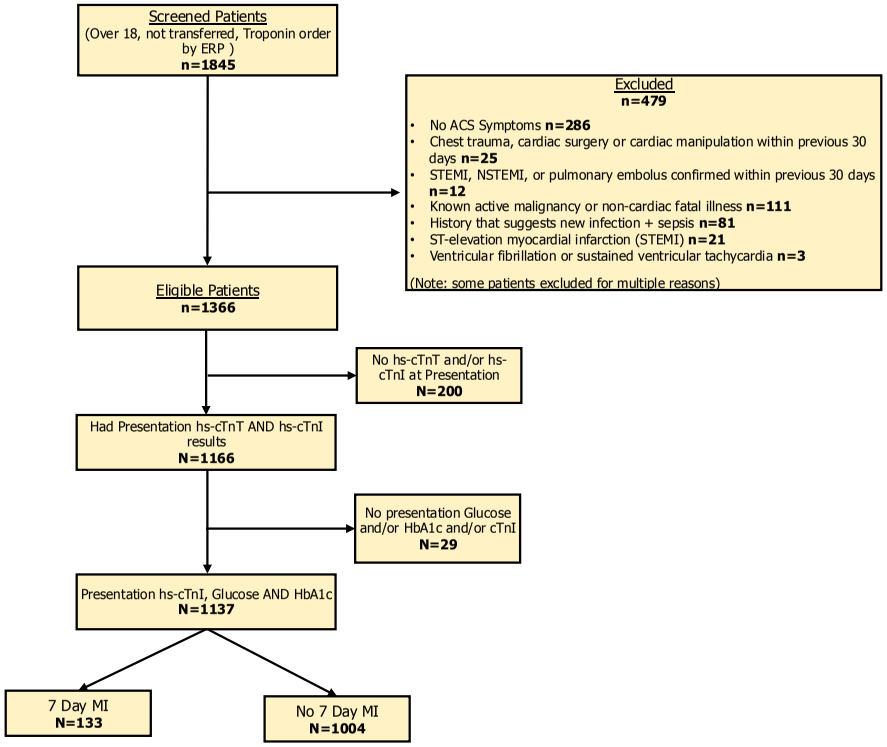
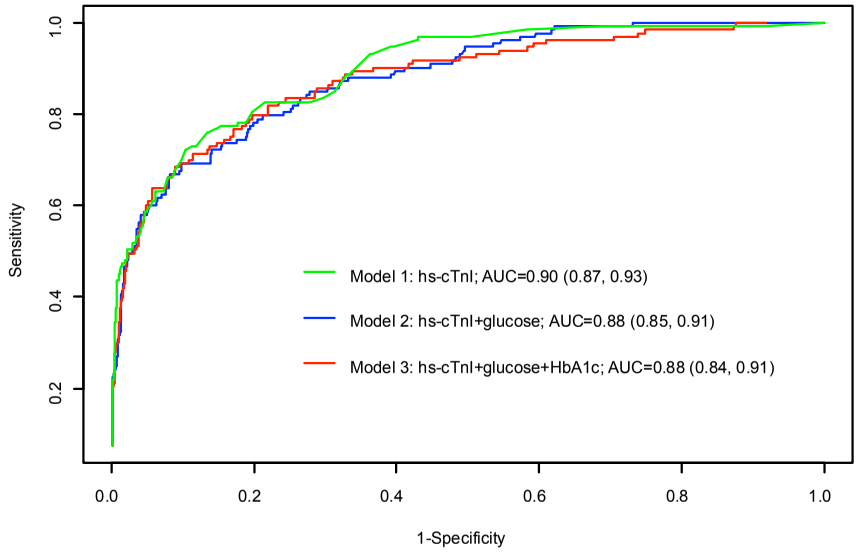
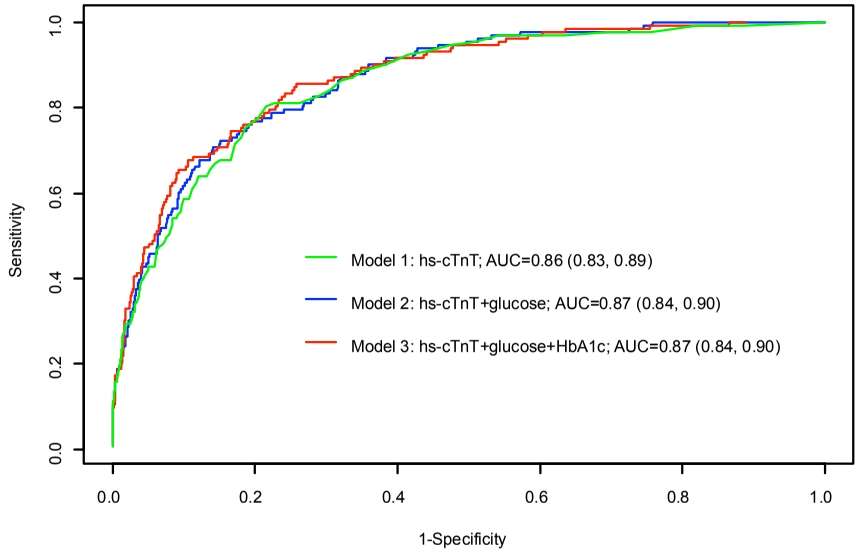


Figure 2a. ROC curve comparing hs-cTnI, hs-cTnI combined with glucose and hs-cTnI combined with glucose and HbA1c



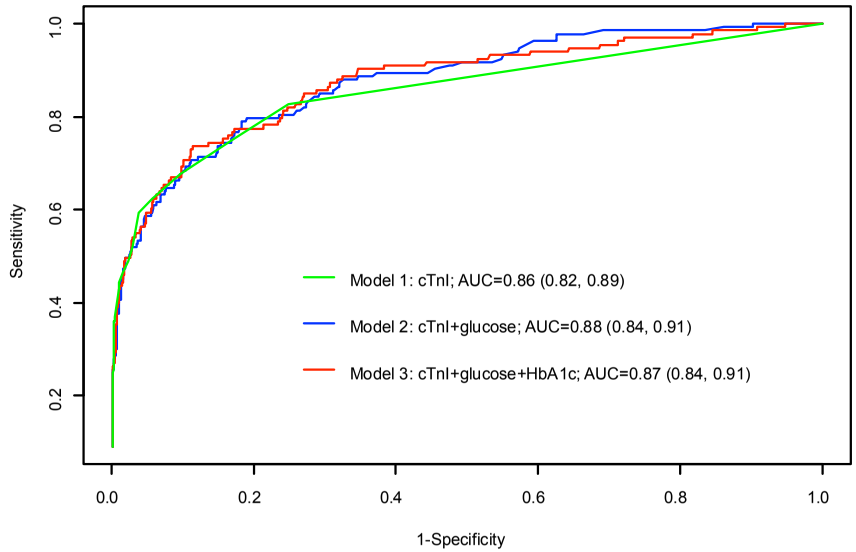
Comparison	AUC Difference (95% CI)	*p*-value
Model 2 vs. Model 1	-0.02 (-0.04, 0.01)	0.19
Model 3 vs. Model 1	-0.02 (-0.04, 0.01)	0.24
Model 3 vs. Model 2	0.00 (-0.01, 0.01)	0.74

Figure 2b. ROC curve comparing hs-cTnT, hs-cTnT combined with glucose and hs-cTnT combined with glucose and HbA1c



Comparison	AUC Difference (95% CI)	*p*-value
Model 2 vs. Model 1	0.01 (-0.01, 0.03)	0.52
Model 3 vs. Model 1	0.01 (-0.01, 0.04)	0.35
Model 3 vs. Model 2	0.01 (-0.01, 0.02)	0.45

Figure 2c. ROC curve comparing cTnI, cTnI combined with glucose and cTnI combined with glucose and HbA1c



Comparison	AUC Difference (95% CI)	*p*-value
Model 2 vs. Model 1	0.02 (-0.01, 0.05)	0.22
Model 3 vs. Model 1	0.02 (-0.02, 0.06)	0.34
Model 3 vs. Model 2	0.00 (-0.02, 0.01)	0.83

Table 1. Baseline Characteristics of cohort (n=1137), comparison of patients with and without MI at seven days

Characteristics	With MI at 7 Days (n=133)	No MI at 7 Days (n=1004)	*p*-values
Age, mean (SD)	73.3 (14.1)	65.8 (16.6)	<0.01
Sex, male (%)	69 (51.9%)	466 (46.4%)	0.235
Presenting Symptoms, n (%)			
Chest Pain	72 (54.1%)	579 (57.7%)	0.44
Arm pain (either arm)	23 (17.3%)	153 (15.2%)	0.54
Jaw pain	8 (6.0%)	28 (2.8%)	0.05
Neck pain	9 (6.8%)	57 (5.7%)	0.61
Back pain	17 (12.8%)	139 (13.8%)	0.74
Abdominal Pain	19 (14.3%)	103 (0.3%)	0.16
SOB	81 (60.9%)	539 (53.7%)	0.12
Dizzy or light headedness	29 (21.8%)	279 (27.8%)	0.14
Nausea and/or vomiting	39 (29.3%)	292 (29.1%)	0.95
Diaphoresis	34 (25.6%)	214 (21.3%)	0.26
Palpitations	9 (6.8%)	108 (10.8%)	0.15
Other	103 (77.4%)	731 (72.8%)	0.26

Cardiovascular Risk factors, n (%)			
Family history of CAD			
Yes	63 (47.4%)	553 (55.1%)	0.02
No	49 (36.8%)	364 (36.3%)	
Don't know	21 (15.8%)	87 (8.7%)	
Hypertension			
Yes	109 (82.0%)	695 (69.2%)	0.01
No	24 (18.1%)	301 (30.0%)	
Don't know	0 (0.0%)	8 (0.8%)	
Hypercholesterolemia			
Yes	91 (68.4%)	585 (58.3%)	0.06
No	39 (29.3%)	401 (39.9%)	
Don't know	3 (2.3%)	18 (1.8%)	
Diabetes			
Yes	53 (39.9%)	280 (27.9%)	0.02
No	79 (59.4%)	711 (70.8%)	
Don't know	1 (0.8%)	13 (1.3%)	
Smoking			
Current	35 (26.3%)	257 (25.6%)	0.03
Former	51 (38.4%)	357 (35.6%)	
Never	38 (28.6%)	364 (36.3%)	
Don't know	9 (6.8%)	26 (2.6%)	

CTAS Level			
1	10 (7.5%)	20 (2.0%)	<0.01
2	79 (59.4%)	530 (52.8%)	
3	44 (33.1%)	440 (43.8%)	
4	0 (0.0%)	14 (1.4%)	
Arrival by EMS, n (%)	83 (62.4%)	423 (42.1%)	<0.01
Cardiovascular History, n (%)			
Previous MI/ACS			
Yes	60 (45.1%)	348 (34.7%)	0.05
No	72 (54.1%)	636 (63.4%)	
Don't know	1 (0.8%)	20 (2.0%)	

Table 2. hs-cTnI Rule-in/Rule-out Algorithms. Sensitivity, Specificity, NPV, PPV expressed as value (95% CI)

hs-cTnI					
Algorithm 1			**Algorithm 2**		
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
hs-cTnI <5ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L	hs-cTnI <5ng/L AND Glucose <119mg/dL AND HbA1c <6.5%	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L
338	731	68	331	738	68
Sensitivity (95%CI) 99.2 (95.4-100)	n/a	**Specificity (95%CI)** 99.0 (98.2-99.5)	**Sensitivity (95%CI)** 99.2 (95.4-100)	n/a	**Specificity (95%CI)** 99.0 (98.2-99.5)
NPV (95%CI) 99.7 (98.2-100)	n/a	**PPV (95%CI)** 85.3 (74.8-92.0)	**NPV (95%CI)** 99.7 (98.2-100)	n/a	**PPV (95%CI)** 85.3 (74.8-92.0)

Algorithm 3				Algorithm 4		
Rule-out	Observe	Rule-in		Rule-out	Observe	Rule-in
hs-cTnI <4ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥82 ng/L AND Glucose ≥198mg/dL		hs-cTnI <5ng/L	Fails to meet In/Out criteria	hs-cTnI ≥64 ng/L
279	842	16		504	541	92
Sensitivity (95%CI) 100.0 (96.6-100)	n/a	**Specificity (95%CI)** 99.9 (99.4-100)		**Sensitivity (95%CI)** 97.0 (92.3-99.1)	n/a	**Specificity (95%CI)** 97.5 (96.3-98.3)
NPV (95%CI) 100.0 (98.4-100)	n/a	**PPV (95%CI)** 93.8 (69.7-100)		**NPV (95%CI)** 99.2 (97.9-99.8)	n/a	**PPV (95%CI)** 72.8 (62.9-80.9)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Table 3. hs-cTnT Rule-in/Rule-out Algorithms. Sensitivity, Specificity, NPV, PPV expressed as value (95% CI)

hs-cTnT					
Algorithm 1			**Algorithm 2**		
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
hs-cTnT <24ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L	hs-cTnT <24ng/L AND Glucose <101mg/dL AND HbA1c <6.5%	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L
261	850	26	252	859	26
Sensitivity (95%CI) 99.2 (95.4-100)	n/a	**Specificity (95%CI)** 99.5 (98.8-99.8)	**Sensitivity (95%CI)** 99.2 (95.4-100)	n/a	**Specificity (95%CI)** 99.5 (98.8-99.8)
NPV (95%CI) 99.6 (97.6-100)	n/a	**PPV (95%CI)** 80.8 (61.7-92.0)	**NPV (95%CI)** 99.6 (97.6-100)	n/a	**PPV (95%CI)** 80.8 (61.7-92.0)

Algorithm 3				Algorithm 4		
Rule-out	Observe	Rule-in		Rule-out	Observe	Rule-in
hs-cTnT <14ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥198mg/dL		hs-cTnT <14ng/L	Fails to meet In/Out criteria	hs-cTnT ≥52 ng/L
207	922	8		597	399	141
Sensitivity (95%CI) 100.0 (96.6-100)	n/a	**Specificity (95%CI)** 100.0 (99.5-100)		**Sensitivity (95%CI)** 92.5 (86.6-96.0)	n/a	**Specificity (95%CI)** 92.5 (90.7-94.0)
NPV (95%CI) 100.0 (62.8-100)	n/a	**PPV (95%CI)** 100 (62.8-100)		**NPV (95%CI)** 98.3 (96.9-99.1)	n/a	**PPV (95%CI)** 46.8 (38.8-55.0)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Table 4. cTnI Rule-in/Rule-out Algorithms. Sensitivity, Specificity, NPV, PPV expressed as value (95% CI)

cTnI					
Algorithm 1			**Algorithm 2**		
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL	cTnI <0.01ug/L AND Glucose <101mg/dL AND HbA1c <6.5%	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL
257	824	56	249	832	56
Sensitivity (95%CI) 99.2 (95.4-100)	n/a	**Specificity (95%CI)** 99.3 (98.5-99.7)	**Sensitivity (95%CI)** 99.2 (95.4-100)	n/a	**Specificity (95%CI)** 99.3 (98.5-99.7)
NPV (95%CI) 99.6 (97.6-100)	n/a	**PPV (95%CI)** 87.5 (76.1-94.1)	**NPV (95%CI)** 99.6 (97.6-100)	n/a	**PPV (95%CI)** 87.5 (76.1-94.1)

Algorithm 3				Algorithm 4		
Rule-out	Observe	Rule-in		Rule-out	Observe	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥198mg/dL		cTnI <0.01ug/L	Fails to meet In/Out criteria	cTnI ≥0.3 ug/L
257	866	14		777	329	31
Sensitivity (95%CI) 99.2 (95.4-100)	n/a	**Specificity (95%CI)** 100.0 (99.5-100)		**Sensitivity (95%CI)** 82.7 (75.3-88.3)	n/a	**Specificity (95%CI)** 99.8 (99.2-100)
NPV (95%CI) 99.6 (97.6-100)	n/a	**PPV (95%CI)** 100.0 (74.9-100)		**NPV (95%CI)** 97.0 (95.6-98.0)	n/a	**PPV (95%CI)** 93.5 (78.3-99.2)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Contribution Statement

Colleen Shortt developed the idea for this paper, alongside Dr. Peter Kavsak. She participated in data collection, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Jinhui Ma participated the statistical analyses, as well as the interpretation of data and manuscript revisions.

Natasha Clayton participated in data collection, as well as the interpretation of data and manuscript revisions.

Jonathan Sherbino participated in data collection and outcome adjudication, as well as the interpretation of data and manuscript revisions.

Richard Whitlock helped developed the concept for this study, as well as the interpretation of data and manuscript revisions.

Guillaume Pare helped developed the concept for this study, as well as the interpretation of data and manuscript revisions.

Stephen Hill contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

Matthew McQueen contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

Shamir R. Mehta contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

PJ Devereaux provided methodological and clinical expertise. He also participated in interpretation of data and manuscript revision.

Andrew Worster contributed to the original study design in which these data were sourced. He participated in data collection, interpretation of the data, and manuscript revisions.

Peter Kavsak contributed to the original study design in which these data were sourced. As well as participating in developing the idea for the study, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Chapter 5

Economic considerations of early rule-in/rule-out algorithms for the diagnosis of myocardial infarction in the emergency department using cardiac troponin and glycemic biomarkers

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Introduction

Emergency departments in Canada account for an ever increasing share of total healthcare expenditures (Canadian Institute for Health Information 2005). In addition, ED visits for chest pain represent a disproportionate number of the total visits to the ED (Canadian Institute for Health Information 2012) and therefore represents a large financial burden. Given the utility of incorporating additional testing, such as glucose, into an early rule-in/out algorithm for MI and MI/CV death at seven days and of HbA1c in the identification of previously unknown diabetes, the economic implications of such an approach must be taken into consideration. I therefore compared the health services cost of each different algorithm from chapter 4. I report that a rule-in/rule-out algorithm

incorporating glucose with cTn at presentation is the most cost effective method in comparison to the other algorithms under investigation, as well as fitting pre-specified criteria for safety. Incorporating HbA1c into these algorithms increased the overall cost but did not miss any additional patients.

Our findings highlight an opportunity to screen patients for diabetes, a well-known CVD risk-factor and may, therefore, have a large impact on not only healthcare spending but overall patient health and safety. In addition, our rule-in/out algorithms may decrease the overall time spent in the ED due to early decision making and, therefore, decrease ED congestion and reduce costs.

Article

Economic considerations of early rule-in/rule-out algorithms for the diagnosis of myocardial infarction in the emergency department using cardiac troponin and glycemic biomarkers

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Keywords: cost; cost-effectiveness; high-sensitivity cardiac troponin; glucose; Hemoglobin A1c; acute coronary syndrome; emergency department; rule-in/rule-out

Abbreviations: ED= Emergency Department; ACS= Acute Coronary Syndrome; MI= Myocardial Infarction; cTn= cardiac troponin; hs-cTn= High-sensitivity Cardiac Troponin; HbA1c= hemoglobin A1c; ROMI-3= Rule-out MI in 3 hours; NPV= Negative Predictive Value; PPV= Positive Predictive Value; ESC= European Society of Cardiology; OCCI= Ontario Case Costing Initiative; CVD= cardiovascular disease

Abstract

BACKGROUND: We have previously demonstrated the utility of a rule-in/rule-out strategy for myocardial infarction (MI) using glycemic biomarkers in combination with cardiac troponin in the emergency department (ED). Given that the cost of assessing patients with possible MI in the ED is increasing, we sought to compare the health services cost of our previously identified early rule-in/rule-out approaches for MI among patients who present to the ED with symptoms suggestive of acute coronary syndrome (ACS).

METHODS: We compared the cost differences between different rule-in/rule-out strategies for MI using presentation cardiac troponin I (cTnI), high-sensitivity cTnI (hs-cTnI), high-sensitivity cardiac troponin T (hscTnT), glucose, and/or hemoglobin A1c (HbA1c) in 1137 ED patients (7-day MI n 133) as per our previously defined algorithms and compared them with the European Society of Cardiology (ESC) 0-h algorithm cutoffs. Costs associated with each decision model were obtained from site-specific sources (length of stay) and provincial sources (Ontario Case Costing Initiative).

RESULTS: Algorithms incorporating cardiac troponin and glucose for early rule-in/rule-out were the most cost effective and clinically safest methods (i.e., 1 MI missed) for early decision making, with hs-cTnI and glucose yielding lower costs compared to cTnI and glucose, despite the higher

price for the hs-cTnI test. The addition of HbA1c to the algorithms increased the cost of these algorithms but did not miss any additional patients with MI. Applying the ESC 0-h algorithm-cutoffs for hs-cTnI and hs-cTnT were the most costly.

CONCLUSIONS: Rule-in/rule-out algorithms incorporating presentation glucose with high-sensitivity cardiac troponin are the safest and most cost-effective options as compared to the ESC 0-h algorithm-cutoffs.

Introduction

Hospital spending in Canada has steadily increased over the years (1). In 2008/2009, 160 Canadian emergency departments (ED) had approximately 5.4 million visits, costing $960 million (2). From 2004/2005–2008/2009 the number of visits, as well as the overall cost increased 6% and 28%, respectively (2). Despite increased funding, EDs continue to be burdened by overcrowding and long wait times (2). Acute coronary syndrome (ACS) in particular represents a large hospital burden. It is estimated that $1.6 billion dollars in direct healthcare costs are spent each year to treat ACS in Canada (3). Chest pain (CP) and abdominal pain, common complaints associated with ACS (4), account for 2 of the top 3 reasons Canadians visit the ED (5) with those in the higher age categories spending significantly more time in the ED than those who are younger (6). Furthermore, patients with more serious complaints, such as CP, can spend up to 12h in the ED thus further contributing to ED overcrowding (2).

Current guidelines for the diagnosis of myocardial infarction (MI) include serial measurements of cardiac troponin over several hours (4). Such serial measurements may increase costs, and ED length of stay (LOS). We have previously demonstrated the utility of using glucose in combination with high-sensitivity cardiac troponin I (hs-cTnI) and high-sensitivity cardiac troponin T (hs-cTnT) for early decision making in the ED

(7). We have expanded on these findings by demonstrating that glycemic biomarkers and cardiac troponin combinations can be used for the early rule-in/rule-out of MI (8). Several other early rule-in/rule-out protocols have been suggested (9 –12), each with their own merits. However, given that healthcare resources are scarce, the economic advantages and disadvantages of these types of approaches must be taken into consideration. Therefore, we sought to compare the health services cost between our previously identified early rule-in/rule-out approaches using cardiac troponin, glucose and/or hemoglobin A1c (HbA1c) for MI at 7 days among patients who present to the ED with symptoms suggestive of ACS to any of 3 adult hospitals in a Canadian city of more than half a million residents.

Methods

Data Collection and Outcome

This study was an a priori secondary data economic analysis of a prospective multicenter observational cohort study conducted across a Canadian city [Optimum Troponin Cutoffs for ACS in the ED (ROMI-3); Clinical Trials.gov identifier: NCT01994577]. The cohort used in this analysis has been previously described (8). Briefly, it comprised 1137 patients who presented to the ED with symptoms suggestive of ACS who had cTnI (Abbott ARCHITECT), hs-cTnI (Abbott ARCHITECT), hscTnT

(Roche Elecsys), glucose, and HbA1c measured. The clinical outcome for this analysis was MI at 7 days using the presentation samples with different testing algorithms as previously described (8).

Decision Tree

A decision analytical model (see Fig. 1) was constructed and populated using each of our previously reported early rule-in/rule-out algorithms (see Table 1 for algorithm details) (8). For each of the algorithms, we applied 4 different criteria:

Algorithm 1. The combination of analytes with the most patients ruled in and ruled out.

Algorithm 2. The combination of analytes that allows for highest rule-in/rule-out while incorporating HbA1c measurements.

Algorithm 3. The combination of analytes with the highest sensitivity and negative predictive value (NPV; rule-out arm) and the highest specificity and positive predictive value (PPV; rule-in arm).

Algorithm 4. The combination of 0-h high sensitivity cardiac troponin cutoffs similar to the APACE/ ADAPT studies, which used multiple measurements.

All branches of the decision analytical model were assigned probabilities based on the patient cohort of ROMI-3. Estimated probabilities were the risk of patients having low (dual or triple negative

i.e., all criteria for early rule-out are met), high (dual or triple positive i.e., all criteria for early rule-in are met) or intermediate risk (failed to meet rule-in/rule-out criteria). We also conducted additional analyses using the 0-h cutoffs from the European Society of Cardiology (ESC) 0/1h algorithms for the high-sensitivity cardiac troponin assays (13).

Costing

The costs incurred by each decision arm were compiled from several Canadian specific databases and resources. The Ontario Case Costing Initiative (OCCI) was used for admission costing, as well as ED costs. Four costing arms were retrieved. First, a visit to the ED with a final ED diagnosis of MI; second, an ED visit with a final diagnosis of “other CP” or “CP unspecified” (common discharge diagnosis for patients who undergo investigation for MI in the ED but ultimately are discharged home) for patients who are discharged home; third, hospital admission with a final diagnosis of MI (all discharge diagnoses with MI were included in this definition); fourth, hospital admission with a final discharge diagnosis of other CP or CP unspecified for patients who are admitted to hospital for MI but ultimately discharged without a diagnosis of MI. All costs from OCCI include all direct and indirect costs associated with providing care and were reported as averages. These costs were from OCCI fiscal year

2010/2011, and were adjusted for inflation (source: Stats Can) (14) for 2016.

ED LOS data was retrieved from the electronic patient data available from Hamilton Health Sciences. The average LOS was calculated for those who were admitted (mean LOS 7.9 h) and those who were not (mean LOS 6.7 h) from the ROMI-3 study. Using the LOS data and OCCI data, cost per hour of ED visit was determined.

All laboratory costs were provided by laboratory personnel and based on the listed costs for these services in 2016. Costs include reagent costs, analyzer costs, technologist time and all other direct costs associated with the tests.

The cost of a missed MI (i.e., an MI incorrectly ruled out at presentation) was quantified as the cost to reassess these patients. This cost includes the original cost of the ED visit (ED visit with a final diagnosis of other CP/CP unspecified), the cost of a return ED visit with an ED discharge diagnosis of MI and a hospital admission with a final diagnosis of MI. This is likely an underestimation of the true cost of a missed MI; however, the true cost of a missed MI is beyond the scope of this project. The details of the individual costs are listed in Table 2.

The health resources consumed by a patient who was ruled in include the specific algorithm laboratory costs, 1h of ED time at the average cost for an hour for a patient visit to the ED with a final ED

diagnosis of MI and either the admission cost for a patient with a discharge diagnosis of MI or other CP/CP unspecified depending on final diagnosis. The healthcare resources consumed by a patient who was ruled out include the specific algorithm laboratory costs, 1 h of ED time at the average cost for an hour for a patient to visit the ED with a final ED diagnosis of other CP/CP unspecified, depending on final diagnosis and if an MI was missed then the cost to reassess and admit a patient for MI. The health resources consumed by a patient who was required to stay for observation included the specific algorithm laboratory costs, the full cost for either a visit to the ED with a final ED diagnosis of other CP/CP unspecified or a final ED diagnosis of MI and admission for an MI, depending on final diagnosis.

All analyses were conducted from the perspective of the Ontario public healthcare payer. Assumptions of the study included: those who must stay for observation followed a similar workup to what is currently recommended (i.e., repeat cardiac troponin measurement at 3 h to detect a rise/fall pattern) (4); patients in the observe zone with an MI were detected and admitted, and those without an MI were discharged; all results for each test were returned within the same timeframe; early rule-out patients did not need any further follow-up for their CP; therefore, their resource utilization stopped after the early rule-out; all patients were triaged and seen in the same amount of time; those ruled out but later had

an MI returned within 7 days of their index visit and therefore, incurred extra costs to be reassessed. A one-way sensitivity analysis was also conducted by varying healthcare costs by ±10% across all high-sensitivity cardiac troponin algorithms with Tornado plots (see Figs. 1– 8 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol63/issue1.

Results

Baseline characteristics of this population have been previously described (8). A total of 1137 cases fit our inclusion/exclusion criteria and were included in the analyses. Of these, 133 had an adjudicated outcome of MI at 7 days. The diagnostic performance of the ESC 0-h algorithm-cutoffs for hs-cTnI and hs-cTnT are presented in online Supplemental Table 1.

Hs-cTnI Algorithms

The disposition for patients in each decision model for hs-cTnI for this cohort can be seen in Table 3, as well as the costs and number of MIs detected by each algorithm.

The hs-cTnI algorithm with the lowest cost was algorithm 4 with a total cost of $1 921 432.12. The cost of a missed MI (4 missed) accounted for almost 3% of the total cost, a cost that is more than 3 times that of the

other hs-cTnI algorithms with respect to missed MIs. Furthermore, the cost of an incorrectly ruled-in MI accounts for almost 11% of the total cost of admission. The total cost to observe patients (i.e., observational arm) using this algorithm was $1 012 414.85, with those who were subsequently discharged from the ED accounting for 22% of these costs. The average cost per patient using this method was $1689.91.

Algorithm 1 had a total cost of $1 956 633.36. The cost of a missed MI using this algorithm was <1% of the total costs. The cost of an incorrectly ruled-in MI accounted for 5.4% of the total cost of admission. The total cost to observe patients using algorithm 1 was $1 251 609.04, with those who were subsequently discharged accounting for 25% of these costs. The average cost per patient using this method was $1720.87. In comparison to algorithm 1, algorithm 2 had an additional cost of HbA1c for every patient. The total cost of this algorithm was $1 969 511.40, a difference of $12 878.04, changing the average cost per patient to $1732.20 due to the extra testing, as well as the 7 patients who would be required to stay for observation, rather than ruled out early. The cost to observe these patients was $1 261 535.12, with those who were subsequently discharged from the ED accounting for 25% of these costs.

Lastly, algorithm 3 had a total cost of $2 034 574.46. This method did not discharge home any patients with an MI; however, a large portion

of costs (91%) were used for the observational zone patients making the average cost per patient $1789.42.

Applying the ESC 0-h algorithm-cutoffs revealed that this method missed the same number of MIs as algorithms 1 and 2, however it cost significantly more at a total cost of $2 085 178.85 or a total cost per patient of $1833.93. Using algorithm 3, for which no MIs were missed, was also more cost-effective than applying the ESC 0-h algorithm-cutoffs.

One-way sensitivity analysis revealed that our conclusions were insensitive to a 10% variation in any of the cost components of the algorithms with the exception of the cost of an admission with a discharge diagnosis of MI for algorithms 1–3. The incremental cost between algorithms 1–3 and ESC varied from $151 689.73, $152 877.73 and $250 435.01, respectively if the cost of admission with a discharge diagnosis of MI was $11 507 to -$124 490.70, -$123 302.70 and -$27 837.70, respectively if the cost was $9415 (see online Supplemental Figs. 1– 4).

Hs-cTnT Algorithms

The disposition for patients in each decision model for hs-cTnT, along with total costs can be seen in Table 4. Unlike the hs-cTnI algorithms, the algorithm with the lowest overall cost was algorithm 1 (includes hs-cTnT and glucose) with a total cost of $2 043 442.09. The cost of a missed MI was <1% of the total costs, whereas the cost of an

incorrectly ruled-in MI was 7.3% of the total costs of admission. The total cost to observe patients using this algorithm was $1 761 766.85, with those who were subsequently discharged accounting for 20% of these costs. The average cost per patient using algorithm 1 was $1797.22. Like hs-cTnI algorithm 2, hs-cTnT algorithm 2 had an additional cost of HbA1c for every patient. The total cost of this method was $2 057 075.86, a difference of $13 633.77, thus making the average cost per patient $1809.21 owing to the extra testing, as well as the 9 patients who would be required to stay for observation, rather than ruled out early. The cost to observe these patients was $1 773 720.24, with those who were subsequently discharged from the ED accounting for 20% of the total observation costs.

Algorithm 4 had total cost of $2 051 781.27. The cost of missing an outcome accounts for 6.8% of the total costs, a cost that was almost 10 times that of the other hs-cTnT algorithms with respect to a missed MI. Furthermore, the cost of an incorrectly ruled-in MI accounted for more than a quarter of all costs, incurring large costs for the observation of true positives–over half a million dollars. The total cost to observe patients using this algorithm was only $885 109.11, with those who were subsequently discharged with no MI accounting for 18% of these costs. The average cost per patient using algorithm 4 was $1804.56.

Algorithm 3 had a total cost of $2 072 533.03. Although no extra costs were incurred for a missed MI, the cost of observing such a large number of patients accounted for almost 95% of the total costs, making the total cost per patient $1822.81.

Similar to the hs-cTnI algorithms, the ESC algorithm for hs-cTnT was the most expensive method with a total cost of $2 206 738.83 or $1940.84 per patient. This method had the same MI miss rate as algorithms 1 and 2, and was more expensive than algorithm 3, which did not miss any MIs.

One-way sensitivity analysis revealed that our conclusions were insensitive to a 10% variation in any of the cost components of the algorithm with the exception of the cost of an admission with a discharge diagnosis of MI for algorithm 3. The incremental cost between hs-cTnT algorithm 3 and ESC varied from $254 386.22 if the cost of admission with a discharge diagnosis of MI was $11 507 to -$23 886.49 if the cost was $9415 (see online Supplemental Figs. 5– 8).

cTnI Algorithms

The disposition and costs for patients in each decision model for cTnI can be seen in Table 5.

Similar to the hs-cTnI algorithm, the decision tree with the lowest overall cost was algorithm 4, with a total cost of $1 850 945.93, or

$1627.92 per patient. The cost of missing an outcome was 17.4% of the total costs, a cost that is 25% more than that of the other algorithms with respect to missed MIs, whereas the cost of incorrectly ruling in a patient was only 2.2% of the total costs of admission. The cost to observe patients using this algorithm was $1 146 659.74, with those who were subsequently discharged from the ED without an MI accounting for 10% of these costs.

Algorithm 1 had the second lowest total cost at $1 993 516.30. The cost of a missed MI using this algorithm was <1% of the total costs. The cost of an incorrectly ruled-in MI accounted for 4.5% of the total cost of admission. Observation of patients using algorithm 1 cost $1 404 104.76, with those who were subsequently discharged from the ED without an MI accounting for almost 25% of these costs. The average cost per patient using this method was $1753.31. Like the hs-cardiac troponin algorithm 1, algorithm 2 had an additional cost of HbA1c for every patient. The total cost of this method was $2 006 772.21, a difference of $13 255.91, changing the average cost per patient to $1764.97 due to the extra testing, as well as the 8 patients who would be required to stay for observation, rather than ruled out early. The total cost to observe patients using this algorithm was $1 415 330.00, with those who were subsequently discharged without an MI accounting for 25% of these costs.

Algorithm 3 had a total cost of $2 041 124.78. This method still missed 1 MI patient and a large portion of costs (91%) were used for the observational zone making the average cost per patient $1795.18.

As the ESC 0-h algorithm-cutoffs are for hs-cardiac troponin assays only, no comparison was performed for the cTnI algorithms.

Discussion

Using our previously reported early rule-in/rule-out algorithms, we describe the economic implications of different diagnostic strategies for patients presenting to the ED with symptoms suggestive of ACS.

The most clinically acceptable and cost-effective method for ruling in and ruling out MI in the ED at presentation is algorithm 1 for all cardiac troponin assays, which includes the measurement of glucose in these algorithms. Although using algorithm 4, cardiac troponin alone, provided the lowest cost option for hs-cTnI and cTnI, the safety of these algorithms exceeded acceptable standards (i.e., sensitivity for MI <99%) (11) with the number of patients with an MI who would be discharged home (i.e., missed MIs) being too high. However, for hs-cTnT, the most cost effective algorithm was algorithm 1, which included glucose as opposed to hs-cTnT alone, likely due to a large number of patients incorrectly ruled in at presentation. Comparing all 3 cardiac troponin assays for algorithms 1–3, algorithms that incorporated hs-cTnI were the most cost effective in our

study. Not surprisingly, the cost to observe patients was lowest when using algorithm 4 for all cardiac troponin assays given the large number of patients who were ruled out, however, as previously mentioned this method ruled out a large number of patients who subsequently had an MI and is therefore not likely to be safe. Using algorithm 1 for all the cardiac troponin assays had the second lowest overall cost for observation, further solidifying the conclusion that our early rule-in/rule-out algorithm using cardiac troponin and glucose is the most cost-effective and clinically acceptable. Our conclusions are also insensitive to costing variation of ±10%, with the exception of the cost of admission with a discharge diagnosis of MI, likely due to the large relative importance of this parameter in these models.

As expected, the addition of HbA1c increased the overall cost of the algorithm; however, as previously reported, the addition of HbA1c to decision tree/algorithm 1 identified diabetes in 50 patients who had previously unknown diabetes in this cohort (8). The identification of diabetes in patients with previously unknown diabetes may have considerable health benefits and therefore cost saving opportunities. For example, the cost difference between hs-cTnI algorithm 1 and hs-cTnI algorithm 2 was $12 878.04, $257.56 per new case of diabetes identified or an extra $11.33 per patient to screen for diabetes. Although the lifetime cost saving opportunity was not taken into account in this paper, it is

important to note the implications of such an approach for both health benefits as well as economic savings. The Canadian Diabetes Association (CDA) estimates that at least 700 000 people in Canada have diabetes and do not know it (15) and by the year 2020 they estimate that 3.7 million Canadians will have diabetes and account for 3.5% of public healthcare spending (15). Evidence suggests that early intervention can help prevent diabetic complications (15–18). Therefore, with these data/information it might be reasonable to begin to evaluate a screening program for diabetes in the ED for patients presenting with ACS. However, additional studies assessing the prevalence of diabetes and different interventions in patients presenting with symptoms suggestive of ACS in the ED are needed to more thoroughly assess the impact of this additional testing. It is also important to comment on algorithm 3 for hs-cTnI and hs-cTnT because these algorithms do not miss a single MI and are more cost effective than employing the ESC 0-h algorithm-cutoffs. It is also noteworthy that despite the higher price for the hs-cTnI test as compared to the cTnI test on the Abbott ARCHITECT platform, the most cost-effective algorithms include hs-cTnI testing. These data further support hs-cTnI as a clinically superior assay and in these algorithms more cost-effective than cTnI.

Limitations of this study are noted. The cost estimates were not based on specific costs associated with our research centers but rather

reflect the Ontario provincial cost of rendering services to patients similar to those in our study. Although this method is not specific to the city in which the study was conducted, it does make the results more generalizable. We have also likely underestimated the cost of a missed MI because we have only taken into consideration the direct cost of reassessing those patients under our algorithm. Given that patients with missed MIs are at higher risk for mortality and major cardiovascular events (19), we have likely underestimated the cost saving and life-saving opportunity of the algorithms. There are also likely quality-of-life implications for missing an MI and possible medicolegal issues as well, but such analyses are beyond the scope of this project. Further, the cost implications of these algorithms for patients with diagnostic ECGs at ED presentation were not evaluated in this study and require further investigation. Lastly, the sensitivity analysis should be interpreted with caution, because it is unlikely that only 1 parameter would change at a time in “real-world” settings.

Conclusion

Use of glycemic biomarkers with cardiac troponin has both clinical and economic implications that may help reduce the ED burden for patients with possible ACS. Both patients and the healthcare system may

benefit from early rule-in/rule-out strategies encompassing high sensitivity cardiac troponin and glucose.

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Table 1. Rule-in/Rule-out algorithms (from Shortt, 2016) (8)

hs-cTnI			
Method	**Rule-Out**	**Observe**	**Rule-in**
Algorithm 1	hs-cTnI <5ng/L AND Glucose <6.6mmol/L	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L
Algorithm 2	hs-cTnI <5ng/L AND Glucose <6.6mmol/L AND HbA1c <6.5%	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L
Algorithm 3	hs-cTnI <4ng/L AND Glucose <6.6mmol/L	Fails to meet In/Out criteria	hs-cTnI≥82 ng/L AND Glucose ≥11mmolL
Algorithm 4	hs-cTnI <5ng/L	Fails to meet In/Out criteria	hs-cTnI≥64 ng/L
ESC 0h cutoffs	hs-cTnI <2ng/L	Fails to meet In/Out criteria	hs-cTnI ≥52 ng/L
hs-cTnT			
Method	**Rule-Out**	**Observe**	**Rule-in**
Algorithm 1	hs-cTnT<24ng/L AND Glucose <5.6mmol/L	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L
Algorithm 2	hs-cTnT<24ng/L AND Glucose <5.6mmol/L AND HbA1c <6.5%	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L
Algorithm 3	hs-cTnT <14ng/L AND Glucose <5.6mmol/L	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥11mmol/L
Algorithm 4	hs-cTnT <14ng/L	Fails to meet In/Out criteria	hs-cTnT ≥52 ng/L
ESC 0h cutoffs	hs-cTnT <5ng/L	Fails to meet In/Out Criteria	hs-cTnT ≥52 ng/L

cTnI			
Method	**Rule-Out**	**Observe**	**Rule-in**
Algorithm 1	cTnI <0.01ng/L AND Glucose <5.6mmol/L	Fails to meet In/Out criteria	cTnI ≥0.09 ng/L AND Glucose ≥5.6mmol/L
Algorithm 2	cTnI <0.01ng/L AND Glucose <5.6mmol/L AND HbA1c <6.5%	Fails to meet In/Out criteria	cTnI ≥0.09 ng/L AND Glucose ≥5.6mmol/L
Algorithm 3	cTnI <0.01ng/L AND Glucose <5.6mmol/L	Fails to meet In/Out criteria	cTnI ≥0.09 ng/L AND Glucose ≥11mmol/L
Algorithm 4	cTnI <0.01ng/L	Fails to meet In/Out criteria	cTnI ≥0.3 ng/L

Table 2. Health services cost

Service	**Cost**	**Median LOS (hours)**	**Cost/Hour**
hs-cTnI	$20.00		
hs-cTnT	$20.00		
cTnI	$18.00		
Glucose	$5.00		
HbA1c	$9.00		
Admit, Discharge Diagnosis of MI	$10,461.38		
Admit, Discharge Diagnosis of other CP or CP unspecified	$3,234.63		
ED visit, Discharge Diagnosis of MI	$2,261.92	7.9	$286.32
ED visit, Discharge Diagnosis of other CP or CP unspecified	$444.16	6.7	$66.29
Untreated Outcome	$13,891.67		

Table 3. Patient disposition using hs-cTnI rule-in/rule-out algorithms with total costs

hs-cTnI						
Method	**Disposition**	**Criteria**	**Outcome**	**N**	**Cost per Patient**	**Cost**
1	**Rule-in**	hs-cTnI ≥99 ng/L	7 Day MI	58	$10,772.70	$624,816.54
			No 7 Day MI	10	$3,545.95	$35,459.49
	Observe	Fails to meet In/Out criteria	7 Day MI	74	$12,748.30	$943,374.20
			No 7 Day MI	657	$469.16	$308,234.84
	Rule-out	hs-cTnI <5ng/L AND Glucose <6.6mmol/L	7 Day MI	1	$13,982.96	$13,982.96
			No 7 Day MI	337	$91.29	$30,765.33
Total						$1,956,633.36

2	**Rule-in**	hs-cTnI ≥99 ng/L	7 Day MI	58	$10,781.70	$625,338.54
			No 7 Day MI	10	$3,554.95	$35,549.49
	Observe	Fails to meet In/Out criteria	7 Day MI	74	$12,757.30	$944,040.20
			No 7 Day MI	664	$478.16	$317,494.92
	Rule-out	hs-cTnI <5ng/L AND Glucose <6.6mmol/L AND HbA1c <6.5%	7 Day MI	1	$13,991.96	$13,991.96
			No 7 Day MI	330	$100.29	$33,096.29
Total						$1,969,511.40

3	**Rule-in**	hs-cTnI ≥82 ng/L AND Glucose ≥11mmol/L	7 Day MI	15	$10,772.70	$161,590.48
			No 7 Day MI	1	$3,545.95	$3,545.95
	Observe	Fails to meet In/Out criteria	7 Day MI	118	$12,748.30	$1,504,299.40
			No 7 Day MI	724	$469.16	$339,668.22
	Rule-out	hs-cTnI <4ng/L AND Glucose <6.6mmol/L	7 Day MI	0	n/a	$0
			No 7 Day MI	279	$91.29	$25,470.41
Total						$2,034,574.46

4	**Rule-in**	hs-cTnI ≥64 ng/L	7 Day MI	67	$10,767.70	$721,435.83
			No 7 Day MI	25	$3,540.95	$88,523.72
	Observe	Fails to meet In/Out criteria	7 Day MI	62	$12,743.30	$790,084.60
			No 7 Day MI	479	$464.16	$222,330.25
	Rule-out	hs-cTnI <5ng/L	7 Day MI	4	$13,977.96	$55,911.83
			No 7 Day MI	500	$86.29	$43,145.90
	Total					$1,921,432.12

ESC 0h	**Rule-in**	hs-cTnI ≥52 ng/L	7 Day MI	71	$10,767.70	$764,506.63
			No 7 Day MI	36	$3,540.95	$127,474.16
	Observe	Fails to meet In/Out criteria	7 Day MI	62	$12,743.30	$790,084.60
			No 7 Day MI	809	$464.16	$375,501.40
	Rule-out	hs-cTnI <2ng/L	7 Day MI	1	$13,977.96	$13,977.96
			No 7 Day MI	158	$86.29	$13,634.10
	Total					$2,085,178.85

Table 4. Patient disposition using hs-cTnT rule-in/rule-out algorithms with total costs

hs-cTnT						
Method	**Disposition**	**Criteria**	**Outcome**	**N**	**Cost per Patient**	**Cost**
1	**Rule-in**	hs-cTnT ≥206 ng/L	7 Day MI	21	$10,772.70	$226,226.68
			No 7 Day MI	5	$3,545.95	$17,729.74
	Observe	Fails to meet In/Out criteria	7 Day MI	111	$12,748.30	$1,415,061.30
			No 7 Day MI	739	$469.16	$346,705.55
	Rule-out	Hs-cTnT <24ng/L AND Glucose <5.6mmol/L	7 Day MI	1	$13,982.96	$13,982.96
			No 7 Day MI	260	$91.29	$23,735.87
Total						$2,043,442.09

2	**Rule-in**	hs-cTnT ≥206 ng/L	7 Day MI	21	$10,781.70	$226,415.68
			No 7 Day MI	5	$3,554.95	$17,774.74
	Observe	Fails to meet In/Out criteria	7 Day MI	111	$12,757.30	$1,416,060.30
			No 7 Day MI	748	$478.16	$357,659.94
	Rule-out	hs-cTnT <24ng/L AND Glucose <5.6mmol/L AND HbA1c <6.5%	7 Day MI	1	$13,991.96	$13,991.96
			No 7 Day MI	251	$100.29	$25,173.24
Total						$2,057,075.86

3	**Rule-in**	hs-cTnT ≥206 ng/L AND Glucose ≥11mmol/L	7 Day MI	8	$10,772.70	$86,181.59
			No 7 Day MI	0	n/a	$-
	Observe	Fails to meet In/Out criteria	7 Day MI	125	$12,748.30	$1,593,537.50
			No 7 Day MI	797	$469.16	$373,916.54
	Rule-out	hs-cTnT <14ng/L AND Glucose <5.6mmol/L	7 Day MI	0	n/a	$-
			No 7 Day MI	207	$91.29	$18,897.40
Total						$2,072,533.03

4	**Rule-in**	hs-cTnT ≥52 ng/L	7 Day MI	66	$10,767.70	$710,668.13
			No 7 Day MI	75	$3,540.95	$265,571.17
	Observe	Fails to meet In/Out criteria	7 Day MI	57	$12,743.30	$726,368.10
			No 7 Day MI	342	$464.16	$158,741.01
	Rule-out	hs-cTnT <14ng/L	7 Day MI	10	$13,977.96	$139,779.57
			No 7 Day MI	587	$86.29	$50,653.28
	Total					$2,051,781.27

ESC 0h	**Rule-in**	hs-cTnT ≥52 ng/L	7 Day MI	66	$10,767.70	$710,668.13
			No 7 Day MI	75	$3,540.95	$265,571.17
	Observe	Fails to meet In/Out criteria	7 Day MI	67	$12,743.30	$853,801.10
			No 7 Day MI	748	$464.16	$347,187.94
	Rule-out	hs-cTnT <5ng/L	7 Day MI	1	$13,977.96	$13,977.96
			No 7 Day MI	180	$86.29	$15,532.52
	Total					$2,206,738.83

Table 5. Patient disposition using cTnI rule-in/rule-out algorithms with total costs

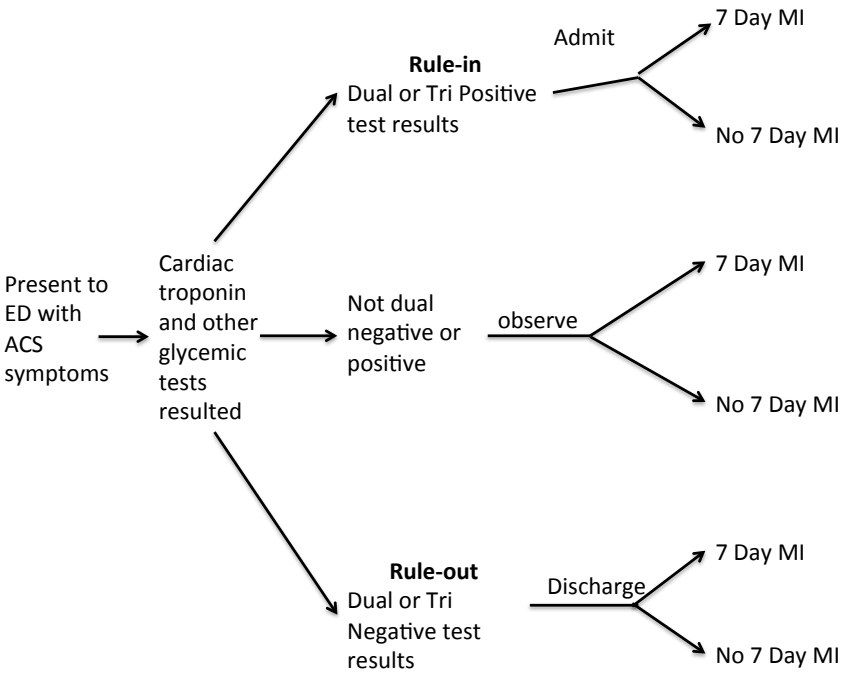
cTnI						
Method	**Disposition**	**Criteria**	**Outcome**	**N**	**Cost per Patient**	**Cost**
1	**Rule-in**	cTnI ≥0.09 ng/L AND Glucose ≥5.6mmol/L	7 Day MI	49	$10,770.70	$527,764.25
			No 7 Day MI	7	$3,543.95	$24,807.64
	Observe	Fails to meet In/Out criteria	7 Day MI	83	$12,746.30	$1,057,942.90
			No 7 Day MI	741	$467.16	$346,161.86
	Rule-out	cTnI <0.01ng/L AND Glucose <5.6mmol/L	7 Day MI	1	$13,980.96	$13,980.96
			No 7 Day MI	256	$89.29	$22,858.70
Total						$1,993,516.30

2	**Rule-in**	cTnI ≥0.09 ng/L AND Glucose ≥5.6mmol/L	7 Day MI	49	$10,779.70	$528,205.25
			No 7 Day MI	7	$3,552.95	$24,870.64
	Observe	Fails to meet In/Out criteria	7 Day MI	83	$12,755.30	$1,058,689.90
			No 7 Day MI	749	$476.16	$356,640.10
	Rule-out	cTnI <0.01ng/L AND Glucose <5.6mmol/L AND HbA1c <6.5%	7 Day MI	1	$13,989.96	$13,989.96
			No 7 Day MI	248	$98.29	$24,376.36
Total						$2,006,772.21

3	**Rule-in**	cTnI ≥0.09 ng/L AND Glucose ≥11mmol/L	7 Day MI	14	$10,770.70	$150,789.79
			No 7 Day MI	0	n/a	$-
	Observe	Fails to meet In/Out criteria	7 Day MI	118	$12,746.30	$1,504,063.40
			No 7 Day MI	748	$467.16	$349,431.94
	Rule-out	cTnI <0.01ng/L AND Glucose <5.6mmol/L	7 Day MI	1	$13,980.96	$13,980.96
			No 7 Day MI	256	$89.29	$22,858.70
Total						$2,041,124.78

4	**Rule-in**	cTnI ≥0.3 ng/L	7 Day MI	29	$10,765.70	$312,205.27
			No 7 Day MI	2	$3,538.95	$7,077.90
	Observe	Fails to meet In/Out criteria	7 Day MI	81	$12,741.30	$1,032,045.30
			No 7 Day MI	248	$462.16	$114,614.44
	Rule-out	cTnI <0.01ng/L	7 Day MI	23	$13,975.96	$321,447.01
			No 7 Day MI	754	$84.29	$63,556.01
	Total					$1,850,945.93

Figure 1. Flow of patients in the emergency department



Contribution Statement

Colleen Shortt developed the idea for this paper, alongside Dr. Peter Kavsak. She participated in data collection, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Feng Xie participated in the statistical analyses, as well as the interpretation of data and manuscript revisions.

Jinhui Ma participated in the statistical analyses, as well as the interpretation of data and manuscript revisions.

Natasha Clayton participated in data collection, as well as the interpretation of data and manuscript revisions.

Jonathan Sherbino participated in data collection and outcome adjudication, as well as the interpretation of data and manuscript revisions.

Richard Whitlock helped developed the concept for this study, as well as the interpretation of data and manuscript revisions.

Guillaume Pare helped developed the concept for this study, as well as the interpretation of data and manuscript revisions.

Stephen Hill contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

Matthew McQueen contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

Shamir R. Mehta contributed to the original study design in which these data were sourced. He also participated in interpretation of data and manuscript revision

PJ Devereaux provided methodological and clinical expertise. He also participated in interpretation of data and manuscript revision.

Andrew Worster contributed to the original study design in which these data were sourced. He participated in data collection, interpretation of the data, and manuscript revisions.

Peter Kavsak contributed to the original study design in which these data were sourced. As well as participating in developing the idea for the study, statistical analyses, interpretation of the data, writing the first draft of the manuscript, as well as revisions.

Chapter 6: Conclusion

6.1 Overview

This doctoral thesis explored the concept, from inception to testing, of an early rule-in and rule-out algorithm for MI. These algorithms were also applied to two different outcomes; MI or CV death and ACS or CV death within the first week for patients presenting to the ED with symptoms of ACS. The original studies in this thesis highlight that glycemic biomarkers, specifically glucose, in combination with cTn may be useful for early decision-making in the ED for patients who present with symptoms of ACS. In addition, this thesis demonstrates that this approach is a cost effective solution that may help with ED overcrowding and spending.

6.2 Implications and Future Direction

6.2.1 Acute Coronary Syndrome and Hyperglycemia

This thesis has demonstrated that glucose can improve the risk assessment capabilities of cTn through identification of low-risk and high-risk subgroups. This is consistent with the current evidence that diabetes and/or patients with dysglycemia tend to fare poorly in a variety of health settings including surgery (Viana et al. 2014), acute heart failure (Seferović et al. 2014; de Miguel-Yanes et al. 2015), sepsis (Ali et al. 2008), and other critically ill patients (Viana et al. 2014). Future studies

regarding early-decision making should aim to explore how well these algorithms compare to other common decision-making tools and guidelines or even how they can be combined. For example, current guidelines recommend the use of change criteria when using only cTn (Thygesen et al. 2012) with European guidelines endorsing a 1 hour protocol for rule-in/out of MI (Roffi et al. 2016). There are also indications of sex-specific differences in cTn concentrations (Shah et al. 2015; Apple et al. 2003; Cullen et al. 2016), two concepts not explored in this thesis. In addition, future research should aim to prospectively validate these results and determine feasibility with respect to healthcare costs. One large (n=1412) study out of Australia and New Zealand did evaluate a dual panel approach using a “low-normal” hs-cTnI (<4ng/L) and a “normal” glucose (<5.6mmol/L) concentration (Greenslade et al. 2015) and found similar results to the paper presented in chapter 3, which further supports our argument for the use of glucose in a multi-marker approach designed for early decision making.

Previous literature has explored whether hyperglycaemia is a marker or mediator of adverse cardiovascular events and while I provide evidence that it is useful for the identification of vulnerable patients, no further conclusions can be made regarding this issue from my data. Future studies should continue to investigate the specific mechanisms regarding hyperglycemia and adverse outcomes in ACS patients. Further, at

present, I do not believe the evidence presented in this thesis is sufficient to recommend intense glucose management in patients presenting with symptom of ACS given the complex nature of ACS treatment, the uncertainty if glucose is a mediator of adverse outcomes or simply a marker, and the conflicting evidence regarding tight glucose control. Clarity on this issue will allow for continued advancements in early-decision making algorithms, as well as how to appropriately treat hyperglycemia in these patients, if at all. Some groups have attempted to do so. In 1995, the DIGAMI study (Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction study) reported that intensive glucose-insulin treatment reduced long-term mortality in diabetic patients with AMI (Malmberg et al. 1995). However, when DIGAMI-2 was subsequently conducted it failed to replicate the original study's results and showed no significant improvement in mortality in those receiving intense glucose-insulin treatment (Malmberg et al. 2005). A 2013 study published in JAMA internal medicine aimed to determine if strict treatment of hyperglycemia would limit infarct size in ACS patients. They found that rapid (almost) normalization of blood glucose could be achieved in ACS patients, however this reduction in glucose concentrations was not accompanied by a reduction in infarct size and the rate of death or a second MI was actually increased (de Mulder et al. 2013).

6.2.2 Diabetes Screening in Emergency Departments

The burden of diabetes has increased with the growing prevalence of diabetes worldwide since 1980; therefore there is an increasing need for appropriate interventions, coupled with early detection (Ezzati 2016). Additionally, the risk for hospitalization for patients with diabetes, undiagnosed diabetes and prediabetes is significantly higher compared to those without diabetes, of which the most common reason for hospitalization is cardiovascular disease (Schneider et al. 2016). Our rule-in/rule-out algorithm 2 from chapter 4 has identified an avenue for the detection of undiagnosed diabetes in chest pain patients. This may represent an unique opportunity to prevent or delay some of the major issues associated with diabetes such as cardiovascular disease, renal disease, non-traumatic limb amputation and the associated costs (Canadian Diabetes Association 2013).

Literature has recommended that improvement of glycemic control among diabetics and the prevention of progression to overt diabetes for those in the prediabetes category is necessary in order to reduce hospital burden (Schneider et al. 2016; Canadian Diabetes Association 2013). An ED screening program we may be able to identify those who would greatly benefit from intense interventions and allow for early implementation. Currently the ADA recommends eight key areas to focus on for diabetic

patients and include “diagnosis, glycemic targets, medical management, hypoglycemia, cardiovascular risk factor management, microvascular disease screening and management, and inpatient diabetes management”(Chamberlain et al. 2016).

There are many options with respect to appropriate interventions for diabetic and pre-diabetic patients, however many studies recommend a focus on overall risk factor reduction. Evidence suggests that integrative health coaching (IHC), a type of coaching focused on personal goal determination and sustainable behavioral changes (i.e., focused on a personal sense of motivation and not just diabetes related goals), can significantly impact HbA1c, as well as medication adherence (Wolever and Dreusicke 2016). There is also evidence that meeting more of the American Heart Association’s ideal cardiovascular health (ICH) components (total cholesterol, blood pressure, dietary intake, smoking, physical activity and BMI) may lower a patient’s risk for diabetes over a medium follow-up of 11.1 years (Joseph et al. 2016), therefore target screening of those who present to the ED with symptoms of ACS may be the ideal population to make a large impact. This study notes that attainment of even four of the ICH components could significantly affect diabetes development (Joseph et al. 2016). Evidence also suggests a role for glycemic control in diabetic patients in order to decrease a patient’s average HbA1c value (Patel et al. 2008), to reduce complications such as

diabetic retinopathy progression (The Action to Control Cardiovascular Risk in Diabetes Follow-On (ACCORDION) Eye Study Group 2016), and other microvascular complications (Patel et al. 2008). Further, the use of very low caloric diets may help to reduce fasting plasma glucose concentrations and potentially put diabetes into remission (characterized as improved acute insulin secretion, and improved hepatic insulin sensitivity) for at least six months, particularly in those who have a shorter diabetes duration (another argument for early identification) (Steven et al. 2016). Caution should be noted however in intense glucose control as it is also associated with increased rates of hypoglycemia and hospitalization (Patel et al. 2008).

The Community Preventive Service Task Force further recommends programs with a combination of diet and physical activity for those at high-risk for diabetes and diabetic complications. They note that studies show this approach is cost-effective to implement (Pronk and Remington 2015).

Future work should include investigation into the feasibility and cost of an ED screening program for diabetes in patients presenting to the ED with symptoms suggestive of ACS. This work should focus on the health services costs associated with the increased testing as well as follow-up for patients with newly diagnosed diabetes. In addition, the cost savings of

identifying diabetes in its early stages should be examined, as well as the quality of life implications.

6.4 Limitations

In this thesis, I present data suggesting a role of glycemic markers in risk assessment of patients who present to the ED with symptoms of ACS. However, there are some limitations of each study that must be considered. Chapter 2 shows data on small study that was exploratory in nature, therefore a significant relationship between GPBB and adverse outcomes is difficult to determine. Furthermore, the small sample size makes the group less generalizable, along with the constraints associated with presentation time (i.e., presentation within 6 hours of pain onset). Despite these limitations, these data do identify an avenue for further exploration – glycemic markers.

Chapter 3 presents data that is also exploratory in nature and uses three different study populations. Both verification cohorts are small in nature and have different major endpoints (composite cardiovascular outcome) than the derivation cohort (all-cause mortality). However, these data are exploratory in nature and help provide the ground-work for our future work.

Chapter 4 presents data on a rule-in/rule-out algorithm for MI using glycemic biomarkers. While all MIs were rigorously adjudicated in this study, it is prone to incorporation bias, which may have led to

overestimation of diagnostic accuracy. Unfortunately, this limitation is unavoidable given that cTn is the reference standard in which to define MI. In addition, we investigated a large number of cutoffs and cutoff combinations with a limited number of events (MI=133), which may increase the risk of a chance finding. However, in this scenario our goal was to identify the most appropriate cut-off rather than compare each cut-off. We are also unable to test for diabetes using other methods such as oral glucose tolerance test (OGTT) for verification as the inherent nature of the ED makes this unfeasible (Danaei et al. 2015). In this chapter, it was also shown that the performance of hs-cTn in combination with glucose is not sufficient to rule-out ACS or CV death at seven days and therefore likely requires further investigation and/or another approach using alternative biomarkers. Unfortunately, further analysis were beyond the scope of this investigation but should be considered in future studies. Of note, in chapter 4 the addition of glucose to cTn does not appear to significantly change the AUC. Although this may raise doubts as to the whether there are meaningful improvements in risk assessment I would note that there are concerns that ROC curves reduce the overall test performance to a single number (Zweig and Campbell 1993) and some of the clinical context may be lost, particularly when what is clinically acceptable [miss rate<1% (Than et al. 2013)] is only a small change in test

performance. In addition, it is also very difficult to move the AUC of an excellent test, such as cardiac troponin in this context (Cook 2007).

Chapter 5 presents data on the health services costs associated with the algorithms presented in chapter 4. In this chapter, it is important to note that the economic data was not specific to each patient in the study but was based on data compiled specifically from hospitals across Ontario. Although these data are not hospital specific it does make it more generalizable. Another limitation is that we do not take into account the cost of the extra testing for patients who fit into the STEMI category. Although these patients do not need the extra testing as their diagnosis can be made using only an ECG, they will likely receive the extra testing if it were to become protocol and thus incur extra costs.

A limitation across studies within this thesis is that we cannot determine if hyperglycemia contributes to a worse outcome or if it is simply a marker of adverse outcomes. As previously mentioned, literature is currently conflicted on this topic which, therefore, requires further investigation. Another limitation across all studies is that we do not take into account the fasting status of each patient. Given the nature of the ED, where patients arrive at all times of day in varying glycemic states this is likely not feasible, however, this would likely lead to an overestimation of positive patients, rather than an under-estimation. We also do not take into consideration sex or age specific cutoffs into our model. Research shows

that sex-specific cutoffs for cardiac troponin significantly change patient outcomes and dispositions, particularly for women (Shah et al. 2015; Gore et al. 2014; Cullen et al. 2016) and that age-specific cutoffs may be useful for reclassifying older patients (Mueller-Hennessen et al. 2016; Gore et al. 2014). However, these studies do suggest cutoffs much higher than our rule-out cutoffs and we are, therefore, not likely to be missing patients due to a lack of consideration for age and sex-specific cutoffs.

Despite the limitations associated with each study, I believe the results provide important information regarding the role of assessing glycemia in ACS patients both for potential diagnoses and for early risk-stratification and decision-making in the ED.

6.5 Summary

The original studies in this thesis provide insight into an early rule-in and rule-out algorithm for MI in patients who present to the ED with symptoms suggestive of ACS. This algorithm may prove to be a useful tool for emergency physicians and may significantly impact the patient experience. Furthermore, identification of previously unknown patients with diabetes may significantly impact the future health of patients and provide an opportunity for earlier intervention.

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Appendix A: Chapter 4 Supplemental Data

Supplemental Methods and Data

Symptoms of ACS:

Symptoms of ACS were adapted from the Heart and Stroke Signs of heart attack and literature (1-3) and included the following symptoms

At least one of:

- Chest pain or discomfort (may involve pressure, tightness or fullness)
- Pain or discomfort in one or both arms
- Pain or discomfort in the jaw
- Pain or discomfort in the neck
- Pain or discomfort in the back
- Pain or discomfort in the abdomen
- Shortness of breath
- Feeling dizzy or lightheaded
- Nauseas and/or vomiting
- Diaphoresis
- Palpitations

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Outcome Definition

All outcomes were independently adjudicated by at least two of the authors and disagreements not resolved by consensus were referred to a third blinded reviewer. All adjudicators were blinded to the research hs-cTn, glucose and HbA1c results.

MI:
MI was diagnosed when at least one cTnI concentration (from all clinically available cTnI results) was above the 99th percentile (i.e., 0.03 ug/L with Abbott ARCHITECT sensitive cTnI assay) with a significant rise/fall (1) (defined as: concentration 0.04 - 0.10 ug/L, the difference/change needs to be at least 0.03 ug/L; with concentrations >0.10 ug/L, the difference/change needs to be at least 20%) (2). Myocardial infarctions after the index presentation (i.e., seven hours after presentation) until 7d post presentation were also diagnosed using ECG findings indicative of new cardiac ischemia (3)

Unstable Angina:
Unstable angina was diagnosed when any of the following criteria were met: a discharge diagnosis of Unstable Angina as per discharge summary and/or admission to hospital with ACS treatment [heparin or low molecular weight heparin, cardiac catheterization resulting in increased treatment (ie. Plavix/ASA) or revascularization] (3)

Cardiovascular Death:
Cardiovascular death was defined as death from a cardiovascular cause, including a revascularization procedure, cardiac arrest, MI, stroke or unknown cause (3)

ACS:
A combination of MI or Unstable Angina

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Supplemental Table 1. Rule-out using only one analyte. N/A= no criteria cut-off could be obtained that met necessary criteria

	Optimal Cutoff Source/Type	Optimal Cutoff	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	LR+	LR-
Abbott hs-cTnI	Manufacturer 99th percentile	26 ng/L	72.2 (64.0-79.1)	89.5 (87.5-91.3)	96.0 (94.6-97.1)	47.8 (41.0-54.6)	6.9	0.31
	ROC derived 99.5% NPV	3 ng/L	99.2 (95.4-100)	31.2 (28.4-34.1)	99.7 (98.0-100)	16.0 (13.7-18.7)	1.44	0.02
	ROC derived 99% Sensitivity	1 ng/L	99.2 (95.4-100)	8.4 (6.8-10.3)	98.8 (93.0-100)	12.5 (10.7-14.7)	1.08	0.09
	ROC derived Max Sens./Spec	15 ng/L	82.7 (75.3-88.3)	78.5 (75.8-80.9)	97.2 (95.8-98.1)	33.7 (28.8-39.0)	3.8	0.22
	ROC derived LR-0.1	7 ng/L	94.7 (89.3-97.6)	60.9 (57.8-638)	98.9 (97.6-99.5)	24.3 (20.8-28.2)	2.4	0.09
	LOD	2 ng/L	99.2 (95.4-100)	15.7 (13.6-18.1)	99.4 (96.2-100)	13.5 (11.5-15.8)	1.18	0.05
	LOB	1 ng/L	99.2 (95.4-100)	8.4 (6.8-10.3)	98.8 (93.0-100)	12.5 (10.7-14.7)	1.1	0.09
	High-STEACs study	5 ng/L	97.0 (92.5-99.2)	49.8 (46.7-52.9)	99.2 (98.0-99.8)	20.4 (17.3-23.7)	1.93	0.06
	PEACE study	4 ng/L	98.5 (94.7-99.8)	41.6 (38.6-44.8)	99.5 (98.3-99.9)	18.3 (15.5-21.3)	1.69	0.04

Roche hs-cTnT	Manufacturer 99th percentile	14 ng/L	92.5 (86.6-96.0)	58.5 (55.4-61.5)	98.3 (96.9-99.1)	22.8 (19.4-26.5)	2.23	0.13
	ROC derived 99.5% NPV	N/A						
	ROC derived 99% Sensitivity	5 ng/L	99.2 (95.4-100)	17.9 (15.7-20.4)	99.4 (96.2-100)	13.8 (11.76-16.1)	1.21	0.04
	ROC derived Max Sens./Spec	24 ng/L	81.2 (73.7-87.0)	77.2 (74.5-79.7)	96.9 (95.4-97.9)	32.0 (29.3-37.2)	3.56	0.24
	ROC derived LR-0.1	12 ng/L	94.7 (89.3-97.6)	52.6 (49.5-55.7)	98.7 (97.3-99.4)	20.9 (17.9-24.4)	2	0.1
	LOD	5 ng/L	99.2 (95.4-100)	17.9 (15.7-20.4)	99.4 (96.2-100)	13.8 (11.76-16.1)	1.21	0.04
	LOB	3 ng/L	99.2 (95.4-100)	11.3 (9.4-13.4)	99.1 (94.7-100)	12.9 (10.9-15.0)	1.12	0.07
	HOPE study	8 ng/L	97.0 (92.3-99.1)	36.5 (33.5-39.5)	98.9 (97.2-99.7)	16.8 (14.0-19.6)	1.53	0.08

Abbott cTnI	Manufacturer 99th percentile	0.03 ug/L	67.7 (59.3-75.0)	90.3 (88.4-92.0)	95.5 (94.0-96.6)	48.1 (41.1-55.3)	7	0.36
	LOD	0.01 ug/L	82.7 (75.3-88.3)	75.1 (72.3-77.7)	97.0 (95.6-98.0)	30.6 (26.0-35.5)	3.32	0.23
	ROC derived 99.5% NPV	N/A						
	ROC derived 99% Sensitivity	N/A						
	ROC derived Max Sens./Spec	0.02 ug/L	75.2 (67.2-81.8)	82.7 (80.2-84.9)	96.2 (94.7-97.3)	36.5 (31.0-42.4)	4.34	0.3
	ROC derived LR-0.1	N/A						
Glucose	ROC derived Max Sens./Spec	119mg/dL	64.7 (56.2-72.3)	60.0 (56.9-63.0)	92.8 (90.1-94.5)	17.6 (14.5-21.3)	1.62	0.59
	ADA Guidelines	101mg/dL	84.2 (77.0-96.0)	32.0 (11.8-16.7)	93.9 (90.8-96.0)	14.1 (11.8-16.7)	1.24	0.49
	ROC derived LR-0.1	65mg/dL	100 (96.6-100)	0.4 (0.12-1.1)	100 (45.1-100)	11.7 (10.0-13.8)	1	0
	WHO Guidelines	126mg/dL	55.6 (47.1-63.1)	68.6 (65.7-71.4)	92.1 (90.0-93.9)	19.0 (15.4-23.2)	1.77	0.65

HbA1c	ROC derived Max Sens./Spec	5.9%	54.9 (46.1-63.1)	58.2 (55.1-61.2)	90.7 (88.2-92.7)	14.8 (11.9-18.2	1.31	0.78
	ADA Guidelines	6.5%	32.3 (25.0-40.7)	80.0 (77.4-82.3)	89.9 (87.8-91.7)	17.6 (13.3-22.9)	1.62	0.85
	ROC derived LR-0.1	4%	100 (96.6-100)	0.2 (<0.01-0.77)	100 (29.0-100)	11.7 (10.0-13.7)	1.002	0

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 2. Rule-in using only one analyte. N/A= no criteria cut-off could be obtained that met necessary criteria

	Optimal Cutoff Source/Type	Optimal Cutoff	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	LR+	LR-
Abbott hs-cTnI	Manufacturer 99th percentile	26 ng/L	72.2 (64.0-79.1)	89.5 (87.5-91.3)	96.0 (94.6-97.1)	47.8 (41.0-54.6)	6.9	0.31
	ROC derived 99% Specificity	99 ng/L	43.6 (35.5-52.1)	99.0 (98.2-99.5)	93.0 (91.3-94.4)	85.3 (74.8-92.0)	43.8	0.57
	ROC derived Max Sens./Spec	15 ng/L	82.7 (75.3-88.3)	78.5 (75.8-80.9)	97.2 (95.8-98.1)	33.7 (28.8-39.0)	3.8	0.22
	ROC derived 80% PPV	82 ng/L	46.6 (38.4-94.6)	98.5 (97.5-99.1)	93.3 (91.6-94.7)	80.5 (70.2-87.9)	31.2	0.54
	ROC derived LR+ 10	36 ng/L	63.2 (54.7-70.9	93.8 (92.2-95.2)	95.1 (93.5-96.3)	57.5 (49.4-65.3)	10.2	0.39
	APACE/ ADAPT study	64 ng/L	50.4 (42.0-58.7)	97.5 (96.3-98.3)	93.7 (92.0-95.0)	72.8 (62.9-80.9)	20.2	0.51

Roche hs-cTnT	Manufacturer 99th percentile	14 ng/L	92.5 (86.6-96.0)	58.5 (55.4-61.5)	98.3 (96.9-99.1)	22.8 (19.4-26.5)	2.2	0.13
	ROC derived 99% Specificity	155 ng/L	19.5 (13.7-27.2)	99.0 (98.2-99.5)	90.3 (88.4-91.9)	72.2 (55.9-84.3)	19.6	0.81
	ROC derived Max Sens./Spec	24 ng/L	81.2 (73.7-87.0)	77.2 (74.5-79.7)	96.9 (95.4-97.9)	32.0 (29.3-37.2)	3.6	0.24
	ROC derived 80% PPV	206 ng/L	15.8 (10.5-23.0)	99.5 (98.8-99.8)	89.9 (88.0-91.6)	80.8 (61.7-92.0)	31.7	0.85
	ROC derived LR+ 10	81 ng/L	34.6 (27.0-43.0)	96.7 (95.4-97.7)	91.8 (90.0-93.3)	58.2 (47.2-68.5)	10.5	0.68
	APACE/ADAPT study	52 ng/L	49.6 (41.3-58.0)	92.5 (90.7-94.0)	93.3 (91.6-94.7)	46.8 (38.8-55.0)	6.6	0.54

Abbott cTnI	Manufacturer 99th percentile	0.03ug/L	67.7 (59.3-75.0)	90.3 (88.4-92.0)	95.5 (94.0-96.6)	48.1 (41.1-55.3)	7.0	0.36
	ROC derived 99% Specificity	0.1 ug/L	42.9 (34.8-51.4)	99.0 (98.2-99.5)	92.9 (91.2-94.3)	85.1 (74.5-91.9)	43.0	0.58
	ROC derived Max Sens./Spec	0.02ug/L	75.2 (67.2-81.8)	82.7 (80.2-84.9)	96.2 (94.7-97.3)	36.5 (31.0-42.4)	4.3	0.30
	ROC derived 80% PPV	0.09ug/L	44.4 (36.2-52.9)	98.9 (98.0-99.4)	93.1 (91.4-94.5)	84.3 (73.8-91.2)	40.5	0.56
	ROC derived LR+ 10	0.04ug/L	62.4 (53.9-70.2)	94.2 (92.6-95.5)	95.0 (93.4-96.2)	58.9 (50.6-66.7)	10.8	0.40
	WHO Guidelines	0.3 ug/L	21.8 (15.6-29.6)	99.8 (99.2-100)	90.6 (88.7-92.2)	93.5 (78.3-99.2)	109.5	0.78
Glucose	ROC derived Max Sens./Spec	119mg/dL	64.7 (56.2-72.3)	60.0 (56.9-63.0)	92.8 (90.5-94.5)	17.6 (14.5-21.3)	1.6	0.59
	ADA Guidelines	101mg/dL	84.2 (77.0-96.0)	32.0 (11.8-16.7)	93.9 (90.8-96.0)	14.1 (11.8-16.7)	1.2	0.49
	ROC derived 80% PPV	598mg/dL	0.9 (0.0-4.6)	100 (99.5-100)	88.4 (86.4-90.1)	100 (16.8-100)	n/a	0.99
	ROC derived LR+ 10	564mg/dL	1.5 (0.1-5.7)	99.9 (99.4-100)	88.4 (86.5-90.2)	66.7 (20.2-94.4)	15.1	0.99
	CDA Guidelines	198mg/dL	27.8 (20.9-36.0)	92.5 (90.7-94.0)	90.6 (88.7-92.2)	33.0 (25.0-42.2)	3.7	0.78
	WHO Guidelines	126mg/dL	55.6 (47.1-63.1)	68.6 (65.7-71.4)	92.1 (90.0-93.9)	19.0 (15.4-23.2)	1.8	0.65

HbA1c	ROC derived Max Sens./Spec	5.9%	54.9 (46.1-63.1)	58.2 (55.1-61.2)	90.7 (88.2-92.7)	14.8 (11.9-18.2	1.3	0.78
	ADA Guidelines	6.5%	32.3 (25.0-40.7)	80.0 (77.4-82.3)	89.9 (87.8-91.7)	17.6 (13.3-22.9)	1.6	0.85
	ROC derived 80% PPV	N/A						
	ROC derived LR+ 10	12%	0.15 (0.07-5.7)	99.9 (99.4-100)	88.4 (86.5-90.2)	66.7 (20.2-94.4)	15.1	0.99

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 3. Hs-cTnI Rule-out combinations

	hs-cTnI Rule-Out										
	Glucose	HbA1c	Criteria	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	LR+	LR-	% correctly deemed low risk	95% CI
Abbott hs-cTnI <1 ng/L	<119mg/dL	n/a	Dual Negative	100 (96.6-100)	6.4 (5.0-8.1)	100 (93.2-100)	12.4 (10.6-14.5)	1.07	0	5.6%	4.4-7.1
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	3.8 (2.8-5.2)	100 (89.1-100)	12.1 (10.3-14.2)	1.04	0	3.3%	2.4-4.6
	<126mg/dL	n/a	Dual Negative	99.2 (95.4-100)	7.1 (5.6-8.8)	98.6 (91.8-100)	12.4 (10.5-14.5)	1.07	0.11	6.2%	5.0-7.8
	n/a	<5.9%	Dual Negative	99.2 (95.4-100)	6.7 (5.3-8.4)	98.5 (91.4-100)	12.3 (10.5-14.5)	1.06	0.11	5.9%	4.7-7.4
	n/a	<6.5%	Dual Negative	99.2 (95.4-100)	7.7 (6.2-9.5)	98.7 (92.4-100)	12.5 (10.6-14.6)	1.08	0.1	6.8%	5.5-8.4
	<119mg/dL	<5.9%	All negative	100 (96.6-100)	5.7 (4.4-7.3)	100 (92.5-100)	12.3 (10.5-14.4)	1.06	0	5.0%	3.9-6.5
	<119mg/dL	<6.5%	All negative	100 (96.6-100)	6.3 (4.9-8.0)	100 (93.1-100)	12.4 (10.5-14.5)	1.07	0	5.5%	4.4-7.0
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	3.6 (2.6-4.9)	100 (88.5-100)	12.1 (10.3-14.1)	1.04	0	3.2%	2.3-4.4
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	3.8 (2.8-5.2)	100 (89.1-100)	12.1 (10.3-14.2)	1.04	0	3.3%	2.4-4.6
	<126mg/dL	<5.9%	All negative	99.2 (95.4-100)	6.2 (4.8-7.9)	98.4 (90.7-100)	12.3 (10.5-14.4)	1.06	0.12	5.5%	4.3-6.9
	<126mg/dL	<6.5%	All negative	99.2 (95.4-100)	7.0 (5.6-8.7)	98.6 (91.7-100)	12.4 (10.5-14.5)	1.07	0.11	6.2%	4.9-7.7

Abbott hs-cTnI <2 ng/L	<119mg/dL	n/a	Dual Negative	100 (96.6-100)	11.0 (9.2-13.0)	100 (96.0-100)	13.0 (11.0-15.2)	1.12	0	9.7%	8.1-11.5
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	6.9 (5.5-8.6)	100 (93.7-100)	12.5 (10.6-14.6)	1.07	0	6.1%	4.8-7.6
	<126mg/dL	n/a	Dual Negative	99.2 (95.4-100)	12.1 (9.0-12.6)	99.2 (95.1-100)	13.0 (11.1-15.2)	1.13	0.06	10.6%	9.0-12.6
	n/a	<5.9%	Dual Negative	99.2 (95.4-100)	11.8 (9.9-13.9)	99.2 (94.9-100)	13.0 (11.0-15.2)	1.13	0.06	10.4%	8.7-12.3
	n/a	<6.5%	Dual Negative	99.2 (95.4-100)	13.8 (11.8-16.1)	99.3 (95.7-100)	13.2 (11.3-15.5)	1.15	0.05	12.2%	10.4-14.3
	<119mg/dL	<5.9%	All negative	100 (96.6-100)	9.7 (8.0-11.7)	100 (95.4-100)	12.8 (10.9-15.0)	1.11	0	8.5%	7.0-10.3
	<119mg/dL	<6.5%	All negative	100 (96.6-100)	10.8 (9.0-12.8)	100 (95.9-100)	12.9 (11.0-15.1)	1.12	0	9.5%	7.9-11.4
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	6.3 (4.9-8.0)	100 (93.1-100)	12.4 (10.5-14.5)	1.07	0	5.5%	4.4-7.0
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	6.9 (5.5-8.6)	100 (93.7-100)	12.5 (10.6-14.6)	1.07	0	6.1%	4.8-7.6
	<126mg/dL	<5.9%	All negative	99.2 (95.4-100)	10.4 (8.6-12.4)	99.0 (94.3-100)	12.8 (10.9-15.0)	1.11	0.07	9.1%	7.6-11.0
	<126mg/dL	<6.5%	All negative	99.2 (95.4-100)	11.9 (10.0-14.0)	99.2 (95.0-100)	13.0 (11.1-15.2)	1.13	0.06	10.5%	8.8-12.4

Abbott hs-cTnI <3 ng/L	<119mg/dL	n/a	Dual Negative	100 (96.6-100)	20.9 (18.5-23.5)	100 (97.8-100)	14.3 (12.2-16.8)	1.26	0	18.5%	16.3-20.8
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	11.8 (9.9-13.9)	100 (96.2-100)	13.1 (11.1-15.3)	1.13	0	10.4%	8.7-12.3
	<126mg/dL	n/a	Dual Negative	99.2 (95.4-100)	23.5 (21.0-26.2)	99.6 (97.4-100)	14.7 (12.5-17.1)	1.3	0.03	20.8%	18.5-23.2
	n/a	<5.9%	Dual Negative	99.2 (95.4-100)	22.6 (20.1-25.3)	99.6 (97.3-100)	14.5 (12.4-17.0)	1.28	0.03	20.0%	17.7-27.0
	n/a	<6.5%	Dual Negative	99.2 (95.4-100)	27.7 (25.0-30.5)	99.6 (97.8-100)	15.4 (13.1-18.0)	1.37	0.03	24.5%	22.0-27.0
	<119mg/dL	<5.9%	All negative	100 (96.6-100)	17.6 (15.4-20.1)	100 (97.4-100)	13.9 (11.8-16.2)	1.21	0	18.1%	16.0-20.5
	<119mg/dL	<6.5%	All negative	100 (96.6-100)	20.5 (18.1-23.1)	100 (97.8-100)	14.3 (12.2-16.7)	1.26	0	18.1%	16.0-20.5
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	10.3 (8.5-12.3)	100 (95.7-100))	12.9 (11.0-15.1)	1.11	0	9.1%	7.5-10.9
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	11.7 (9.8-13.8)	100 (96.2-100)	13.0 (11.1-15.3)	1.13	0	10.3%	8.7-12.2
	<126mg/dL	<5.9%	All negative	99.2 (95.4-100)	19.5 (17.2-22.1)	99.5 (96.9-100)	14.0 (12.0-16.4)	1.23	0.04	17.2%	15.2-19.6
	<126mg/dL	<6.5%	All negative	99.2 (95.4-100)	22.9 (20.4-25.6)	99.6 (97.3-100)	14.6 (12.4-17.0)	1.29	0.03	20.2%	18.0-22.7

Abbott hs-cTnI <4 ng/L	<119mg/dL	n/a	Dual Negative	100 (96.6-100)	27.8 (25.1-30.6)	100 (98.4-100)	15.5 (13.3-18.1)	1.39	0	24.5%	22.1-27.1
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	15.2 (13.1-17.6)	100 (97.1-100)	13.5 (11.5-15.8)	1.18	0	13.5%	11.6-15.6
	<126mg/dL	n/a	Dual Negative	98.5 (94.3-99.9)	31.2 (28.4-34.1)	99.4 (97.6-100)	15.9 (13.6-18.6)	1.43	0.05	27.5%	25.0-30.2
	n/a	<5.9%	Dual Negative	98.5 (94.3-99.9)	28.7 (26.0-31.6)	99.3 (97.4-100)	15.5 (13.2-18.1)	1.38	0.05	25.3%	22.9-27.9
	n/a	<6.5%	Dual Negative	98.5 (94.3-99.9)	36.3 (33.2-39.2)	99.5 (98.0-100)	17.0 (14.5-19.8)	1.54	0.04	31.9%	29.3-34.7
	<119mg/dL	<5.9%	All negative	100 (96.6-100)	22.5 (20.0-25.2)	100 (98.0-100)	14.6 (12.5-17.1)	1.29	0	19.9%	17.7-22.3
	<119mg/dL	<6.5%	All negative	100 (96.6-100)	27.2 (24.5-30.0)	100 (98.3-100)	15.4 (13.1-18.0)	1.37	0	24.0%	21.6-26.6
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	13.1 (11.2-15.4)	100 (96.6-100)	13.2 (11.3-15.5)	1.15	0	11.6%	9.9-13.6
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	15.1 (13.1-17.5)	100 (97.0-100)	13.5 (11.5-15.8)	1.18	0	13.4%	11.5-15.5
	<126mg/dL	<5.9%	All negative	98.5 (94.3-99.9)	24.9 (22.3-27.7)	99.2 (97.0-100)	14.8 (12.6-17.3)	1.31	0.06	22.0%	19.7-24.5
	<126mg/dL	<6.5%	All negative	98.5 (94.3-99.9)	30.2 (27.4-33.1)	99.3 (97.5-100)	15.7 (13.4-18.4)	1.41	0.05	26.6%	24.2-29.3

Abbott hs-cTnI <5 ng/L	<119mg/dL	n/a	Dual Negative	99.2 (95.4-100)	33.6 (30.7-36.6)	99.7 (98.2-100)	16.5 (14.1-19.3)	1.49	0.02	29.6%	27.1-32.4
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	18.4 (16.2-21.0)	100 (97.6-100)	14.0 (11.9-16.3)	1.23	0	16.3%	14.2-18.5
	<126mg/dL	n/a	Dual Negative	97.7 (93.3-99.5)	37.5 (34.6-40.6)	99.2 (97.6-99.8)	17.2 (14.7-20.0)	1.57	0.6	33.2%	30.5-36.0
	n/a	<5.9%	Dual Negative	97.0 (92.3-99.1)	34.3 (31.4-37.3)	98.9 (97.0-99.7)	16.3 (13.9-19.1)	1.48	0.09	30.3%	27.7-33.0
	n/a	<6.5%	Dual Negative	97.0 (92.3-99.1)	43.3 (40.3-46.4)	99.1 (97.6-99.7)	18.5 (15.8-21.5)	1.71	0.07	38.3%	35.5-41.1
	<119mg/dL	<5.9%	All negative	99.2 (95.4-100)	27.1 (24.4-29.9)	99.6 (97.7-100)	15.3 (13.0-17.8)	1.36	0.03	23.9%	21.5-26.5
	<119mg/dL	<6.5%	All negative	99.2 (95.4-100)	32.9 (30.0-35.8)	99.7 (98.1-100)	16.4 (14.0-19.1)	1.48	0.02	29.0%	26.5-31.7
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	15.9 (13.8-18.3)	100 (97.2-100)	13.6 (11.6-15.9)	1.19	0	14.1%	12.2-16.2
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	18.2 (16.0-20.7)	100 (97.5-100)	13.9 (11.9-16.3)	1.22	0	16.1%	14.1-18.4
	<126mg/dL	<5.9%	All negative	97.7 (93.3-99.5)	29.9 (27.1-32.8)	99.0 (97.0-99.8)	15.6 (13.3-18.2)	1.39	0.08	26.4%	23.9-29.0
	<126mg/dL	<6.5%	All negative	97.7 (93.3-99.5)	36.4 (33.4-39.4)	99.2 (97.5-99.8)	16.9 (14.4-19.7)	1.54	0.06	32.1%	29.5-34.9

Abbott hs-cTnI <7 ng/L	<119mg/dL	n/a	Dual Negative	98.5 (94.3-99.9)	39.8 (36.9-42.9)	99.5 (98.1-100)	17.8 (15.2-20.8)	1.64	0.04	35.2%	32.5-38.0
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	21.7 (19.3-24.4)	100 (97.9-100)	14.5 (12.3-16.9)	1.28	0	19.2%	17.0-21.6
	<126mg/dL	n/a	Dual Negative	97.0 (92.3-99.1)	44.6 (41.6-47.7)	99.1 (97.7-99.7)	18.8 (16.1-21.9)	1.75	0.07	39.4%	36.6-42.3
	n/a	<5.9%	Dual Negative	96.2 (91.3-98.6)	40.2 (37.3-43.3)	98.8 (97.1-99.6)	17.6 (15.0-20.5)	1.61	0.09	35.5%	32.8-38.4
	n/a	<6.5%	Dual Negative	95.5 (90.3-98.1)	51.6 (48.5-54.7)	98.9 (97.5-99.5)	20.7 (17.7-24.1)	1.97	0.09	45.6%	42.7-48.5
	<119mg/dL	<5.9%	All negative	98.5 (94.3-99.9)	31.5 (28.7-34.4)	99.4 (97.6-100)	16.0 (13.6-18.7)	1.44	0.05	27.8%	25.3-30.5
	<119mg/dL	<6.5%	All negative	98.5 (94.3-99.9)	38.6 (35.7-41.7)	99.5 (98.0-100)	17.5 (15.0-20.4)	1.61	0.04	34.1%	31.4-36.9
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	18.4 (16.2-21.0)	100 (97.6-100)	14.0 (11.9-16.3)	1.23	0	16.3%	14.2-18.5
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	21.3 (18.9-24.0)	100 (97.9-100)	14.4 (12.3-16.8)	1.27	0	18.8%	16.7-21.2
	<126mg/dL	<5.9%	All negative	97.0 (92.3-99.1)	34.5 (31.6-37.5)	98.9 (97.0-99.7)	16.4 (14.0-19.1)	1.48	0.09	30.4%	27.8-33.2
	<126mg/dL	<6.5%	All negative	97.0 (92.3-99.1)	42.7 (39.7-45.8)	99.1 (97.6-99.7)	18.3 (15.6-21.4)	1.69	0.07	37.7%	35.0-40.6

Abbott hs-cTnI <15 ng/L	<119mg/dL	n/a	Dual Negative	94.7 (89.3-97.6)	49.2 (46.1-52.3)	98.6 (97.1-99.4)	19.8 (16.9-23.1)	1.87	0.11	43.4%	40.6-46.4
	<101mg/dL	n/a	Dual Negative	99.2 (95.4-100)	26.5 (23.9-29.3)	99.6 (97.7-100)	15.2 (12.9-17.7)	1.35	0.03	23.4%	21.0-25.9
	<126mg/dL	n/a	Dual Negative	93.2 (87.5-96.6)	56.1 (53.0-59.1)	98.4 (97.0-99.2)	21.9 (18.7-25.6)	2.12	0.12	49.5%	46.6-52.4
	n/a	<5.9%	Dual Negative	91.7 (85.7-95.5)	48.6 (45.5-51.7)	97.8 (96.1-98.8)	19.1 (16.3-22.4)	1.79	0.17	42.9%	40.1-45.8
	n/a	<6.5%	Dual Negative	87.2 (80.4-92.0)	64.6 (61.6-67.5)	97.4 (95.2-98.4)	24.6 (21.0-28.7)	2.47	0.2	57.1%	54.2-59.9
	<119mg/dL	<5.9%	All negative	96.2 (91.3-98.6)	37.6 (34.7-40.7)	98.7 (96.9-99.5)	17.0 (14.5-19.8)	1.54	0.1	33.2%	30.6-36.0
	<119mg/dL	<6.5%	All negative	94.7 (89.3-97.6)	47.2 (44.1-50.3)	98.5 (97.0-99.4)	19.2 (16.4-22.4)	1.8	0.11	41.7%	38.9-44.6
	<101mg/dL	<5.9%	All negative	99.2 (95.4-100)	21.4 (19.0-24.1)	99.5 (97.2-100)	14.3 (12.2-16.8)	1.26	0.04	18.9%	16.7-21.3
	<101mg/dL	<6.5%	All negative	99.2 (95.4-100)	25.6 (23.0-28.4)	99.6 (97.6-100)	15.0 (12.8-17.5)	1.33	0.03	22.6%	20.3-25.1
	<126mg/dL	<5.9%	All negative	94.7 (89.3-97.6)	41.4 (38.4-44.5)	98.3 (96.6-99.3)	17.6 (15.0-20.6)	1.62	0.13	36.6%	33.8-39.4
	<126mg/dL	<6.5%	All negative	93.2 (87.5-96.6)	52.9 (49.8-56.0)	98.3 (96.8-99.2)	20.8 (17.7-24.2)	1.99	0.13	46.7%	43.8-49.6

Abbott hs-cTnI <26 ng/L	<119mg/dL	n/a	Dual Negative	92.5 (86.6-96.0)	55.1 (52.0-58.1)	98.2 (52.0-58.1)	21.4 (18.3-25.0)	2.06	0.14	48.6%	45.7-51.5
	<101mg/dL	n/a	Dual Negative	97.7 (93.3-99.5)	29.6 (26.9-32.5)	99.0 (97.0-99.8)	15.5 (13.2-18.2)	1.39	0.08	26.1%	23.7-28.8
	<126mg/dL	n/a	Dual Negative	90.2 (83.9-94.3)	62.9 (59.9-65.9)	98.0 (96.6-98.9)	24.4 (20.8-28.4)	2.44	0.16	55.6%	52.7-58.5
	n/a	<5.9%	Dual Negative	88.7 (82.1-93.2)	54.4 (51.3-57.4)	97.3 (95.6-98.4)	20.5 (17.4-24.0)	1.95	0.21	48.0%	45.1-50.9
	n/a	<6.5%	Dual Negative	80.5 (72.8-86.4)	73.1 (70.3-75.8)	96.6 (95.0-97.7)	28.4 (24.1-33.1)	2.99	0.27	64.6%	61.7-67.3
	<119mg/dL	<5.9%	All negative	96.2 (91.3-98.6)	41.9 (38.9-45.0)	98.8 (97.2-99.6)	18.0 (15.4-21.0)	1.66	0.09	37.0%	34.3-39.9
	<119mg/dL	<6.5%	All negative	92.5 (86.6-96.0)	52.7 (49.6-55.8)	98.1 (96.6-99.0)	20.6 (17.5-24.0)	1.96	0.14	46.5%	43.6-49.4
	<101mg/dL	<5.9%	All negative	99.2 (95.4-100)	23.7 (21.2-26.4)	99.6 (97.4-100)	14.7 (12.5-17.2)	1.3	0.03	20.9%	18.7-23.4
	<101mg/dL	<6.5%	All negative	97.7 (93.3-99.5)	28.5 (25.8-31.4)	99.0 (96.9-99.8)	15.3 (13.1-17.9)	1.37	0.08	25.2%	22.7-27.8
	<126mg/dL	<5.9%	All negative	94.0 (88.4-97.1)	46.2 (43.2-49.3)	98.3 (96.6-99.2)	18.8 (16.0-22.0)	1.75	0.13	40.8%	38.0-43.7
	<126mg/dL	<6.5%	All negative	90.2 (96.3-98.8)	59.3 (56.2-62.3)	97.9 (96.3-98.8)	22.7 (19.3-26.5)	2.22	0.17	52.3%	49.4-55.2

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 4. Hs-cTnI Rule-in combinations

hs-cTnI Rule-In											
	Glucose	HbA1c	Criteria	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	LR+	LR-	% correctly deemed high risk	95% CI
Abbott hs-cTnI ≥15 ng/L	≥119mg/dL	n/a	Dual Positive	52.6 (44.2-60.9)	89.2 (87.2-91.0)	93.4 (91.7-94.8)	39.3 (32.4-46.7)	4.89	0.53	6.2%	4.9-7.7
	≥101mg/dL	n/a	Dual Positive	67.7 (59.3-75.0)	84.0 (81.6-86.1)	95.1 (93.5-96.4)	35.9 (30.2-42.0)	42.2	0.39	7.9%	6.5-9.6
	≥126mg/dL	n/a	Dual Positive	45.1 (36.9-53.6)	91.0 (89.1-92.7)	92.6 (90.8-94.1)	40.0 (32.5-48.0)	5.03	0.60	5.3%	4.1-6.7
	≥198mg/dL	n/a	Dual Positive	24.1 (17.5-32.0)	98.2 (97.2-98.9)	90.7 (88.8-92.3)	64.0 (50.1-75.9)	13.4	0.77	2.8%	2.0-4.0
	n/a	≥5.9%	Dual Positive	45.9 (37.6-54.3)	88.0 (85.9-89.9)	92.5 (90.6-94.0)	33.7 (27.2-40.9)	3.84	0.62	5.4%	4.2-6.8
	n/a	≥6.5%	Dual Positive	27.8 (20.9-36.0)	93.8 (92.2-95.2)	90.8 (88.8-92.4)	37.4 (28.5-47.2)	4.51	0.77	3.3%	2.4-4.5
	≥119mg/dL	≥5.9%	All Positive	36.1 (28.4-44.6)	91.8 (90.0-93.4)	91.6 (89.7-93.1)	36.9 (29.1-45.5)	4.42	0.7	4.2%	3.2-5.6
	≥119mg/dL	≥6.5%	All Positive	24.8 (18.2-32.8)	94.9 (93.4-96.1)	90.5 (88.6-92.1)	39.3 (29.5-50.0)	4.89	0.79	2.9%	2.1-4.1
	≥101mg/dL	≥5.9%	All Positive	41.4 (33.3-49.9)	89.7 (87.7-91.5)	92.0 (90.2-93.6)	34.8 (27.8-42.5)	4.03	0.65	4.8%	3.7-6.3
	≥101mg/dL	≥6.5%	All Positive	27.1 (20.2-35.2)	94.4 (92.8-95.7)	90.7 (88.8-92.3)	39.1 (29.8-49.4)	4.85	0.77	3.2%	2.3-4.4
	≥126mg/dL	≥5.9%	All Positive	33.8 (26.3-42.2)	92.9 (91.2-94.4)	91.4 (89.5-93.0)	38.8 (30.4-47.9)	4.78	0.71	4.0%	3.0-5.3
	≥126mg/dL	≥6.5%	All Positive	24.8 (18.2-32.8)	95.3 (93.8-96.5)	90.5 (88.6-92.2)	41.3 (31.1-52.2)	5.30	0.79	2.9%	2.1-4.1
	≥198mg/dL	≥5.9%	All Positive	21.8 (15.6-29.6)	98.3 (97.3-99.0)	90.5 (88.6-92.1)	63.0 (48.6-75.5)	12.9	0.80	2.6%	1.8-3.7
	≥198mg/dL	≥6.5%	All Positive	18.8 (13.0-26.3)	98.4 (97.4-99.0)	90.1 (88.2-91.8)	61.0 (45.7-74.4)	11.8	0.83	2.2%	1.5-3.2

Abbott hs-cTnI ≥26 ng/L	≥119mg/dL	n/a	Dual Positive	44.4 (36.2-52.9)	94.4 (92.8-95.7)	92.8 (91.0-94.2)	51.3 (42.3-60.3)	8.00	0.59	5.2%	4.0-6.6
	≥101mg/dL	n/a	Dual Positive	58.6 (50.2-66.7)	91.9 (90.1-93.5)	94.4 (92.7-95.7)	49.1 (41.4-56.8)	7.27	0.45	6.9%	5.5-8.5
	≥126mg/dL	n/a	Dual Positive	37.6 (29.8-46.1)	95.2 (93.7-96.4)	92.0 (90.2-93.5)	51.0 (41.3-60.7)	7.86	0.66	4.4%	3.3-5.8
	≥198mg/dL	n/a	Dual Positive	20.3 (14.3-28.0)	99.0 (98.2-99.5)	90.4 (88.5-92.0)	73.0 (56.9-84.8)	20.4	0.81	2.4%	1.6-3.5
	n/a	≥5.9%	Dual Positive	38.3 (30.5-46.8)	93.3 (91.6-94.7)	92.0 (90.1-93.5)	43.2 (34.6-52.2)	5.75	0.66	4.5%	3.4-5.9
	n/a	≥6.5%	Dual Positive	24.1 (17.6-32.0)	96.4 (95.1-97.4)	90.6 (88.6-92.2)	47.1 (35.7-58.8)	6.71	0.79	2.8%	2.0-4.0
	≥119mg/dL	≥5.9%	All Positive	30.8 (23.6-39.1)	95.5 (94.0-96.7)	91.2 (89.4-92.8)	47.7 (37.5-58.1)	6.88	0.72	3.6%	2.7-4.9
	≥119mg/dL	≥6.5%	All Positive	21.1 (14.9-28.8)	97.1 (95.9-98.0)	90.3 (88.4-91.9)	49.1 (36.6-61.7)	7.29	0.81	2.5%	1.7-3.6
	≥101mg/dL	≥5.9%	All Positive	35.3 (27.7-43.8)	94.2 (92.6-95.5)	91.7 (89.8-93.2)	44.8 (35.6-54.3)	6.12	0.69	4.1%	3.1-5.5
	≥101mg/dL	≥6.5%	All Positive	23.3 (16.9-31.2)	96.8 (95.5-97.8)	90.5 (88.6-92.1)	49.2 (37.3-61.2)	7.31	0.79	2.7%	1.9-3.9
	≥126mg/dL	≥5.9%	All Positive	28.6 (21.6-36.8)	96.1 (94.7-97.2)	91.0 (89.2-92.6)	49.4 (38.5-60.3)	7.36	0.74	3.3%	2.4-4.6
	≥126mg/dL	≥6.5%	All Positive	21.1 (14.9-28.8)	97.4 (96.2-98.2)	90.3 (88.4-91.9)	51.9 (38.9-64.6)	8.13	0.81	2.5%	1.7-3.6
	≥198mg/dL	≥5.9%	All Positive	18.8 (13.0-26.3)	99.0 (98.2-99.5)	90.2 (88.3-91.8)	71.4 (54.8-83.8)	18.9	0.82	2.2%	1.5-3.2
	≥198mg/dL	≥6.5%	All Positive	15.8 (10.5-23.0)	99.1 (98.3-99.6)	89.9 (88.0-91.5)	70.0 (52.0-83.5)	17.6	0.85	1.8%	1.2-2.8

Abbott hs-cTnI ≥36 ng/L	≥119mg/dL	n/a	Dual Positive	39.1 (31.2-47.6)	96.7 (95.4-97.7)	92.3 (90.5-93.8)	61.2 (50.5-70.9)	11.9	0.63	4.6%	3.5-6.0
	≥101mg/dL	n/a	Dual Positive	52.6 (44.2-60.9)	95.3 (93.8-96.5)	93.8 (92.2-95.2)	59.8 (50.8-68.3)	11.2	0.5	6.2%	4.9-7.7
	≥126mg/dL	n/a	Dual Positive	33.1 (25.7-41.5)	97.2 (96.0-98.1)	91.6 (89.8-93.2)	61.1 (49.6-71.6)	11.9	0.69	3.9%	2.9-5.2
	≥198mg/dL	n/a	Dual Positive	18.0 (12.4-25.5)	99.5 (98.8-99.8)	90.2 (88.3-91.8)	82.8 (65.0-92.9)	36.2	0.82	2.1%	1.4-3.1
	n/a	≥5.9%	Dual Positive	33.8 (26.3-42.2)	96.0 (94.6-97.1)	91.6 (89.8-93.2)	52.9 (42.4-63.2)	8.49	0.69	4.0%	3.0-5.3
	n/a	≥6.5%	Dual Positive	22.6 (16.2-30.4)	97.8 (96.7-98.6)	90.5 (88.6-92.1)	57.7 (44.2-70.1)	10.3	0.79	2.6%	1.8-3.8
	≥119mg/dL	≥5.9%	All Positive	27.1 (20.2-35.2)	97.5 (96.3-98.3)	91.0 (89.1-92.6)	59.0 (46.5-70.5)	10.9	0.75	3.2%	2.3-4.4
	≥119mg/dL	≥6.5%	All Positive	19.5 (13.7-27.2)	98.3 (97.3-99.0)	90.2 (88.3-91.9)	60.5 (45.6-73.7)	11.6	0.82	2.3%	1.6-3.4
	≥101mg/dL	≥5.9%	All Positive	31.6 (24.3-39.9)	96.6 (95.3-97.6)	91.4 (89.6-93.0)	55.3 (44.1-65.9)	9.33	0.71	3.7%	2.7-5.0
	≥101mg/dL	≥6.5%	All Positive	21.8 (15.6-29.6)	98.0 (96.9-98.7)	90.4 (88.5-92.1)	59.2 (45.2-71.8)	10.9	0.8	2.6%	1.8-3.7
	≥126mg/dL	≥5.9%	All Positive	25.6 (18.9-33.6)	97.8 (96.7-98.6)	90.8 (89.0-92.4)	60.7 (47.6-72.4)	11.7	0.76	3.0%	2.1-4.2
	≥126mg/dL	≥6.5%	All Positive	19.5 (13.7-27.2)	98.4 (97.4-99.0)	90.2 (88.3-91.9)	61.9 (46.8-75.0)	12.3	0.82	2.3%	1.6-3.3
	≥198mg/dL	≥5.9%	All Positive	16.5 (11.1-23.8)	99.5 (98.8-99.8)	90.0 (88.1-91.6)	81.5 (62.8-92.3)	33.2	0.84	1.9%	1.3-2.9
	≥198mg/dL	≥6.5%	All Positive	14.3 (9.3-21.3)	99.5 (98.8-99.8)	89.8 (87.8-91.4)	79.2 (59.1-91.2)	28.7	0.86	1.7%	1.1-2.6

Abbott hs-cTnI ≥64 ng/L	≥119mg/dL	n/a	Dual Positive	30.1 (22.9-38.4)	98.7 (97.8-99.3)	91.4 (89.6-93.0)	75.5 (62.3-85.2)	23.2	0.71	3.5%	2.6-4.8
	≥101mg/dL	n/a	Dual Positive	42.1 (34.1-50.6)	98.2 (97.2-98.9)	92.8 (91.0-94.2)	75.7 (64.7-84.1)	23.5	0.59	4.9%	3.8-6.4
	≥126mg/dL	n/a	Dual Positive	25.6 (18.9-33.6)	98.7 (97.8-99.3)	90.9 (89.1-92.5)	72.3 (58.1-83.2)	19.7	0.75	3.0%	2.1-4.2
	≥198mg/dL	n/a	Dual Positive	12.8 (8.0-19.6)	99.9 (99.4-100)	89.6 (87.7-91.3)	94.4 (72.4-100)	128.3	0.87	1.5%	0.9-2.4
	n/a	≥5.9%	Dual Positive	25.6 (18.9-33.6)	98.5 (97.5-99.1)	90.9 (89.0-92.5)	69.4 (55.4-80.6)	17.1	0.76	3.0%	2.1-4.2
	n/a	≥6.5%	Dual Positive	15.8 (10.5-23.0)	99.1 (98.3-99.6)	89.9 (88.0-91.5)	70.0 (52.0-83.5)	17.6	0.85	1.8%	1.2-2.8
	≥119mg/dL	≥5.9%	All Positive	19.5 (13.7-27.2)	99.0 (98.2-99.5)	90.3 (88.4-91.9)	72.2 (55.9-84.3)	19.6	0.81	2.3%	1.6-3.3
	≥119mg/dL	≥6.5%	All Positive	12.8 (8.0-19.6)	99.2 (98.4-99.6)	89.6 (87.6-91.2)	68.0 (48.3-82.9)	16.0	0.88	1.5%	0.9-2.4
	≥101mg/dL	≥5.9%	All Positive	24.1 (17.6-32.0)	98.8 (97.9-99.3)	90.8 (88.9-92.3)	72.7 (58.0-83.8)	20.1	0.77	2.8%	2.0-4.0
	≥101mg/dL	≥6.5%	All Positive	15.0 (9.9-22.2)	99.2 (98.4-99.6)	89.8 (87.9-91.5)	71.4 (52.8-84.9)	18.9	0.86	1.8%	1.1-2.7
	≥126mg/dL	≥5.9%	All Positive	18.8 (13.0-26.3)	99.0 (98.2-99.5)	90.2 (88.3-91.8)	71.4 (54.8-83.8)	18.9	0.82	2.2%	1.5-3.2
	≥126mg/dL	≥6.5%	All Positive	12.8 (8.0-19.6)	99.2 (98.4-99.6)	89.6 (87.6-91.2)	68.0 (48.3-82.9)	16.0	0.88	1.5%	0.9-2.4
	≥198mg/dL	≥5.9%	All Positive	11.3 (6.9-17.9)	99.9 (99.4-100)	89.5 (87.5-91.1)	93.8 (69.7-100)	113.2	0.89	1.3%	0.8-2.2
	≥198mg/dL	≥6.5%	All Positive	9.0 (5.1-15.2)	99.9 (99.4-100)	89.2 (87.3-90.9)	92.3 (64.6-100)	90.6	0.91	1.1%	0.6-1.9

Abbott hs-cTnI ≥82 ng/L	≥119mg/dL	n/a	Dual Positive	27.8 (20.9-36.0)	99.3 (98.5-99.7)	91.2 (89.4-92.8)	84.1 (70.3-92.4)	39.9	0.73	3.3%	2.4-4.5
	≥101mg/dL	n/a	Dual Positive	38.3 (30.5-46.8)	99.0 (98.2-99.5)	92.4 (90.6-93.8)	83.6 (72.2-91.0)	38.5	0.62	4.5%	3.4-5.9
	≥126mg/dL	n/a	Dual Positive	23.3 (16.9-31.2)	99.3 (98.5-99.7)	90.7 (88.9-92.3)	81.6 (66.3-91.1)	33.4	0.77	2.7%	1.9-3.9
	≥198mg/dL	n/a	Dual Positive	11.3 (6.9-17.9)	99.9 (99.4-100)	89.5 (87.5-91.1)	93.8 (69.7-100)	113.2	0.89	1.3%	0.8-2.2
	n/a	≥5.9%	Dual Positive	23.3 (16.9-31.2)	99.1 (98.3-99.6)	90.7 (88.8-92.3)	77.5 (62.3-87.9)	26	0.77	2.7%	1.9-3.9
	n/a	≥6.5%	Dual Positive	14.3 (9.3-21.3)	99.4 (98.7-99.8)	89.7 (87.8-91.4)	76.0 (56.3-88.8)	23.9	0.86	1.7%	1.1-2.6
	≥119mg/dL	≥5.9%	All Positive	18.0 (12.4-25.5)	99.3 (98.5-99.7)	90.1 (88.2-91.8)	77.4 (59.9-88.9)	25.9	0.83	2.1%	1.4-3.1
	≥119mg/dL	≥6.5%	All Positive	12.0 (7.4-18.8)	99.4 (98.7-99.8)	89.5 (87.6-91.2)	72.7 (51.6-87.1)	20.1	0.89	1.4%	0.9-2.3
	≥101mg/dL	≥5.9%	All Positive	21.8 (15.6-29.6)	99.2 (98.4-99.6)	90.5 (88.7-92.1)	78.4 (62.6-88.9)	27.4	0.79	2.6%	1.8-3.7
	≥101mg/dL	≥6.5%	All Positive	13.5 (8.7-20.5)	99.4 (98.7-99.8)	89.7 (87.7-91.3)	75.0 (54.8-88.3)	22.7	0.87	1.6%	1.0-2.5
	≥126mg/dL	≥5.9%	All Positive	17.3 (11.8-24.7)	99.3 (98.5-99.7)	90.1 (88.2-91.7)	76.7 (58.8-88.5)	24.8	0.83	2.0%	1.3-3.0
	≥126mg/dL	≥6.5%	All Positive	12.0 (7.4-18.8)	99.4 (98.7-99.8)	89.5 (87.6-91.2)	72.7 (51.6-87.1)	20.1	0.89	1.4%	0.9-2.3
	≥198mg/dL	≥5.9%	All Positive	10.5 (6.3-17.0)	99.9 (99.4-100)	89.4 (87.5-91.1)	93.3 (68.2-100)	105.7	0.90	1.2%	0.7-2.1
	≥198mg/dL	≥6.5%	All Positive	8.3 (4.5-14.3)	99.9 (99.4-100)	89.2 (87.2-90.9)	91.7 (62.5-100)	83.0	0.92	1.0%	0.5-1.8

Abbott hs-cTnI ≥99 ng/L	≥119mg/dL	n/a	Dual Positive	26.3 (19.5-34.4)	99.5 (98.8-99.8)	91.1 (89.2-92.6)	87.5 (73.4-95.0)	52.8	0.74	3.1%	2.2-4.3
	≥101mg/dL	n/a	Dual Positive	36.1 (28.4-44.6)	99.4 (98.7-99.8)	92.2 (90.4-93.6)	88.9 (77.5-95.2)	60.4	0.64	4.2%	3.2-5.6
	≥126mg/dL	n/a	Dual Positive	21.8 (15.6-29.6)	99.5 (98.8-99.8)	90.6 (88.7-92.2)	85.3 (69.4-94.0)	43.8	0.79	2.6%	1.8-3.7
	≥198mg/dL	n/a	Dual Positive	10.5 (6.3-17.0)	99.9 (99.4-100)	89.4 (87.5-91.1)	93.3 (68.2-100)	105.7	0.90	1.2%	0.7-2.1
	n/a	≥5.9%	Dual Positive	21.1 (14.9-28.8)	99.3 (98.5-99.7)	90.5 (88.6-92.1)	80.0 (63.8-90.3)	30.2	0.80	2.5%	1.7-3.6
	n/a	≥6.5%	Dual Positive	12.8 (8.0-19.6)	99.6 (98.9-99.9)	89.6 (87.7-91.3)	81.0 (59.4-92.9)	32.1	0.88	1.5%	0.9-2.4
	≥119mg/dL	≥5.9%	All Positive	16.5 (11.1-23.8)	99.5 (98.8-99.8)	90.0 (88.1-91.6)	81.5 (62.8-92.3)	33.2	0.84	1.9%	1.3-2.9
	≥119mg/dL	≥6.5%	All Positive	11.3 (6.9-17.9)	99.6 (98.9-99.9)	89.4 (87.5-91.1)	78.9 (56.1-92.1)	28.3	0.89	1.3%	0.8-2.2
	≥101mg/dL	≥5.9%	All Positive	19.5 (13.7-27.2)	99.4 (98.7-99.8)	90.3 (88.4-91.9)	81.3 (64.3-91.5)	32.7	0.81	2.3%	1.6-3.3
	≥101mg/dL	≥6.5%	All Positive	12.0 (7.4-18.8)	99.6 (98.9-99.9)	89.5 (87.6-91.2)	80.0 (57.8-92.5)	30.2	0.88	1.4%	0.9-2.3
	≥126mg/dL	≥5.9%	All Positive	15.8 (10.5-23.0)	99.5 (98.8-99.8)	89.9 (88.0-91.6)	80.8 (61.7-92.0)	31.7	0.85	1.8%	1.2-2.8
	≥126mg/dL	≥6.5%	All Positive	11.3 (6.9-17.9)	99.6 (98.9-99.9)	89.4 (87.5-91.1)	78.9 (56.1-92.1)	28.3	0.89	1.3%	0.8-2.2
	≥198mg/dL	≥5.9%	All Positive	9.8 (5.7-16.1)	99.9 (99.4-100)	89.3 (87.4-91.0)	92.9 (66.5-100)	98.1	0.90	1.1%	0.7-2.0
	≥198mg/dL	≥6.5%	All Positive	8.3 (4.5-14.3)	99.9 (99.4-100)	89.2 (87.2-90.9)	91.7 (62.5-100)	83.0	0.92	1.0%	0.5-1.8

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 5. Hs-cTnT Rule-out combinations

	hs-cTnT Rule-Out										
	Glucose	HbA1c	Criteria	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	LR+	LR-	% correctly deemed low risk	95% CI
Roche hs-cTnT <3 ng/L	<119mg/dL	n/a	Dual Negative	100 (96.6-100)	9.3 (7.6-11.2)	100 (95.2-100)	12.7 (10.9-14.9)	1.1	0	8.2%	6.7-9.9
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	6.1 (4.8-7.7)	100 (92.9-100)	12.4 (10.5-14.5)	1.07	0	5.4%	4.2-6.8
	<126mg/dL	n/a	Dual Negative	99.2 (95.4-100)	9.8 (8.1-11.8)	99.0 (94.0-100)	12.7 (10.8-14.9)	1.1	0.08	8.6%	7.1-10.4
	n/a	<5.9%	Dual Negative	99.2 (95.4-100)	9.8 (8.1-11.8)	99.0 (94.0-100)	12.7 (10.8-14.9)	1.1	0.08	8.6%	7.1-10.4
	n/a	<6.5%	Dual Negative	99.2 (95.4-100)	10.8 (9.0-12.8)	99.1 (94.5-100)	12.8 (10.9-15.0)	1.11	0.07	9.5%	7.9-11.4
	<119mg/dL	<5.9%	All negative	100 (96.6-100)	8.4 (6.8-10.3)	100 (94.8-100)	12.6 (10.8-14.8)	1.09	0	7.4%	6.0-9.1
	<119mg/dL	<6.5%	All negative	100 (96.6-100)	9.2 (7.5-11.1)	100 (95.2-100)	12.7 (10.8-14.9)	1.1	0	8.1%	6.6-9.8
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	5.7 (4.4-7.3)	100 (92.5-100)	12.3 (10.5-14.4)	1.06	0	5.4%	4.2-6.8
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	6.1 (4.8-7.7)	100 (92.9-100)	12.4 (10.5-14.5)	1.07	0	5.4%	4.2-6.8
	<126mg/dL	<5.9%	All negative	99.2 (95.4-100)	8.9 (7.3-10.8)	98.9 (93.4-100)	12.6 (10.7-14.8)	1.09	0.09	7.8%	6.4-9.5
	<126mg/dL	<6.5%	All negative	99.2 (95.4-100)	9.7 (8.0-11.7)	99.0 (93.9-100)	12.7 (10.8-14.9)	1.1	0.08	8.5%	7.0-10.3

Roche hs-cTnT <5 ng/L	<119mg/dL	n/a	Dual Negative	100 (96.6-100)	13.6 (11.7-15.9)	100 (96.7-100)	13.3 (11.3-15.6)	1.16	0	12.0%	10.3-14.1
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	8.4 (6.8-10.3)	100 (94.8-100)	12.6 (10.8-14.8)	1.09	0	7.4%	6.0-9.1
	<126mg/dL	n/a	Dual Negative	99.2 (95.4-100)	14.6 (12.6-17.0)	99.3 (95.9-100)	13.3 (11.4-15.6)	1.16	0.05	12.9%	11.1-15.0
	n/a	<5.9%	Dual Negative	99.2 (95.4-100)	15.1 (13.1-17.5)	99.3 (96.0-100)	13.4 (11.4-15.7)	1.17	0.05	13.4%	11.5-15.5
	n/a	<6.5%	Dual Negative	99.2 (95.4-100)	16.7 (14.6-19.2)	99.4 (96.4-100)	13.6 (11.6-16.0)	1.19	0.05	14.8%	12.8-17.0
	<119mg/dL	<5.9%	All negative	100 (96.6-100)	12.5 (10.6-14.8)	100 (96.4-100)	13.2 (11.2-15.4)	1.14	0	11.1%	9.4-13.0
	<119mg/dL	<6.5%	All negative	100 (96.6-100)	13.5 (11.6-15.8)	100 (96.7-100)	13.3 (11.3-15.5)	1.16	0	12.0%	10.2-14.0
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	8.0 (6.4-9.8)	100 (94.5-100)	12.6 (10.7-14.7)	1.09	0	7.0%	5.7-8.7
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	8.4 (6.8-10.3)	100 (94.8-100)	12.6 (10.8-14.8)	1.09	0	7.4%	6.0-9.1
	<126mg/dL	<5.9%	All negative	99.2 (95.4-100)	13.2 (11.3-15.5)	99.3 (95.5-100)	13.2 (11.2-15.4)	1.14	0.06	11.7%	10.0-13.7
	<126mg/dL	<6.5%	All negative	99.2 (95.4-100)	14.3 (12.3-16.7)	99.3 (95.8-100)	13.3 (11.3-15.6)	1.16	0.05	12.7%	10.9-14.7

Roche hs-cTnT <8 ng/L	<119mg/dL	n/a	Dual Negative	98.5 (94.3-99.9)	25.2 (22.6-28.0)	99.2 (97.0-100)	14.9 (12.7-17.4)	1.32	0.06	22.3%	19.9-24.8
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	14.6 (12.6-17.0)	100 (96.9-100)	13.4 (11.5-15.7)	1.17	0	12.9%	11.1-15.0
	<126mg/dL	n/a	Dual Negative	97.7 (93.3-99.5)	28.4 (25.7-31.3)	99.0 (96.8-99.8)	15.3 (13.0-17.9)	1.37	0.08	25.1%	22.6-27.7
	n/a	<5.9%	Dual Negative	97.0 (92.3-99.1)	26.8 (24.1-29.6)	98.5 (96.2-99.6)	14.9 (12.7-17.5)	1.33	0.11	23.7%	21.3-26.2
	n/a	<6.5%	Dual Negative	97.0 (92.3-99.1)	32.6 (29.7-35.5)	98.8 (96.8-99.6)	16.0 (13.6-18.7)	1.44	0.09	28.8%	26.2-31.5
	<119mg/dL	<5.9%	All negative	98.5 (94.3-99.9)	21.4 (19.0-24.1)	99.1 (96.5-100)	14.2 (12.1-16.7)	1.25	0.07	18.9%	16.7-21.3
	<119mg/dL	<6.5%	All negative	98.5 (94.3-99.9)	24.8 (22.2-27.6)	99.2 (97.0-100)	14.8 (12.6-17.3)	1.31	0.06	21.9%	19.6-24.4
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	13.2 (11.3-15.5)	100 (96.6-100)	13.2 (11.3-15.5)	1.15	0	11.7%	10.0-13.7
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	14.6 (12.6-17.0)	100 (96.9-100)	13.4 (11.5-15.7)	1.17	0	12.9%	11.1-15.0
	<126mg/dL	<5.9%	All negative	97.7 (93.3-99.5)	23.4 (20.9-26.1)	98.7 (96.1-99.7)	14.5 (12.3-16.9)	1.28	0.1	20.7%	18.4-23.1
	<126mg/dL	<6.5%	All negative	97.7 (93.3-99.5)	27.6 (24.9-30.4)	98.9 (96.8-99.8)	15.2 (12.9-17.7)	1.35	0.08	24.4%	22.0-26.9

Roche hs-cTnT <12 ng/L	<119mg/dL	n/a	Dual Negative	98.5 (94.3-99.9)	35.2 (32.3-38.2)	99.4 (97.8-100)	16.8 (14.3-19.5)	1.52	0.04	31.0%	28.4-33.8
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	19.2 (16.9-21.8)	100 (97.7-100)	14.1 (12.0-16.5)	1.24	0	17.0%	14.9-19.3
	<126mg/dL	n/a	Dual Negative	97.7 (93.3-99.5)	39.5 (36.6-42.6)	99.3 (97.7-99.9)	17.6 (15.1-20.6)	1.62	0.06	34.9%	32.2-37.7
	n/a	<5.9%	Dual Negative	95.5 (90.3-98.1)	36.6 (33.6-39.6)	98.4 (96.5-99.4)	16.6 (14.2-19.4)	1.51	0.12	32.3%	29.6-35.1
	n/a	<6.5%	Dual Negative	95.5 (90.3-98.1)	45.8 (42.8-48.9)	98.7 (97.2-99.5)	18.9 (16.1-22.1)	1.76	0.1	40.5%	37.6-43.3
	<119mg/dL	<5.9%	All negative	98.5 (94.3-99.9)	28.7 (26.0-31.6)	99.3 (97.4-100)	15.5 (13.2-18.1)	1.38	0.05	25.3%	22.9-27.9
	<119mg/dL	<6.5%	All negative	98.5 (94.3-99.9)	34.5 (31.6-37.5)	99.4 (97.8-100)	16.6 (14.2-19.4)	1.50	0.04	30.4%	27.8-33.2
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	16.7 (14.6-19.2)	100 (97.3-100)	13.7 (11.7-16.0)	1.2	0	14.8%	12.8-17.0
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	19.0 (16.7-21.6)	100 (97.6-100)	14.1 (12.0-16.4)	1.24	0	16.8%	14.7-19.1
	<126mg/dL	<5.9%	All negative	97.7 (93.3-99.5)	31.6 (28.8-34.5)	99.1 (97.2-99.8)	15.9 (13.6-18.6)	1.43	0.07	27.9%	25.4-30.6
	<126mg/dL	<6.5%	All negative	97.7 (93.3-99.5)	38.3 (35.4-41.4)	99.2 (97.6-99.9)	17.4 (14.8-20.2)	1.59	0.06	33.9%	31.2-36.7

Roche hs-cTnT <14 ng/L	<119mg/dL	n/a	Dual Negative	97.7 (93.3-99.5)	38.4 (35.5-41.5)	99.2 (97.7-99.9)	17.4 (14.8-20.3)	1.59	0.06	33.9%	31.3-36.8
	<101mg/dL	n/a	Dual Negative	100 (96.6-100)	20.6 (18.2-23.2)	100 (97.8-100)	14.3 (12.2-16.7)	1.26	0	18.2%	16.1-20.6
	<126mg/dL	n/a	Dual Negative	97.0 (92.3-99.1)	43.6 (40.6-46.7)	99.1 (97.6-99.7)	18.6 (15.8-21.6)	1.72	0.07	38.5%	35.7-41.4
	n/a	<5.9%	Dual Negative	94.7 (89.3-97.6)	39.2 (36.3-42.3)	98.3 (96.4-99.2)	17.1 (14.6-20.0)	1.56	0.13	34.7%	31.9-37.5
	n/a	<6.5%	Dual Negative	94.0 (88.4-97.1)	49.9 (46.8-53.0)	98.4 (96.9-99.3)	19.9 (17.0-23.2)	1.88	0.12	44.1%	41.2-47.0
	<119mg/dL	<5.9%	All negative	97.7 (93.3-99.5)	15.8 (13.5-18.5)	99.0 (97.1-99.8)	15.8 (13.5-18.5)	1.42	0.07	27.4%	24.8-35.9
	<119mg/dL	<6.5%	All negative	97.7 (93.3-99.5)	37.5 (34.5-40.5)	99.2 (97.6-99.8)	17.2 (14.6-20.0)	1.56	0.06	33.1%	30.4-35.9
	<101mg/dL	<5.9%	All negative	100 (96.6-100)	17.8 (15.6-20.3)	100 (97.5-100)	13.9 (11.8-16.2)	1.22	0	15.7%	13.7-18.0
	<101mg/dL	<6.5%	All negative	100 (96.6-100)	20.3 (17.9-22.9)	100 (97.8-100)	14.3 (12.2-16.7)	1.26	0	17.9%	15.8-20.3
	<126mg/dL	<5.9%	All negative	97.0 (92.3-99.1)	34.2 (31.3-37.2)	98.8 (97.0-99.7)	16.3 (13.9-19.1)	1.47	0.09	30.2%	27.6-32.9
	<126mg/dL	<6.5%	All negative	97.0 (92.3-99.1)	41.8 (38.8-44.9)	99.1 (97.5-99.7)	18.1 (15.4-21.1)	1.67	0.07	36.9%	34.2-39.8

Roche hs-cTnT <24 ng/L	<119mg/dL	n/a	Dual Negative	95.5 (90.3-98.1)	48.4 (45.3-51.5)	98.8 (97.3-99.5)	19.7 (16.8-22.9)	1.85	0.09	42.7%	39.9-45.6
	<101mg/dL	n/a	Dual Negative	99.2 (95.4-100)	25.9 (23.3-28.7)	99.6 (97.6-100)	15.1 (12.9-17.6)	1.34	0.03	22.9%	20.5-25.4
	<126mg/dL	n/a	Dual Negative	94.0 (88.4-97.1)	54.9 (51.8-57.9)	98.6 (97.2-99.3)	21.6 (18.5-25.2)	2.08	0.11	48.5%	45.6-51.4
	n/a	<5.9%	Dual Negative	91.0 (84.8-94.9)	48.8 (45.7-51.9)	97.6 (95.8-98.7)	19.1 (16.2-22.3)	1.78	0.19	43.1%	40.3-46.0
	n/a	<6.5%	Dual Negative	86.5 (79.5-91.4)	64.1 (61.1-67.1)	97.3 (95.7-98.3)	24.2 (20.6-28.3)	2.41	0.21	56.6%	53.7-59.5
	<119mg/dL	<5.9%	All negative	96.2 (91.3-98.6)	37.6 (34.7-40.7)	98.7 (96.9-99.5)	17.0 (14.5-19.8)	1.54	0.1	33.2%	30.6-36.0
	<119mg/dL	<6.5%	All negative	95.5 (90.3-98.1)	46.5 (43.5-49.6)	98.7 (97.2-99.5)	19.1 (16.3-22.3)	1.79	0.1	41.1%	38.3-44.0
	<101mg/dL	<5.9%	All negative	99.2 (95.4-100)	21.2 (18.8-23.9)	99.5 (97.1-100)	14.3 (12.2-16.7)	1.26	0.04	18.7%	16.6-21.1
	<101mg/dL	<6.5%	All negative	99.2 (95.4-100)	25.0 (22.4-27.8)	99.6 (97.6-100)	14.9 (12.7-17.4)	1.32	0.03	22.1%	19.7-24.6
	<126mg/dL	<5.9%	All negative	94.7 (89.3-97.6)	41.4 (38.4-44.5)	98.3 (96.6-99.3)	17.6 (15.0-20.6)	1.62	0.13	36.6%	33.8-39.4
	<126mg/dL	<6.5%	All negative	94.0 (88.4-97.1)	51.9 (48.8-55.0)	98.5 (97.0-99.3)	20.6 (17.5-24.0)	1.95	0.12	45.8%	43.0-48.7

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 6. Hs-cTnT Rule-in combinations

	hs-cTnT Rule-In										
	Glucose	HbA1c	Criteria	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	LR+	LR-	% correctly deemed high risk	95% CI
Roche hs-cTnT ≥14 ng/L	≥119mg/dL	n/a	Dual Positive	59.4 (50.9-67.4)	80.0 (77.4-82.3)	93.7 (91.9-95.2)	28.2 (23.3-33.8)	2.97	0.51	6.9%	5.6-8.6
	≥101mg/dL	n/a	Dual Positive	76.7 (68.8-83.1)	69.8 (66.9-72.6)	95.8 (94.0-97.0)	25.2 (21.2-29.6)	2.54	0.33	9.0%	7.4-10.8
	≥126mg/dL	n/a	Dual Positive	51.1 (42.7-59.5)	83.5 (81.0-85.6)	92.8 (90.9-94.3)	29.1 (23.6-35.2)	3.09	0.59	6.0%	4.7-7.5
	≥198mg/dL	n/a	Dual Positive	25.6 (18.9-33.6)	96.3 (95.0-97.3)	90.7 (88.8-92.3)	47.9 (36.7-59.3)	6.94	0.77	3.0%	2.1-4.2
	n/a	≥5.9%	Dual Positive	52.6 (44.2-60.9)	77.4 (74.7-80.0)	92.5 (90.5-94.1)	23.6 (19.1-28.7)	2.33	0.61	6.2%	4.9-7.7
	n/a	≥6.5%	Dual Positive	30.8 (23.6-39.1)	88.5 (86.4-90.4)	90.6 (88.6-92.3)	26.3 (20.0-33.7)	2.69	0.78	3.6%	2.7-4.9
	≥119mg/dL	≥5.9%	All Positive	41.4 (33.3-49.9)	85.3 (82.9-87.3)	91.6 (89.7-93.3)	27.1 (21.4-33.6)	2.81	0.69	4.8%	3.7-6.3
	≥119mg/dL	≥6.5%	All Positive	27.8 (20.9-36.0)	90.6 (88.7-92.3)	90.5 (88.5-92.1)	28.2 (21.2-36.5)	2.97	0.80	3.3%	2.4-4.5
	≥101mg/dL	≥5.9%	All Positive	48.1 (39.8-56.5)	81.4 (78.9-83.7)	92.2 (90.3-93.8)	25.5 (20.5-31.2)	2.58	0.64	5.6%	4.4-7.1
	≥101mg/dL	≥6.5%	All Positive	30.1 (22.9-38.4)	89.7 (87.7-91.5)	90.6 (88.7-92.3)	28.0 (21.2-35.9)	2.93	0.78	3.5%	2.6-4.8
	≥126mg/dL	≥5.9%	All Positive	39.1 (31.2-47.6)	87.5 (85.3-89.4)	91.6 (98.6-93.2)	29.2 (23.0-36.3)	3.12	0.70	4.6%	3.5-6.0
	≥126mg/dL	≥6.5%	All Positive	27.8 (20.9-36.0)	91.4 (89.5-93.0)	90.5 (88.6-92.2)	30.1 (22.7-38.7)	3.25	0.80	3.3%	2.4-4.5
	≥198mg/dL	≥5.9%	All Positive	23.3 (16.9-31.2)	96.5 (95.2-97.5)	90.5 (88.6-92.1)	47.0 (35.4-58.8)	6.69	0.80	2.7%	1.9-3.9
	≥198mg/dL	≥6.5%	All Positive	19.5 (13.7-27.2)	96.7 (95.4-97.7)	90.1 (88.1-91.7)	44.1 (32.2-56.7)	5.95	0.83	2.3%	1.6-3.3

Roche hs-cTnT ≥24 ng/L	≥119mg/dL	n/a	Dual Positive	50.4 (42.0-58.7)	88.7 (86.6-90.6)	93.1 (91.3-94.6)	37.2 (30.5-44.5)	4.48	0.56	5.9%	4.7-7.4
	≥101mg/dL	n/a	Dual Positive	66.2 (57.8-73.7)	83.3 (80.8-85.5)	94.9 (93.2-96.2)	34.4 (28.8-40.4)	3.95	0.41	7.7%	6.3-9.5
	≥126mg/dL	n/a	Dual Positive	42.9 (34.8-51.4)	90.9 (89.0-92.6)	92.3 (90.5-93.8)	38.5 (31.1-46.6)	4.73	0.63	5.0%	3.9-6.5
	≥198mg/dL	n/a	Dual Positive	22.6 (16.2-30.4)	97.7 (96.6-98.5)	90.5 (88.6-92.1)	56.6 (43.3-69.1)	9.85	0.79	2.6%	1.8-3.8
	n/a	≥5.9%	Dual Positive	45.1 (36.9-53.6)	86.6 (84.3-88.5)	92.3 (90.4-93.8)	30.8 (24.7-37.6)	3.36	0.63	5.3%	4.1-6.7
	n/a	≥6.5%	Dual Positive	27.1 (20.2-35.2)	93.0 (91.3-94.5)	90.6 (88.7-92.2)	34.0 (25.6-43.4)	3.88	0.78	3.2%	2.3-4.4
	≥119mg/dL	≥5.9%	All Positive	34.6 (27.0-43.0)	91.1 (89.2-92.8)	91.3 (89.4-92.9)	34.1 (26.6-42.4)	3.90	0.72	4.0%	3.0-5.4
	≥119mg/dL	≥6.5%	All Positive	24.1 (17.6-32.0)	94.2 (92.6-95.5)	90.4 (88.4-92.0)	35.6 (26.4-45.9)	4.17	0.81	2.8%	2.0-4.0
	≥101mg/dL	≥5.9%	All Positive	40.6 (32.6-49.1)	88.6 (86.5-90.5)	91.8 (90.0-93.4)	32.1 (25.5-39.6)	3.58	0.67	4.7%	3.7-6.2
	≥101mg/dL	≥6.5%	All Positive	26.3 (19.5-34.4)	93.6 (91.9-95.0)	90.6 (88.6-92.2)	35.4 (26.6-45.2)	4.13	0.79	3.1%	2.2-4.3
	≥126mg/dL	≥5.9%	All Positive	32.3 (25.0-40.7)	92.6 (90.8-94.1)	91.2 (89.3-92.8)	36.8 (28.6-45.8)	4.39	0.73	3.8%	2.8-5.1
	≥126mg/dL	≥6.5%	All Positive	24.1 (17.6-32.0)	94.7 (93.2-96.0)	90.4 (88.5-92.0)	37.6 (28.1-48.3)	4.56	0.80	2.8%	2.0-4.0
	≥198mg/dL	≥5.9%	All Positive	21.1 (14.9-28.8)	97.8 (96.7-98.6)	90.3 (88.4-92.0)	56.0 (42.3-68.8)	9.61	0.81	2.5%	1.7-3.6
	≥198mg/dL	≥6.5%	All Positive	18.0 (12.4-25.5)	97.9 (96.8-98.7)	90.0 (88.1-91.7)	53.3 (39.1-67.1)	8.63	0.84	2.1%	1.4-3.1

Roche hs-cTnT ≥52 ng/L	≥119mg/dL	n/a	Dual Positive	30.8 (23.6-39.1)	95.9 (94.5-97.0)	91.3 (89.4-92.8)	50.0 (39.4-60.6)	7.55	0.72	3.6%	2.7-4.9
	≥101mg/dL	n/a	Dual Positive	42.1 (34.1-50.6)	94.2 (92.6-95.5)	92.5 (90.7-93.9)	49.1 (40.1-58.2)	7.29	0.61	4.9%	3.8-6.4
	≥126mg/dL	n/a	Dual Positive	27.1 (20.2-35.2)	96.3 (95.0-97.3)	90.9 (89.0-92.5)	49.3 (38.2-60.5)	7.35	0.76	3.2%	2.3-4.4
	≥198mg/dL	n/a	Dual Positive	13.5 (8.7-20.5)	98.8 (97.9-99.3)	89.6 (87.7-91.3)	60.0 (42.3-75.4)	11.32	0.88	1.6%	1.0-2.5
	n/a	≥5.9%	Dual Positive	27.8 (20.9-36.0)	94.8 (93.3-96.0)	90.8 (88.9-92.5)	41.6 (31.9-52.0)	5.37	0.76	3.3%	2.4-4.5
	n/a	≥6.5%	Dual Positive	19.5 (13.7-27.2)	96.9 (95.6-97.8)	90.1 (88.2-91.7)	45.6 (33.4-58.4)	6.33	0.83	2.3%	1.6-3.3
	≥119mg/dL	≥5.9%	All Positive	21.8 (15.6-29.6)	96.4 (95.1-97.4)	90.3 (88.4-91.9)	44.6 (33.2-56.7)	6.08	0.81	2.6%	1.8-3.7
	≥119mg/dL	≥6.5%	All Positive	16.5 (11.1-23.8)	97.4 (96.2-98.2)	89.8 (87.9-91.5)	45.8 (32.6-59.7)	6.39	0.86	1.9%	1.3-2.9
	≥101mg/dL	≥5.9%	All Positive	26.3 (19.5-34.4)	95.7 (94.3-96.8)	90.7 (88.8-92.4)	44.9 (34.3-55.9)	6.14	0.77	3.1%	2.2-4.3
	≥101mg/dL	≥6.5%	All Positive	18.8 (13.0-26.3)	97.2 (96.0-98.1)	90.0 (88.1-91.7)	47.2 (34.4-60.3)	6.74	0.84	2.2%	1.5-3.2
	≥126mg/dL	≥5.9%	All Positive	21.8 (15.6-29.6)	96.8 (95.5-97.8)	90.3 (88.4-92.0)	47.5 (35.5-59.8)	6.84	0.81	2.6%	1.8-3.7
	≥126mg/dL	≥6.5%	All Positive	16.5 (11.1-23.8)	97.7 (96.6-98.5)	89.8 (87.9-91.5)	48.9 (35.0-63.0)	7.22	0.85	1.9%	1.3-2.9
	≥198mg/dL	≥5.9%	All Positive	12.0 (7.4-18.8)	98.8 (97.9-99.3)	89.4 (87.5-91.1)	57.1 (39.1-73.5)	10.07	0.89	1.4%	0.9-2.3
	≥198mg/dL	≥6.5%	All Positive	10.5 (6.3-17.0)	98.9 (98.0-99.4)	89.3 (87.3-91.0)	56.0 (37.1-73.4)	9.61	0.91	1.2%	0.7-2.1

Roche hs-cTnT ≥81 ng/L	≥119mg/dL	n/a	Dual Positive	21.8 (15.6-29.6)	97.8 (96.7-98.6)	90.4 (88.5-92.0)	56.9 (43.3-69.5)	9.95	0.80	2.6%	1.8-3.7
	≥101mg/dL	n/a	Dual Positive	28.6 (21.6-36.8)	97.4 (96.2-98.2)	91.1 (89.3-92.7)	59.4 (47.1-70.6)	11.03	0.73	3.3%	2.4-4.6
	≥126mg/dL	n/a	Dual Positive	18.8 (13.0-26.3)	97.8 (96.7-98.6)	90.1 (88.2-91.7)	53.2 (39.2-66.7)	8.58	0.83	2.2%	1.5-3.2
	≥198mg/dL	n/a	Dual Positive	9.8 (6.3-17.8)	99.4 (98.7-99.8)	89.3 (87.3-91.0)	68.4 (45.8-84.8)	16.36	0.91	1.1%	0.7-2.0
	n/a	≥5.9%	Dual Positive	18.8 (13.0-26.3)	97.6 (96.5-98.4)	90.1 (88.2-91.7)	51.0 (37.5-64.4)	7.86	0.83	2.2%	1.5-3.2
	n/a	≥6.5%	Dual Positive	13.5 (8.7-20.5)	98.3 (97.3-99.0)	89.6 (87.6-91.2)	51.4 (35.6-67.0)	7.99	0.88	1.6%	1.0-2.5
	≥119mg/dL	≥5.9%	All Positive	15.0 (9.9-22.2)	98.2 (97.2-98.9)	89.7 (87.8-91.4)	52.6 (37.3-67.5)	8.39	0.87	1.8%	1.1-2.7
	≥119mg/dL	≥6.5%	All Positive	11.3 (6.9-17.9)	98.5 (97.5-99.1)	89.3 (87.4-91.0)	50.0 (33.2-66.9)	7.55	0.90	1.3%	0.8-2.2
	≥101mg/dL	≥5.9%	All Positive	18.0 (12.4-25.5)	98.1 (97.0-98.8)	90.0 (88.1-91.7)	55.8 (41.1-69.6)	9.54	0.84	2.1%	1.4-3.1
	≥101mg/dL	≥6.5%	All Positive	12.8 (8.0-19.6)	98.5 (97.5-99.1)	89.5 (87.6-91.2)	53.1 (36.5-69.1)	8.56	0.89	1.5%	0.9-2.4
	≥126mg/dL	≥5.9%	All Positive	15.0 (9.9-22.2)	98.2 (97.2-98.9)	89.7 (87.8-91.4)	52.6 (37.3-67.5)	8.39	0.87	1.8%	1.1-2.7
	≥126mg/dL	≥6.5%	All Positive	11.3 (6.9-17.9)	98.5 (97.5-99.1)	89.3 (87.4-91.0)	50.0 (33.2-66.9)	7.55	0.90	1.3%	0.8-2.2
	≥198mg/dL	≥5.9%	All Positive	9.0 (5.1-15.2)	99.4 (98.7-99.8)	89.2 (87.2-90.9)	66.7 (43.6-83.9)	15.10	0.92	1.1%	0.6-1.9
	≥198mg/dL	≥6.5%	All Positive	8.3 (4.5-14.3)	99.4 (98.7-99.8)	89.1 (87.1-90.8)	64.7 (41.2-82.8)	13.84	0.92	1.0%	0.5-1.8

Roche hs-cTnT ≥155 ng/L	≥119mg/dL	n/a	Dual Positive	12.8 (8.0-19.6)	99.1 (98.3-99.6)	89.6 (87.6-91.2)	65.4 (46.1-80.7)	14.26	0.88	1.5%	0.9-2.4
	≥101mg/dL	n/a	Dual Positive	17.3 (11.8-24.7)	99.1 (98.3-99.6)	90.0 (88.1-91.7)	71.9 (54.5-84.6)	19.29	0.84	2.0%	1.3-3.0
	≥126mg/dL	n/a	Dual Positive	11.3 (6.9-17.9)	99.1 (98.3-99.6)	89.4 (87.5-91.1)	62.5 (42.6-78.9)	12.58	0.90	1.3%	0.8-2.2
	≥198mg/dL	n/a	Dual Positive	6.8 (3.4-12.5)	99.9 (99.4-100)	89.0 (87.0-90.7)	90.0 (57.4-100)	67.94	0.93	0.8%	0.4-1.5
	n/a	≥5.9%	Dual Positive	10.5 (6.3-17.0)	99.3 (98.5-99.7)	89.3 (87.4-91.0)	66.7 (45.2-83.0)	15.10	0.90	1.2%	0.7-2.1
	n/a	≥6.5%	Dual Positive	7.5 (4.0-13.4)	99.5 (98.8-99.8)	89.0 (87.1-90.7)	66.7 (41.5-85.0)	15.10	0.93	0.9%	0.5-1.6
	≥119mg/dL	≥5.9%	All Positive	8.3 (4.5-14.3)	99.4 (98.7-99.8)	89.1 (87.1-90.8)	64.7 (41.2-82.8)	13.84	0.92	1.0%	0.5-1.8
	≥119mg/dL	≥6.5%	All Positive	6.8 (3.4-12.5)	99.6 (98.9-99.9)	89.0 (87.0-90.7)	69.2 (42.0-87.7)	16.99	0.94	0.8%	0.4-1.5
	≥101mg/dL	≥5.9%	All Positive	9.8 (5.7-16.1)	99.4 (98.7-99.8)	89.3 (87.3-91.0)	68.4 (45.8-84.8)	16.36	0.91	1.1%	0.7-2.0
	≥101mg/dL	≥6.5%	All Positive	6.8 (3.4-12.5)	99.6 (98.9-99.9)	89.0 (87.0-90.7)	69.2 (42.0-87.7)	16.99	0.94	0.8%	0.4-1.5
	≥126mg/dL	≥5.9%	All Positive	8.3 (4.5-14.3)	99.4 (98.7-99.8)	89.1 (87.1-90.8)	64.7 (41.2-82.8)	13.84	0.92	1.0%	0.5-1.8
	≥126mg/dL	≥6.5%	All Positive	6.8 (3.4-12.5)	99.6 (98.9-99.9)	89.0 (87.0-90.7)	69.2 (42.0-87.7)	16.99	0.94	0.8%	0.4-1.5
	≥198mg/dL	≥5.9%	All Positive	6.0 (2.9-11.6)	99.9 (99.4-100)	88.9 (87.0-90.6)	88.9 (54.3-100)	60.39	0.94	0.7%	0.3-1.4
	≥198mg/dL	≥6.5%	All Positive	5.3 (2.4-10.7)	99.9 (99.4-100)	88.8 (86.9-90.6)	87.5 (50.8-99.9)	52.84	0.95	0.6%	0.3-1.3

Roche hs-cTnT ≥206 ng/L	≥119mg/dL	n/a	Dual Positive	9.8 (5.7-16.1)	99.6 (98.9-99.9)	89.3 (87.3-91.0)	76.5 (52.2-91.0)	24.53	0.91	1.1%	0.7-2.0
	≥101mg/dL	n/a	Dual Positive	14.3 (9.3-21.3)	99.6 (98.9-99.9)	89.8 (87.8-91.4)	82.6 (62.3-93.6)	35.86	0.86	1.7%	1.1-2.6
	≥126mg/dL	n/a	Dual Positive	9.0 (5.1-15.2)	99.6 (98.9-99.9)	89.2 (87.3-90.9)	75.0 (50.0-90.3)	22.65	0.91	1.1%	0.6-1.9
	≥198mg/dL	n/a	Dual Positive	6.0 (2.9-11.6)	100 (99.5-100)	88.9 (87.0-90.6)	100 (62.8-100)	n/a	0.94	0.7%	0.3-1.4
	n/a	≥5.9%	Dual Positive	9.8 (5.7-16.1)	99.5 (98.8-99.8)	89.3 (87.3-91.0)	72.2 (48.8-87.8)	19.63	0.91	1.1%	0.7-2.0
	n/a	≥6.5%	Dual Positive	6.8 (3.4-12.5)	99.6 (98.9-99.9)	89.0 (87.0-90.7)	69.2 (42.0-87.7)	16.99	0.94	0.8%	0.4-1.5
	≥119mg/dL	≥5.9%	All Positive	7.5 (4.0-13.4)	99.6 (98.9-99.9)	89.0 (87.1-90.8)	71.4 (45.0-88.7)	18.87	0.93	0.9%	0.5-1.6
	≥119mg/dL	≥6.5%	All Positive	6.0 (2.9-11.6)	99.7 (99.1-99.9)	88.9 (86.9-90.6)	72.7 (42.9-90.8)	20.13	0.94	0.7%	0.3-1.4
	≥101mg/dL	≥5.9%	All Positive	9.0 (5.1-15.2)	99.6 (98.9-99.9)	89.2 (87.3-90.9)	75.0 (50.0-90.3)	22.65	0.91	1.1%	0.6-1.9
	≥101mg/dL	≥6.5%	All Positive	6.0 (2.9-11.6)	99.7 (99.1-99.9)	88.9 (86.9-90.6)	72.7 (42.9-90.8)	20.13	0.94	0.7%	0.3-1.4
	≥126mg/dL	≥5.9%	All Positive	7.5 (4.0-13.4)	99.6 (98.9-99.9)	89.0 (87.1-90.8)	71.4 (45.0-88.7)	18.87	0.93	0.9%	0.5-1.6
	≥126mg/dL	≥6.5%	All Positive	6.0 (2.9-11.6)	99.7 (99.1-99.9)	88.9 (86.9-90.6)	72.7 (42.9-90.8)	20.13	0.94	0.7%	0.3-1.4
	≥198mg/dL	≥5.9%	All Positive	5.3 (2.4-10.7)	100 (99.5-100)	88.8 (86.9-90.6)	100 (59.6-100)	n/a	0.95	0.6%	0.3-1.3
	≥198mg/dL	≥6.5%	All Positive	4.5 (1.9-9.7)	100 (99.5-100)	88.8 (86.8-90.5)	100 (55.7-100)	n/a	0.96	0.5%	0.2-1.2

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 7. cTnI Rule-out combinations

cTnI Rule-Out											
	Glucose	HbA1c	Criteria	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	LR+	LR-	% correctly deemed low risk	95% CI
Abbott cTnI <0.01 ug/L	<119mg/dL	n/a	Dual Negative	94.7 (89.3-97.6)	46.5 (43.5-49.6)	98.5 (96.9-99.4)	19.0 (16.2-22.2)	1.77	0.11	41.1%	38.3-44.0
	<101mg/dL	n/a	Dual Negative	99.2 (95.4-100)	25.5 (22.9-28.3)	99.6 (97.6-100)	15.0 (12.8-17.5)	1.33	0.03	22.5%	20.2-25.0
	<126mg/dL	n/a	Dual Negative	93.2 (87.5-96.6)	53.3 (50.2-56.4)	98.3 (96.8-99.2)	20.9 (17.8-24.4)	2.0	0.13	47.1%	44.2-45.0
	n/a	<5.9%	Dual Negative	91.0 (84.8-94.9)	46.6 (43.6-49.7)	97.5 (95.6-98.6)	18.4 (15.6-21.6)	1.7	0.19	41.2%	38.3-44.1
	n/a	≥6.5%	Dual Negative	86.5 (79.5-91.4)	62.1 (59.0-65.0)	97.2 (95.6-98.3)	23.2 (19.7-27.1)	2.28	0.22	54.8%	51.9-57.7
	<119mg/dL	<5.9%	All negative	96.2 (91.3-98.6)	35.5 (32.6-38.5)	98.6 (96.7-99.5)	16.5 (14.0-19.3)	1.49	0.11	31.3%	28.7-34.1
	<119mg/dL	<6.5%	All negative	94.7 (89.3-97.6)	44.6 (41.6-47.7)	98.5 (96.8-99.3)	18.5 (15.7-21.6)	1.71	0.12	39.4%	36.6-42.3
	<101mg/dL	<5.9%	All negative	99.2 (95.4-100)	20.6 (18.2-23.2)	99.5 (97.1-100)	14.2 (12.1-16.6)	1.25	0.04	18.2%	16.1-20.6
	<101mg/dL	<6.5%	All negative	99.2 (95.4-100)	24.7 (22.1-27.5)	99.6 (97.5-100)	14.9 (12.7-17.4)	1.32	0.03	21.8%	19.5-24.3
	<126mg/dL	<5.9%	All negative	94.7 (89.3-97.6)	39.4 (36.5-42.5)	98.3 (96.4-99.2)	17.2 (14.6-20.1)	1.56	0.13	34.8%	31.1-37.7
	<126mg/dL	<6.5%	All negative	93.2 (87.5-96.6)	50.3 (47.2-53.4)	98.2 (96.7-99.1)	19.9 (17.0-23.2)	1.88	0.14	44.4%	41.6-47.3

Abbott cTnI <0.02 ug/L	<119mg/dL	n/a	Dual Negative	93.2 (87.5-96.6)	50.8 (47.7-53.9)	98.3 (96.7-99.1)	20.1 (17.1-23.4)	1.9	0.13	44.9%	42.0-47.8
	<101mg/dL	n/a	Dual Negative	98.5 (94.3-99.9)	27.6 (24.9-30.4)	99.3 (97.3-100)	15.3 (13.0-17.8)	1.36	0.06	24.4%	22.0-26.9
	<126mg/dL	n/a	Dual Negative	91.7 (85.7-95.5)	58.4 (55.3-61.4)	98.2 (96.7-99.0)	22.6 (19.3-26.3)	2.2	0.14	51.5%	48.6-54.4
	n/a	<5.9%	Dual Negative	90.2 (83.9-94.3)	50.1 (47.0-53.2)	97.5 (95.7-98.6)	19.3 (16.4-22.6)	1.81	0.2	44.2%	41.4-47.1
	n/a	<6.5%	Dual Negative	82.7 (75.3-88.3)	67.5 (64.6-70.4)	96.7 (95.1-97.8)	25.2 (21.4-29.5)	2.55	0.26	59.6%	56.7-62.5
	<119mg/dL	<5.9%	All negative	96.2 (91.3-98.6)	38.5 (35.6-41.6)	98.7 (97.0-99.5)	17.2 (14.6-20.1)	1.57	0.1	34.0%	31.3-36.8
	<119mg/dL	<6.5%	All negative	93.2 (87.5-96.6)	48.6 (45.5-51.7)	98.2 (96.5-99.1)	19.4 (16.5-22.6)	1.81	0.14	42.9%	40.1-45.8
	<101mg/dL	<5.9%	All negative	99.2 (95.4-100)	22.0 (19.6-24.7)	99.5 (97.2-100)	14.4 (12.3-16.9)	1.27	0.03	19.4%	17.2-21.8
	<101mg/dL	<6.5%	All negative	98.5 (94.3-100)	26.5 (23.9-29.3)	99.3 (97.1-100)	15.1 (12.8-17.6)	1.34	0.06	23.4%	21.0-25.9
	<126mg/dL	<5.9%	All negative	94.7 (89.3-97.6)	42.7 (39.7-45.8)	98.4 (96.7-99.3)	18.0 (15.3-21.0)	1.65	0.12	37.7%	35.0-40.6
	<126mg/dL	<6.5%	All negative	91.7 (85.7-95.5)	54.9 (51.8-57.9)	98.0 (96.5-99.0)	21.2 (18.1-24.8)	2.03	0.15	48.5%	45.6-51.4

Abbott cTnI <0.03 ug/L	<119mg/dL	n/a	Dual Negative	90.2 (83.9-94.3)	55.0 (51.9-58.0)	97.7 (96.1-98.7)	21.0 (17.8-24.5)	2.0	0.18	48.5%	45.7-51.5
	<101mg/dL	n/a	Dual Negative	96.2 (91.3-98.6)	29.6 (26.8-32.5)	98.3 (96.1-99.4)	15.3 (13.0-17.9)	1.37	0.13	26.1%	23.7-28.8
	<126mg/dL	n/a	Dual Negative	88.7 (82.1-93.2)	63.0 (60.0-66.0)	97.7 (96.2-98.6)	24.1 (20.5-28.1)	2.4	0.18	55.7%	52.8-58.5
	n/a	<5.9%	Dual Negative	86.5 (79.5-91.4)	54.5 (51.4-57.5)	96.8 (95.0-98.0)	20.1 (17.0-23.6)	1.9	0.25	48.1%	45.2-51.0
	n/a	<6.5%	Dual Negative	77.4 (69.6-87.8)	73.3 (70.5-76.0)	96.1 (94.5-97.3)	27.8 (23.5-32.5)	2.9	0.31	64.7%	61.9-67.5
	<119mg/dL	<5.9%	All negative	94.0 (88.4-97.1)	41.8 (38.8-44.9)	98.1 (96.3-99.1)	17.6 (15.0-20.6)	1.62	0.14	36.9%	34.2-39.8
	<119mg/dL	<6.5%	All negative	90.2 (83.9-94.3)	52.5 (49.4-55.6)	97.6 (95.9-98.6)	20.1 (17.1-23.5)	1.9	0.19	46.4%	43.5-49.3
	<101mg/dL	<5.9%	All negative	97.7 (93.3-99.5)	23.7 (21.2-26.4)	98.8 (96.2-99.8)	14.5 (12.4-17.0)	1.28	0.1	20.9%	18.7-23.4
	<101mg/dL	<6.5%	All negative	96.2 (91.3-98.6)	28.4 (25.7-31.3)	98.3 (95.9-99.4)	15.1 (12.9-17.7)	1.34	0.13	25.1%	22.6-27.7
	<126mg/dL	<5.9%	All negative	92.5 (86.6-96.0)	46.2 (43.2-49.3)	97.9 (96.1-98.9)	18.6 (15.8-21.7)	1.72	0.16	40.8%	38.0-43.7
	<126mg/dL	<6.5%	All negative	88.7 (82.1-93.2)	59.2 (56.1-62.2)	97.5 (95.9-98.5)	22.3 (19.0-26.1)	2.17	0.19	52.2%	49.3-55.1

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 8. cTnI Rule-in combinations

	cTnI Rule-In										
	Glucose	HbA1c	Criteria	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	LR+	LR-	% correctly deemed high risk	95% CI
Abbott cTnI ≥0.03 ug/L	≥119mg /dL	n/a	Dual Positive	42.1 (34.1-50.6)	95.3 (93.8-96.5)	92.6 (90.8-94.0)	54.4 (44.8-63.7)	8.99	0.61	4.9%	3.8-6.4
	≥101mg /dL	n/a	Dual Positive	55.6 (47.2-63.8)	92.7 (91.0-94.2)	94.0 (92.4-95.4)	50.3 (42.4-58.3)	7.65	0.48	6.5%	5.3-8.1
	≥126mg /dL	n/a	Dual Positive	34.6 (27.0-43.0)	95.9 (94.5-97.0)	91.7 (89.9-93.2)	52.9 (42.5-63.0)	8.47	0.68	4.0%	3.0-5.4
	≥198mg /dL	n/a	Dual Positive	18.8 (13.0-26.3)	99.0 (98.2-99.5)	90.2 (88.3-91.8)	71.4 (54.8-83.8)	18.87	0.82	2.2%	1.5-3.2
	n/a	≥5.9%	Dual Positive	36.1 (28.4-44.6)	94.0 (92.4-95.3)	91.7 (89.9-93.3)	44.4 (35.4-53.9)	6.04	0.68	4.2%	3.2-5.6
	n/a	≥6.5%	Dual Positive	22.6 (16.2-30.4)	97.0 (95.8-97.9)	90.4 (88.5-92.1)	50.0 (37.7-62.3)	7.55	0.80	2.6%	1.8-3.8
	≥119mg /dL	≥5.9%	All Positive	28.6 (21.6-36.8)	96.2 (94.8-97.2)	91.0 (89.2-92.6)	50.0 (39.0-61.0)	7.55	0.74	3.3%	2.4-4.6
	≥119mg /dL	≥6.5%	All Positive	19.5 (13.7-27.2)	97.6 (96.5-98.4)	90.2 (88.2-91.8)	52.0 (38.5-65.2)	8.18	0.82	2.3%	1.6-3.3
	≥101mg /dL	≥5.9%	All Positive	33.1 (25.7-41.5)	94.9 (93.4-96.1)	91.5 (89.6-93.0)	46.3 (36.6-56.3)	6.51	0.71	3.9%	2.9-5.2
	≥101mg /dL	≥6.5%	All Positive	21.8 (15.6-29.6)	97.3 (96.1-98.2)	90.4 (88.5-92.0)	51.8 (39.0-64.3)	8.11	0.80	2.6%	1.8-3.7
	≥126mg /dL	≥5.9%	All Positive	26.3 (19.5-34.4)	96.7 (95.4-97.7)	90.8 (89.0-92.4)	51.5 (39.8-63.0)	8.01	0.76	3.1%	2.2-4.3
	≥126mg /dL	≥6.5%	All Positive	19.5 (13.6-27.2)	97.8 (96.7-98.6)	90.2 (88.3-91.8)	54.2 (40.3-67.4)	8.92	0.82	2.3%	1.6-3.3
	≥198mg /dL	≥5.9%	All Positive	17.3 (11.8-24.7)	99.0 (98.2-99.5)	90.0 (88.1-91.7)	69.7 (52.5-82.8)	17.36	0.84	2.0%	1.3-3.0
	≥198mg /dL	≥6.5%	All Positive	14.3 (9.3-21.3)	99.1 (98.3-99.6)	89.7 (87.8-91.4)	67.9 (49.2-82.2)	15.94	0.87	1.7%	2.2-4.3

Abbott cTnI ≥0.1 ug/L	≥119mg /dL	n/a	Dual Positive	24.8 (18.2-32.8)	99.5 (98.8-99.8)	90.9 (89.1-92.5)	86.8 (72.2-94.7)	49.82	0.76	2.9%	2.1-4.1
	≥101mg /dL	n/a	Dual Positive	35.3 (27.7-43.8)	99.3 (98.5-99.7)	92.1 (90.3-93.5)	87.0 (75.3-93.9)	50.69	0.65	4.1%	3.1-5.5
	≥126mg /dL	n/a	Dual Positive	20.3 (14.3-28.0)	99.5 (98.8-99.8)	90.4 (88.5-92.0)	84.4 (67.8-93.6)	40.76	0.80	2.4%	1.6-3.5
	≥198mg /dL	n/a	Dual Positive	9.8 (5.7-16.1)	100 (99.5-100)	89.3 (87.4-91.0)	100 (73.4-100)	n/a	0.90	1.1%	0.7-2.0
	n/a	≥5.9%	Dual Positive	21.1 (14.9-28.8)	99.3 (98.5-99.7)	90.5 (88.6-92.1)	80.0 (63.8-90.3)	30.20	0.80	2.5%	1.7-3.6
	n/a	≥6.5%	Dual Positive	13.5 (8.7-20.5)	99.7 (99.1-99.9)	89.7 (87.8-91.4)	85.7 (64.5-95.9)	45.29	0.87	1.6%	1.0-2.5
	≥119mg /dL	≥5.9%	All Positive	15.8 (10.5-23.0)	99.5 (98.8-99.8)	89.9 (88.0-91.6)	80.8 (61.7-92.0)	31.71	0.85	1.8%	1.2-2.8
	≥119mg /dL	≥6.5%	All Positive	11.3 (6.9-17.9)	99.7 (99.1-99.9)	89.5 (87.5-91.1)	83.3 (60.0-95.0)	37.74	0.89	1.3%	0.8-2.2
	≥101mg /dL	≥5.9%	All Positive	19.5 (13.7-27.2)	99.4 (98.7-99.8)	90.3 (88.4-91.9)	81.3 (64.3-91.5)	32.71	0.81	2.3%	1.6-3.3
	≥101mg /dL	≥6.5%	All Positive	12.8 (8.0-19.6)	99.7 (99.1-99.9)	89.6 (87.7-91.3)	85.0 (63.1-95.6)	42.78	0.88	1.5%	0.9-2.4
	≥126mg /dL	≥5.9%	All Positive	15.0 (9.9-22.2)	99.5 (98.8-99.8)	89.8 (87.9-91.5)	80.0 (60.4-91.6)	30.20	0.85	1.8%	1.1-2.7
	≥126mg /dL	≥6.5%	All Positive	11.3 (6.9-17.9)	99.7 (99.1-99.9)	89.5 (87.5-91.1)	83.3 (60.0-95.0)	37.74	0.89	1.3%	0.8-2.2
	≥198mg /dL	≥5.9%	All Positive	9.0 (5.1-15.2)	100 (99.5-100)	89.2 (87.3-90.9)	100 (71.8-100)	n/a	0.91	1.1%	0.6-1.9
	≥198mg /dL	≥6.5%	All Positive	8.3 (4.5-14.3)	100 (99.5-100)	89.2 (87.2-90.9)	100 (70.0-100)	n/a	0.92	1.0%	0.5-1.8

Abbott cTnI ≥0.02 ug/L	≥119mg /dL	n/a	Dual Positive	46.6 (38.4-55.1)	91.8 (90.0-93.4)	92.8 (91.1-94.3)	43.1 (35.3-51.2)	5.71	0.58	5.5%	4.3-6.9
	≥101mg /dL	n/a	Dual Positive	60.9 (52.4-68.8)	87.1 (84.8-89.0)	94.4 (92.7-95.7)	38.4 (32.1-45.1)	4.70	0.45	7.1%	5.8-8.8
	≥126mg /dL	n/a	Dual Positive	39.1 (31.2-47.6)	92.9 (91.2-94.4)	92.0 (90.2-93.5)	42.3 (33.9-51.1)	5.53	0.66	4.6%	3.5-6.0
	≥198mg /dL	n/a	Dual Positive	21.1 (14.9-28.8)	98.4 (97.4-99.0)	90.4 (88.5-92.0)	63.6 (48.8-76.3)	13.21	0.80	2.5%	1.7-3.6
	n/a	≥5.9%	Dual Positive	39.8 (31.9-48.4)	90.7 (88.8-92.4)	91.9 (90.1-93.5)	36.3 (28.9-44.4)	4.30	0.66	4.7%	3.6-6.1
	n/a	≥6.5%	Dual Positive	24.8 (18.2-32.8)	95.1 (93.6-96.3)	90.5 (88.6-92.2)	40.2 (30.3-51.1)	5.08	0.79	2.9%	2.1-4.1
	≥119mg /dL	≥5.9%	All Positive	31.6 (24.3-39.9)	93.8 (92.2-95.2)	91.2 (89.3-92.8)	40.4 (31.5-50.0)	5.11	0.73	3.7%	2.7-5.0
	≥119mg /dL	≥6.5%	All Positive	21.8 (15.6-29.6)	96.0 (94.6-97.1)	90.3 (88.3-91.9)	42.0 (31.1-53.8)	5.47	0.81	2.6%	1.8-3.7
	≥101mg /dL	≥5.9%	All Positive	36.1 (28.4-44.6)	91.9 (90.1-93.5)	91.6 (89.7-93.1)	37.2 (29.3-45.8)	4.47	0.70	4.2%	3.2-5.6
	≥101mg /dL	≥6.5%	All Positive	24.1 (17.6-32.0)	95.5 (94.0-96.7)	90.5 (88.6-92.1)	41.6 (31.2-52.7)	5.37	0.80	2.8%	2.0-4.0
	≥126mg /dL	≥5.9%	All Positive	29.3 (22.2-37.6)	94.6 (93.0-95.9)	91.0 (89.1-92.6)	41.9 (32.4-52.1)	5.45	0.75	3.4%	2.5-4.7
	≥126mg /dL	≥6.5%	All Positive	21.8 (15.6-29.6)	96.3 (95.0-97.3)	90.3 (88.4-91.9)	43.9 (32.6-55.9)	5.92	0.81	2.6%	1.8-3.7
	≥198mg /dL	≥5.9%	All Positive	19.5 (13.7-27.2)	98.5 (97.5-99.1)	90.2 (88.3-91.9)	63.4 (48.1-76.5)	13.09	0.82	2.3%	1.6-3.3
	≥198mg /dL	≥6.5%	All Positive	16.5 (11.1-23.8)	98.6 (97.7-99.2)	89.9 (88.0-91.6)	61.1 (44.8-75.3)	11.86	0.85	1.9%	1.3-2.9

Abbott cTnI ≥0.04 ug/L	≥119mg /dL	n/a	Dual Positive	37.6 (29.8-46.1)	97.0 (95.8-97.9)	92.1 (90.4-93.6)	62.5 (51.5-72.23)	12.58	0.64	4.4%	3.3-5.8
	≥101mg /dL	n/a	Dual Positive	51.1 (42.7-59.5)	95.7 (94.3-96.8)	93.7 (92.0-95.0)	61.3 (52.0-69.8)	11.94	0.51	6.0%	4.7-7.5
	≥126mg /dL	n/a	Dual Positive	31.6 (24.3-39.9)	97.3 (96.1-98.2)	91.5 (89.7-93.0)	60.9 (49.1-71.5)	11.74	0.70	3.7%	2.7-5.0
	≥198mg /dL	n/a	Dual Positive	17.3 (11.8-24.7)	99.5 (98.8-99.8)	90.1 (88.8-99.8)	82.1 (63.9-92.6)	34.73	0.83	2.0%	1.3-3.0
	n/a	≥5.9%	Dual Positive	33.1 (25.7-41.5)	96.1 (94.7-97.2)	91.6 (89.7-93.1)	53.0 (42.4-63.4)	8.52	0.70	3.9%	2.9-5.2
	n/a	≥6.5%	Dual Positive	21.1 (14.9-28.8)	97.9 (96.8-98.7)	90.3 (88.4-92.0)	57.1 (43.3-70.0)	10.07	0.81	2.5%	1.7-3.6
	≥119mg /dL	≥5.9%	All Positive	25.6 (18.9-33.6)	97.5 (96.3-98.3)	90.8 (88.9-92.4)	57.6 (44.9-69.4)	10.27	0.76	3.0%	2.1-4.2
	≥119mg /dL	≥6.5%	All Positive	18.0 (12.4-25.5)	98.4 (97.4-99.0)	90.1 (98.8-99.8)	60.0 (44.6-73.7)	11.32	0.83	2.1%	1.4-3.1
	≥101mg /dL	≥5.9%	All Positive	30.1 (22.9-38.4)	96.8 (95.5-97.8)	91.3 (89.4-92.8)	55.6 (44.1-66.5)	9.44	0.72	3.5%	2.6-4.8
	≥101mg /dL	≥6.5%	All Positive	20.3 (14.3-28.0)	98.2 (97.2-98.9)	90.3 (88.4-91.9)	60.0 (45.4-73.0)	11.32	0.81	2.4%	1.6-3.5
	≥126mg /dL	≥5.9%	All Positive	24.1 (17.6-32.0)	97.7 (96.6-98.5)	90.7 (88.8-92.3)	58.2 (45.0-70.3)	10.50	0.78	2.8%	2.0-4.0
	≥126mg /dL	≥6.5%	All Positive	18.0 (12.4-25.6)	98.5 (97.5-99.1)	90.1 (88.2-91.7)	61.5 (45.9-75.1)	12.08	0.83	2.1%	1.4-3.1
	≥198mg /dL	≥5.9%	All Positive	15.8 (10.5-23.0)	99.5 (98.8-99.8)	89.9 (88.0-91.6)	80.8 (61.7-92.0)	31.71	0.85	1.8%	1.2-2.8
	≥198mg /dL	≥6.5%	All Positive	13.5 (8.7-20.5)	99.5 (98.8-99.8)	89.7 (87.8-91.3)	78.3 (57.7-90.8)	27.18	0.87	1.6%	1.0-2.5

Abbott cTnI ≥0.09 ug/L	≥119mg /dL	n/a	Dual Positive	26.3 (19.5-34.4)	99.5 (98.8-99.8)	91.1 (89.2-92.6)	87.5 (73.4-95.0)	52.84	0.74	3.1%	2.2-4.3
	≥101mg /dL	n/a	Dual Positive	36.8 (29.1-45.3)	99.3 (98.5-99.7)	92.2 (90.5-93.7)	87.5 (76.1-94.1)	52.84	0.64	4.3%	3.3-5.7
	≥126mg /dL	n/a	Dual Positive	21.8 (15.6-29.6)	99.5 (98.8-99.8)	90.6 (88.7-92.2)	85.3 (69.4-94.0)	43.78	0.79	2.6%	1.8-3.7
	≥198mg /dL	n/a	Dual Positive	10.5 (6.3-17.0)	100 (99.5-100)	89.4 (87.5-91.2)	100 (74.9-100)	n/a	0.90	1.2%	0.7-2.1
	n/a	≥5.9%	Dual Positive	22.6 (16.2-30.4)	99.2 (98.4-99.6)	90.6 (88.7-92.2)	78.9 (63.4-89.2)	28.31	0.78	2.6%	1.8-3.8
	n/a	≥6.5%	Dual Positive	13.5 (8.7-20.5)	99.6 (98.9-99.9)	89.7 (87.8-91.3)	81.8 (60.9-93.3)	33.97	0.87	1.6%	1.0-2.5
	≥119mg /dL	≥5.9%	All Positive	17.3 (11.8-24.7)	99.5 (98.8-99.8)	90.1 (88.2-91.7)	82.1 (63.9-92.6)	34.73	0.83	2.0%	1.3-3.0
	≥119mg /dL	≥6.5%	All Positive	11.3 (6.9-17.9)	99.7 (99.1-99.9)	89.5 (87.5-91.1)	83.3 (60.0-95.0)	37.74	0.89	1.3%	0.8-2.2
	≥101mg /dL	≥5.9%	All Positive	21.1 (14.9-28.8)	99.4 (98.7-99.8)	90.5 (88.6-92.1)	82.4 (66.1-92.0)	35.23	0.79	2.5%	1.7-3.6
	≥101mg /dL	≥6.5%	All Positive	12.8 (8.0-19.6)	99.7 (99.1-99.9)	89.6 (87.7-91.3)	85.0 (63.1-95.6)	42.78	0.88	1.5%	0.9-2.4
	≥126mg /dL	≥5.9%	All Positive	16.5 (11.1-23.8)	99.5 (98.8-99.8)	90.0 (88.1-91.6)	81.5 (62.8-92.3)	33.22	0.84	1.9%	1.3-2.9
	≥126mg /dL	≥6.5%	All Positive	11.3 (6.9-17.9)	99.7 (99.1-99.9)	89.5 (87.5-91.1)	83.3 (60.0-95.0)	37.74	0.89	1.3%	0.8-2.2
	≥198mg /dL	≥5.9%	All Positive	9.8 (5.7-16.1)	100 (99.5-100)	89.3 (87.4-91.0)	100 (73.4-100)	n/a	0.90	1.1%	0.7-2.0
	≥198mg /dL	≥6.5%	All Positive	8.3 (4.5-14.3)	100 (99.5-100)	89.2 (87.2-90.9)	100 (70.0-100)	n/a	0.92	1.0%	0.5-1.8

Abbott cTnI ≥0.3 ug/L	≥119mg /dL	n/a	Dual Positive	14.3 (9.3-21.3)	99.8 (99.2-100)	89.8 (87.9-91.4)	90.5 (69.9-98.6)	71.71	0.86	1.7%	2.2-4.3
	≥101mg /dL	n/a	Dual Positive	19.5 (13.7-27.2)	99.8 (99.2-100)	90.4 (88.5-92.0)	92.9 (76.3-99.1)	98.14	0.81	2.3%	1.6-3.3
	≥126mg /dL	n/a	Dual Positive	11.3 (6.9-17.9)	99.8 (99.2-100)	89.5 (87.5-91.1)	88.2 (64.4-98.0)	56.62	88.90	1.3%	0.8-2.2
	≥198mg /dL	n/a	Dual Positive	6.0 (2.9-11.6)	100 (99.5-100)	88.9 (87.0-90.6)	100 (62.8-100)	n/a	0.94	0.7%	0.3-1.4
	n/a	≥5.9%	Dual Positive	12.8 (8.0-19.6)	99.8 (99.2-100)	89.6 (87.7-91.3)	89.5 (67.4-98.3)	64.17	0.87	1.5%	0.9-2.4
	n/a	≥6.5%	Dual Positive	7.5 (4.0-13.4)	99.9 (99.4-100)	89.1 (87.1-90.8)	90.9 (60.1-100)	75.49	0.93	0.9%	0.5-1.6
	≥119mg /dL	≥5.9%	All Positive	9.0 (5.1-15.2)	99.8 (99.2-100)	89.2 (87.3-90.9)	85.7 (58.8-97.2)	45.29	0.91	1.1%	0.6-1.9
	≥119mg /dL	≥6.5%	All Positive	6.0 (2.9-11.6)	99.9 (99.4-100)	88.9 (86.2-90.0)	88.9 (54.3-100)	60.39	0.94	0.7%	0.3-1.4
	≥101mg /dL	≥5.9%	All Positive	12.0 (7.4-18.8)	99.8 (99.2-100)	89.5 (87.5-91.2)	88.9 (66.0-98.1)	60.39	0.88	1.4%	0.9-2.3
	≥101mg /dL	≥6.5%	All Positive	6.8 (3.4-12.5)	99.9 (99.4-100)	89.0 (87.0-90.7)	90.0 (57.4-100)	67.94	0.93	0.8%	0.4-1.5
	≥126mg /dL	≥5.9%	All Positive	8.3 (4.5-14.3)	99.8 (99.2-100)	89.1 (87.2-90.8)	84.6 (56.5-96.9)	41.52	0.92	1.0%	0.5-1.8
	≥126mg /dL	≥6.5%	All Positive	6.0 (2.9-11.6)	99.9 (99.4-100)	88.9 (86.2-90.0)	88.9 (54.3-100)	60.39	0.94	0.7%	0.3-1.4
	≥198mg /dL	≥5.9%	All Positive	5.3 (2.4-10.7)	100 (99.5-100)	88.8 (86.9-90.6)	100 (59.6-100)	n/a	0.95	0.6%	0.3-1.3
	≥198mg /dL	≥6.5%	All Positive	4.5 (1.9-9.7)	100 (99.5-100)	88.8 (86.8-90.5)	100 (55.7-100)	n/a	0.96	0.5%	0.2-1.2

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 9. hs-cTnI algorithms 1 and 3 and separated by diabetes status. Primary endpoint of MI

Diabetic Patients (n=333)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnI <5ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L	hs-cTnI <4ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥82 ng/L AND Glucose ≥198mg/dL	hs-cTnI <2ng/L	hs-cTnI ≥2
36	269	28	29	291	13	29	304
Sensitivity (95%CI) 100.0% (91.9-100)	n/a	**Specificity (95%CI)** 98.2% (95.8-99.4)	**Sensitivity (95%CI)** 100.0% (91.9-100)	n/a	**Specificity (95%CI)** 99.6% (97.8-100)	**Sensitivity (95%CI)** 100.0% (91.9-100)	**Specificity (95%CI)** 10.4% (7.3-14.5)
NPV (95%CI) 100.0% (88.5-100)	n/a	**PPV (95%CI)** 82.1% (63.9-92.6)	**NPV (95%CI)** 100.0% (86.1-100)	n/a	**PPV (95%CI)** 92.3% (64.6-100)	**NPV (95%CI)** 100.0% (86.1-100)	**PPV (95%CI)** 17.4% (13.6-22.1)
Non-Diabetic Patients (n=804)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnI <5ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L	hs-cTnI <4ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥82 ng/L AND Glucose ≥198mg/dL	hs-cTnI <2ng/L	hs-cTnI ≥2ng/L
302	462	40	250	551	3	130	674
Sensitivity (95%CI) 98.8% (92.6-100)	n/a	**Specificity (95%CI)** 99.3% (98.3-99.8)	**Sensitivity (95%CI)** 100.0% (94.5-100)	n/a	**Specificity (95%CI)** 100.0% (99.4-100)	**Sensitivity (95%CI)** 98.8% (92.6-100)	**Specificity (95%CI)** 17.8% (15.2-20.8)
NPV (95%CI) 99.7% (98.0-100)	n/a	**PPV (95%CI)** 87.5% (73.4-95.0)	**NPV (95%CI)** 100.0% (98.2-100)	n/a	**PPV (95%CI)** 100.0% (38.3-100)	**NPV (95%CI)** 99.2% (95.3-100)	**PPV (95%CI)** 11.7% (9.5-14.4)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 10. hs-cTnT algorithms 1 and 3 and separated by diabetes status. Primary endpoint of MI

hs-cTnT							
Diabetic Patients (n=333)							
Algorithm 1			**Algorithm3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnT <24ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L	hs-cTnT <14ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥198mg/dL	hs-cTnT <5ng/L	hs-cTnT ≥5ng/L
28	291	14	21	306	6	18	315
Sensitivity (95%CI) 100.0% (91.9-100)	n/a	**Specificity (95%CI)** 98.6% (96.3-99.6)	**Sensitivity (95%CI)** 100.0% (91.9-100)	n/a	**Specificity (95%CI)** 100.0% (98.4-100)	**Sensitivity (95%CI)** 100.0% (91.9-100)	**Specificity (95%CI)** 6.4% (4.1-10.0)
NPV (95%CI) 100.0% (7.0-14.1)	n/a	**PPV (95%CI)** 71.4% (45.0-88.7)	**NPV (95%CI)** 100.0% (81.8-100)	n/a	**PPV (95%CI)** 100.0% (55.7-100)	**NPV (95%CI)** 100.0% (79.3-100)	**PPV (95%CI)** 16.8% (13.1-21.4)
Non-Diabetic Patients (n=804)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnT <24ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L	hs-cTnT <14ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥198mg/dL	hs-cTnT <5ng/L	hs-cTnT ≥5ng/L
233	559	12	186	616	2	163	641
Sensitivity (95%CI) 98.8% (92.6-100)	n/a	**Specificity (95%CI)** 99.9% (99.1-100)	**Sensitivity (95%CI)** 100.0% (94.5-100)	n/a	**Specificity (95%CI)** 100.0% (99.4-100)	**Sensitivity (95%CI)** 98.8% (92.6-100)	**Specificity (95%CI)** 22.4% (19.5-25.6)
NPV (95%CI) 99.6% (97.4-100)	n/a	**PPV (95%CI)** 91.7% (62.5-100)	**NPV (95%CI)** 100.0% (97.6-100)	n/a	**PPV (95%CI)** 100.0% (29.0-100)	**NPV (95%CI)** 99.4% (96.3-100)	**PPV (95%CI)** 12.3% (10.0-15.1)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 11. cTnI algorithms 1 and 3 and separated by diabetes status. Primary endpoint of MI

cTnI							
Diabetic Patients (n=333)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL	cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥198mg/dL	cTnI<0.01ug/L	cTnI ≥0.01ug/L
27	279	27	27	295	11	197	136
Sensitivity (95%CI) 100.0% (91.9-100)	n/a	**Specificity (95%CI)** 98.2% (95.8-99.4)	**Sensitivity (95%CI)** 100.0% (91.9-100)	n/a	**Specificity (95%CI)** 100.0% (98.4-100)	**Sensitivity (95%CI)** 88.7% (77.1-95.1)	**Specificity (95%CI)** 68.2% (62.5-73.4)
NPV (95%CI) 100.0% (85.2-100)	n/a	**PPV (95%CI)** 81.5% (62.8-92.3)	**NPV (95%CI)** 100.0% (85.2-100)	n/a	**PPV (95%CI)** 100.0% (70.0-100)	**NPV (95%CI)** 97.0% (93.4-98.8)	**PPV (95%CI)** 34.6% (27.1-42.9)
Non-Diabetic Patients (n=804)							
Criteria 1			**Criteria 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL	cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥198mg/dL	cTnI<0.01ug/L	cTnI ≥0.01ug/L
230	545	29	230	571	2	580	224
Sensitivity (95%CI) 98.8% (92.6-100)	n/a	**Specificity (95%CI)** 99.7% (98.9-100)	**Sensitivity (95%CI)** 98.8% (92.6-100)	n/a	**Specificity (95%CI)** 100.0% (99.4-100)	**Sensitivity (95%CI)** 78.8% (68.5-86.4)	**Specificity (95%CI)** 77.8% (74.6-80.6)
NPV (95%CI) 99.6% (97.3-100)	n/a	**PPV (95%CI)** 93.1% (77.0-99.2)	**NPV (95%CI)** 99.6% (97.3-100)	n/a	**PPV (95%CI)** 100.0% (38.3-100)	**NPV (95%CI)** 97.1% (95.3-98.2)	**PPV (95%CI)** 28.1% (22.6-34.4)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 12. Diagnostic performance of hs-cTnI algorithms 1 and 3 and the LoD for the diagnosis of MI or CV death in all patients and stratified by diabetes status

hs-cTnI (MI or CV Death)								
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnI <5ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L	hs-cTnI <4ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥82 ng/L AND Glucose ≥198mg/dL	hs-cTnI <2ng/L	hs-cTnI ≥2
338	731	68	279	842	16	159	978
Sensitivity (95%CI) 99.3% (95.6-100)	n/a	**Specificity (95%CI)** 99.0% (98.1-99.5)	**Sensitivity (95%CI)** 100.0% (96.7-100)	n/a	**Specificity (95%CI)** 99.9% (99.4-100)	**Sensitivity (95%CI)** 99.3% (95.6-100)	**Specificity (95%CI)** 15.8% (13.7-18.2)
NPV (95%CI) 99.7% (98.2-100)	n/a	**PPV (95%CI)** 85.3% (74.8-92.0)	**NPV (95%CI)** 100.0% (98.4-100)	n/a	**PPV (95%CI)** 93.8% (69.7-100)	**NPV (95%CI)** 99.4% (96.2-100)	**PPV (95%CI)** 13.9% (11.9-16.2)
Diabetic Patients (n=333)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnI <5ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L	hs-cTnI <4ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥82 ng/L AND Glucose ≥198mg/dL	hs-cTnI <2ng/L	hs-cTnI ≥2
36	269	28	29	291	13	29	304
Sensitivity (95%CI) 100.0% (92.2-100)	n/a	**Specificity (95%CI)** 98.2% (95.7-99.4)	**Sensitivity (95%CI)** 100.0% (92.2-100)	n/a	**Specificity (95%CI)** 99.6% (97.8-100)	**Sensitivity (95%CI)** 100.0% (92.2-100)	**Specificity (95%CI)** 10.4% (7.3-14.6)
NPV (95%CI) 100.0% (88.5-100)	n/a	**PPV (95%CI)** 82.1% (63.9-92.6)	**NPV (95%CI)** 100.0% (86.1-100)	n/a	**PPV (95%CI)** 92.3% (64.6-100)	**NPV (95%CI)** 100.0% (86.1-100)	**PPV (95%CI)** 18.1% (14.2-22.8

Non-Diabetic Patients (n=804)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnI <5ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L	hs-cTnI <4ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥82 ng/L AND Glucose ≥198mg/dL	hs-cTnI <2ng/L	hs-cTnI ≥2ng/L
302	462	40	250	551	3	130	674
Sensitivity (95%CI) 98.8% (92.8-100)	n/a	**Specificity (95%CI)** 99.3% (98.3-99.8)	**Sensitivity (95%CI)** 100.0% (94.6-100)	n/a	**Specificity (95%CI)** 100.0% (99.4-100)	**Sensitivity (95%CI)** 98.8% (92.8-100)	**Specificity (95%CI)** 17.9% (15.2-20.8)
NPV (95%CI) 99.7% (98.0-100)	n/a	**PPV (95%CI)** 87.5% (73.4-95.0)	**NPV (95%CI)** 100.0% (98.2-100)	n/a	**PPV (95%CI)** 100.0% (38.3-100)	**NPV (95%CI)** 99.2% (95.3-100)	**PPV (95%CI)** 12.0% (9.8-14.7)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 13. Diagnostic performance of hs-cTnT algorithms 1 and 3 and the LoD for the diagnosis of MI or CV death in all patients and stratified by diabetes status

hs-cTnT (MI or CV Death)								
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnT <24ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L	hs-cTnT <14ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥198mg/dL	hs-cTnT <5ng/L	hs-cTnT ≥5ng/L
261	850	26	207	922	8	181	956
Sensitivity (95%CI) 99.3% (95.6-100)	n/a	**Specificity (95%CI)** 99.5% (98.8-99.8)	**Sensitivity (95%CI)** 100.0% (96.7-100)	n/a	**Specificity (95%CI)** 100.0% (99.5-100)	**Sensitivity (95%CI)** 99.3% (95.5-100)	**Specificity (95%CI)** 18.0% (16.1-21.0)
NPV (95%CI) 99.6% (97.6-100)	n/a	**PPV (95%CI)** 80.8% (61.7-92.0)	**NPV (95%CI)** 100.0% (97.8-100)	n/a	**PPV (95%CI)** 100.0% (62.8-100)	**NPV (95%CI)** 99.4% (95.8-100)	**PPV (95%CI)** 14.2% (15.0-20.0)
Diabetic Patients (n=333)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnT <24ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L	hs-cTnT <14ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥198mg/dL	hs-cTnT <5ng/L	hs-cTnT ≥5ng/L
28	291	14	21	306	6	18	315
Sensitivity (95%CI) 100.0% (92.2-100)	n/a	**Specificity (95%CI)** 98.6% (96.2-99.6)	**Sensitivity (95%CI)** 100.0% (92.2-100)	n/a	**Specificity (95%CI)** 100.0% (98.4-100)	**Sensitivity (95%CI)** 100.0% (92.2-100)	**Specificity (95%CI)** 6.5% (4.1-10.1)
NPV (95%CI) 100.0% (85.7-100)	n/a	**PPV (95%CI)** 71.4% (45.0-88.7)	**NPV (95%CI)** 100.0% (81.8-100)	n/a	**PPV (95%CI)** 100.0% (55.7-100)	**NPV (95%CI)** 100.0% (79.3-100)	**PPV (95%CI)** 17.5% (13.7-22.1)

Non-Diabetic Patients (n=804)								
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnT <24ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L	hs-cTnT <14ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥198mg/dL	hs-cTnT <5ng/L	hs-cTnT ≥5ng/L
233	559	12	186	616	2	163	641
Sensitivity (95%CI) 98.8% (92.8-100)	n/a	**Specificity (95%CI)** 99.9% (99.1-100)	**Sensitivity (95%CI)** 100.0% (94.6-100)	n/a	**Specificity (95%CI)** 100.0% (99.4-100)	**Sensitivity (95%CI)** 98.8% (92.8-100)	**Specificity (95%CI)** 22.4% (19.5-25.6)
NPV (95%CI) 99.6% (97.4-100)	n/a	**PPV (95%CI)** 91.7% (62.5-100)	**NPV (95%CI)** 100.0% (97.6-100)	n/a	**PPV (95%CI)** 100.0% (29.0-100)	**NPV (95%CI)** 99.4% (96.3-100)	**PPV (95%CI)** 12.6% (10.3-15.4)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 14. Diagnostic performance of cTnI algorithms 1 and 3 and the LoD for the diagnosis of MI or CV death in all patients and stratified by diabetes status

cTnI (MI or CV Death)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL	cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥198mg/dL	cTnI<0.01ug/L	cTnI ≥0.01ug/L
257	824	56	257	866	14	777	360
Sensitivity (95%CI) 99.3% (95.6-100)	n/a	**Specificity (95%CI)** 99.3% (98.5-99.7)	**Sensitivity (95%CI)** 99.3% (95.6-100)	n/a	**Specificity (95%CI)** 100.0% (99.5-100)	**Sensitivity (95%CI)** 82.5% (75.2-88.0)	**Specificity (95%CI)** 75.3% (72.5-77.9)
NPV (95%CI) 99.6% (97.6-100)	n/a	**PPV (95%CI)** 87.5% (76.1-94.1)	**NPV (95%CI)** 99.6% (97.6-100)	n/a	**PPV (95%CI)** 100.0% (74.9-100)	**NPV (95%CI)** 96.9% (95.4-97.9)	**PPV (95%CI)** 31.4% (26.8-36.4)
Diabetic Patients (n=333)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL	cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥198mg/dL	cTnI<0.01ug/L	cTnI ≥0.01ug/L
27	279	27	27	295	11	197	136
Sensitivity (95%CI) 100.0% (92.2-100)	n/a	**Specificity (95%CI)** 98.2% (95.7-99.4)	**Sensitivity (95%CI)** 100.0% (92.2-100)	n/a	**Specificity (95%CI)** 100.0% (98.4-100)	**Sensitivity (95%CI)** 87.3% (75.7-94.0)	**Specificity (95%CI)** 68.3% (62.7-73.5)
NPV (95%CI) 100.0% (85.2-100)	n/a	**PPV (95%CI)** 81.5% (62.8-92.3)	**NPV (95%CI)** 100.0% (85.2-100)	n/a	**PPV (95%CI)** 100.0% (70.0-100)	**NPV (95%CI)** 96.4% (92.7-98.4)	**PPV (95%CI)** 35.3% (27.8-43.6)

Non-Diabetic Patients (n=804)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL	cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥198mg/dL	cTnI<0.01ug/L	cTnI ≥0.01ug/L
230	545	29	230	571	3	580	224
Sensitivity (95%CI) 98.8% (92.8-100)	n/a	**Specificity (95%CI)** 99.7% (98.9-100)	**Sensitivity (95%CI)** 98.8% (92.8-100)	n/a	**Specificity (95%CI)** 100.0% (99.4-100)	**Sensitivity (95%CI)** 79.3% (69.2-86.7)	**Specificity (95%CI)** 78.0% (74.8-80.9)
NPV (95%CI) 99.6% (97.3-100)	n/a	**PPV (95%CI)** 93.1% (77.0-99.2)	**NPV (95%CI)** 99.6% (97.3-100)	n/a	**PPV (95%CI)** 100.0% (38.3-100)	**NPV (95%CI)** 97.1% (95.3-98.2)	**PPV (95%CI)** 29.0% (23.5-35.3)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 15. Diagnostic performance of hs-cTnI algorithms 1 and 3 and the LoD for the diagnosis of ACS or CV death in all patients and stratified by diabetes status

hs-cTnI (ACS or CV Death)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnI <5ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L	hs-cTnI <4ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥82 ng/L AND Glucose ≥198mg/dL	hs-cTnI <2ng/L	hs-cTnI ≥2
338	731	68	279	842	16	159	978
Sensitivity (95%CI) 96.5% (92.3-98.5)	n/a	**Specificity (95%CI)** 99.1% (98.2-99.5)	**Sensitivity (95%CI)** 97.6% (93.9-99.3)	n/a	**Specificity (95%CI)** 99.9% (99.4-100)	**Sensitivity (95%CI)** 98.2% (94.7-99.6)	**Specificity (95%CI)** 16.1% (13.9-18.6)
NPV (95%CI) 98.2% (96.1-99.3)	n/a	**PPV (95%CI)** 86.8% (76.5-93.1)	**NPV (95%CI)** 98.6% (96.2-99.6)	n/a	**PPV (95%CI)** 93.8% (69.7-100)	**NPV (95%CI)** 98.1% (94.4-99.6)	**PPV (95%CI)** 17.1% (14.8-19.6)
Diabetic Patients (n=333)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnI <5ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L	hs-cTnI <4ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥82 ng/L AND Glucose ≥198mg/dL	hs-cTnI <2ng/L	hs-cTnI ≥2
36	269	28	29	291	13	29	304
Sensitivity (95%CI) 100.0% (93.6-100)	n/a	**Specificity (95%CI)** 98.1% (95.5-99.3)	**Sensitivity (95%CI)** 100.0% (93.6-100)	n/a	**Specificity (95%CI)** 99.6% (97.7-100)	**Sensitivity (95%CI)** 97.1% (89.3-99.8)	**Specificity (95%CI)** 10.2% (7.1-14.5)
NPV (95%CI) 100.0% (88.5-100)	n/a	**PPV (95%CI)** 82.1% (63.9-92.6)	**NPV (95%CI)** 100.0% (86.1-100)	n/a	**PPV (95%CI)** 92.3% (64.6-100)	**NPV (95%CI)** 93.1% (77.0-99.2)	**PPV (95%CI)** 21.7% (17.4-26.7)

Non-Diabetic Patients (n=804)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnI <5ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥99 ng/L	hs-cTnI <4ng/L AND Glucose <119mg/dL	Fails to meet In/Out criteria	hs-cTnI ≥82 ng/L AND Glucose ≥198mg/dL	hs-cTnI <2ng/L	hs-cTnI ≥2ng/L
302	462	40	250	551	3	130	674
Sensitivity (95%CI) 94.1% (87.5-97.5)	n/a	**Specificity (95%CI)** 99.4% (98.5-99.8)	**Sensitivity (95%CI)** 96.1% (90.0-98.8)	n/a	**Specificity (95%CI)** 100.0% (38.3-100)	**Sensitivity (95%CI)** 99.0% (94.1-100)	**Specificity (95%CI)** 18.4% (15.7-21.4)
NPV (95%CI) 98.0% (95.6-99.2)	n/a	**PPV (95%CI)** 90.0% (76.4-96.6)	**NPV (95%CI)** 98.4% (95.8-99.5)	n/a	**PPV (95%CI)** 100.0% (99.3-100)	**NPV (95%CI)** 99.2% (95.3-100)	**PPV (95%CI)** 15.0% (12.5-17.9)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 16. Diagnostic performance of hs-cTnT algorithms 1 and 3 and the LoD for the diagnosis of ACS or CV death in all patients and stratified by diabetes status

hs-cTnT (ACS or CV Death)								
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnT <24ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L	hs-cTnT <14ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥198mg/dL	hs-cTnT <5ng/L	hs-cTnT ≥5ng/L
261	850	26	207	922	8	181	956
Sensitivity (95%CI) 95.3% (90.8-97.7)	n/a	**Specificity (95%CI)** 99.5% (98.8-99.8)	**Sensitivity (95%CI)** 98.2% (94.7-99.6)	n/a	**Specificity (95%CI)** 100.0% (99.5-100)	**Sensitivity (95%CI)** 98.8% (95.5-100)	**Specificity (95%CI)** 18.5% (16.2-21.1)
NPV (95%CI) 96.9% (94.0-98.5)	n/a	**PPV (95%CI)** 80.8% (61.7-92.0)	**NPV (95%CI)** 98.6% (95.6-99.7)	n/a	**PPV (95%CI)** 100.0% (62.8-100)	**NPV (95%CI)** 98.9% (95.8-100)	**PPV (95%CI)** 17.6% (15.3-20.1)
Diabetic Patients (n=333)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnT <24ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L	hs-cTnT <14ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥198mg/dL	hs-cTnT <5ng/L	hs-cTnT ≥5ng/L
28	291	14	21	306	6	18	315
Sensitivity (95%CI) 98.5% (91.4-100)	n/a	**Specificity (95%CI)** 98.5% (96.0-99.6)	**Sensitivity (95%CI)** 100.0% (93.6-100)	n/a	**Specificity (95%CI)** 100.0% (98.3-100)	**Sensitivity (95%CI)** 100.0% (93.6-100)	**Specificity (95%CI)** 6.8% (4.3-10.5)
NPV (95%CI) 96.4% (80.8-100)	n/a	**PPV (95%CI)** 71.4% (45.0-88.7)	**NPV (95%CI)** 100.0% (81.8-100)	n/a	**PPV (95%CI)** 100.0% (55.7-100)	**NPV (95%CI)** 100.0% (79.3-100)	**PPV (95%CI)** 21.6% (17.4-26.5)

Non-Diabetic Patients (n=804)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
hs-cTnT <24ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L	hs-cTnT <14ng/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	hs-cTnT ≥206 ng/L AND Glucose ≥198mg/dL	hs-cTnT <5ng/L	hs-cTnT ≥5ng/L
233	559	12	186	616	2	163	641
Sensitivity (95%CI) 93.1% (86.3-96.9)	n/a	**Specificity (95%CI)** 99.9% (99.1-100)	**Sensitivity (95%CI)** 97.1% (91.3-99.4)	n/a	**Specificity (95%CI)** 100.0% (99.3-100)	**Sensitivity (95%CI)** 98.0% (92.7-99.9)	**Specificity (95%CI)** 22.9% (20.0-26.2)
NPV (95%CI) 97.0% (93.8-98.7)	n/a	**PPV (95%CI)** 91.7% (62.5-100)	**NPV (95%CI)** 98.4% (95.2-99.7)	n/a	**PPV (95%CI)** 100.0% (29.0-100)	**NPV (95%CI)** 98.8% (95.4-100)	**PPV (95%CI)** 15.6% (13.0-18.6)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Supplemental Table 17. Diagnostic performance of cTnI algorithms 1 and 3 and the LoD for the diagnosis of ACS or CV death in all patients and stratified by diabetes status

cTnI (ACS or CV Death)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL	cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥198mg/dL	cTnI<0.01ug/L	cTnI ≥0.01ug/L
257	824	56	257	866	14	777	360
Sensitivity (95%CI) 97.1% (93.1-98.9)	n/a	**Specificity (95%CI)** 99.3% (98.5-99.7)	**Sensitivity (95%CI)** 97.1% (93.1-98.9)	n/a	**Specificity (95%CI)** 100.0% (99.5-100)	**Sensitivity (95%CI)** 74.1% (67.0-80.1)	**Specificity (95%CI)** 75.8% (73.0-78.4)
NPV (95%CI) 98.1% (95.4-99.3)	n/a	**PPV (95%CI)** 87.5% (76.1-94.1)	**NPV (95%CI)** 98.1% (95.4-99.3)	n/a	**PPV (95%CI)** 100.0% (74.9-100)	**NPV (95%CI)** 94.3% (92.5-95.8)	**PPV (95%CI)** 35.0% (30.3-40.1)
Diabetic Patients (n=333)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL	cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥198mg/dL	cTnI<0.01ug/L	cTnI ≥0.01ug/L
27	279	27	27	295	11	197	136
Sensitivity (95%CI) 100.0% (93.6-100)	n/a	**Specificity (95%CI)** 98.1% (95.5-99.3)	**Sensitivity (95%CI)** 100.0% (93.6-100)	n/a	**Specificity (95%CI)** 100.0% (98.3-100)	**Sensitivity (95%CI)** 75.0% (63.5-83.9)	**Specificity (95%CI)** 67.9% (62.1-73.3)
NPV (95%CI) 100.0% (85.2-100)	n/a	**PPV (95%CI)** 81.5% (62.8-92.3)	**NPV (95%CI)** 100.0% (85.2-100)	n/a	**PPV (95%CI)** 100.0% (70.0-100)	**NPV (95%CI)** 91.4% (86.5-94.6)	**PPV (95%CI)** 37.5% (29.8-45.9)

Non-Diabetic Patients (n=804)							
Algorithm 1			**Algorithm 3**			**LoD**	
Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in	Rule-out	Rule-in
cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥101mg/dL	cTnI <0.01ug/L AND Glucose <101mg/dL	Fails to meet In/Out criteria	cTnI ≥0.09 ug/L AND Glucose ≥198mg/dL	cTnI<0.01ug/L	cTnI ≥0.01ug/L
230	545	29	230	571	3	580	224
Sensitivity (95%CI) 95.1% (88.8-98.2)	n/a	**Specificity (95%CI)** 99.7% (98.9-100)	**Sensitivity (95%CI)** 95.1% (88.8-98.2)	n/a	**Specificity (95%CI)** 100.0% (99.3-100)	**Sensitivity (95%CI)** 73.5% (64.2-81.2)	**Specificity (95%CI)** 78.8% (75.6-81.6)
NPV (95%CI) 97.8% (94.9-99.2)	n/a	**PPV (95%CI)** 93.1% (77.0-99.2)	**NPV (95%CI)** 97.8% (94.9-99.2)	n/a	**PPV (95%CI)** 100.0% (38.3-100)	**NPV (95%CI)** 95.3% (93.3-96.8)	**PPV (95%CI)** 33.5% (27.6-39.9)

*Glucose values are presented as mg/dL and can be converted to mmol/L using a conversion factor of 0.0555

Appendix B: Chapter 5 Supplemental Data

Supplementary

Table 1. Diagnostic performance of using ESC 0 hour algorithm cutoffs

hs-cTnI ESC 0/1hr			hs-cTnT ESC 0/1hr		
Sensitivity	99.2%	(95.4 to 100)	Sensitivity	99.2%	(95.4 to 100)
Specificity	96.4%	(95.1 to 97.4)	Specificity	92.5%	(90.7 to 94.0)
NPV	99.4%	(96.2 to 100)	NPV	99.4%	(96.6 to 100)
PPV	66.4%	(57.0 to 74.6)	PPV	46.8%	(38.8 to 55.0)

Figure 1. Tornado plot comparing the incremental cost of hs-cTnI algorithm 1 to the ESC 0 hour algorithm cutoffs when costs of algorithm 1 are varied +10% (high) and -10% (low). The Y axis indicates the base cost (ie. The cost of the ESC 0 hour algorithm). Cost labels indicate the cost per patient when varied and any costs in brackets indicate a negative number. Abbreviations: Dx=discharge diagnosis, CP NYD= final diagnosis of "Other CP" or "CP Unspecified"

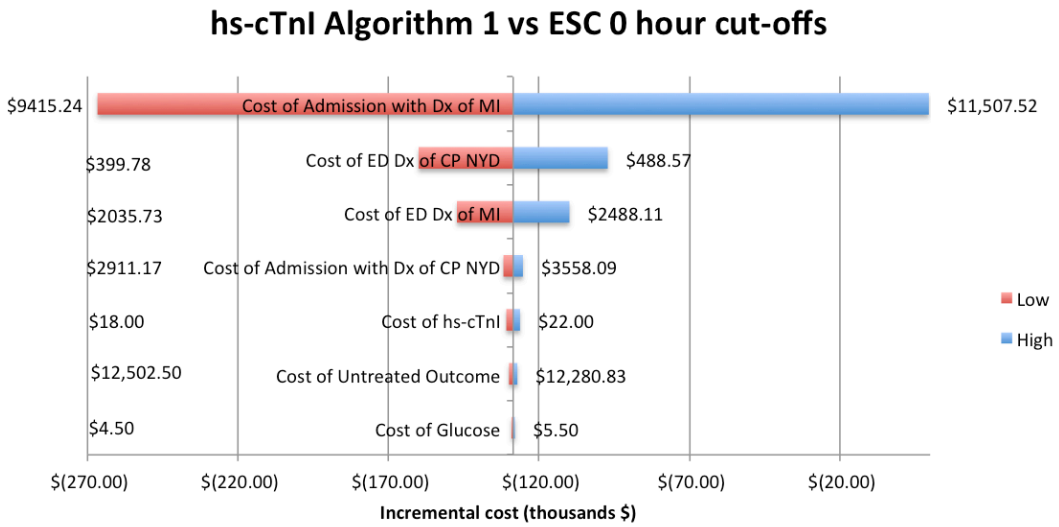


Figure 2. Tornado plot comparing the incremental cost of hs-cTnI algorithm 2 to the ESC 0 hour algorithm cutoffs when costs of algorithm 2 are varied +10% (high) and -10% (low). The Y axis indicates the base cost (ie. The cost of the ESC 0 hour algorithm). Cost labels indicate the cost per patient when varied and any costs in brackets indicate a negative number. Abbreviations: Dx=discharge diagnosis, CP NYD= final diagnosis of "Other CP" or "CP Unspecified"Abbreviations: Dx=discharge diagnosis, CP NYD= final diagnosis of "Other CP" or "CP Unspecified"

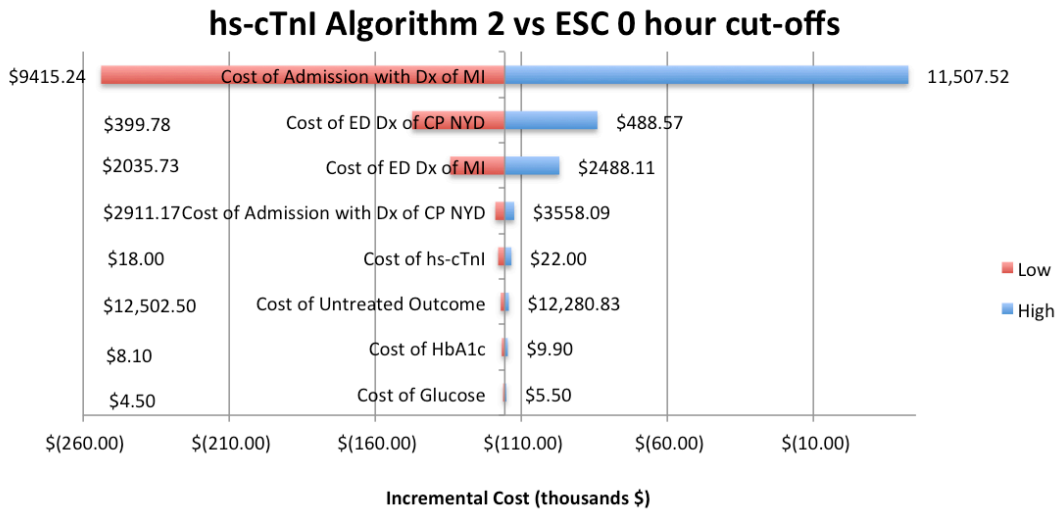


Figure 3. Tornado plot comparing the incremental cost of hs-cTnI algorithm 3 to the ESC 0 hour algorithm cutoffs when costs of algorithm 3 are varied +10% (high) and -10% (low). The Y axis indicates the base cost (ie. The cost of the ESC 0 hour algorithm). Cost labels indicate the cost per patient when varied and any costs in brackets indicate a negative number. Abbreviations: Dx=discharge diagnosis, CP NYD= final diagnosis of "Other CP" or "CP Unspecified"

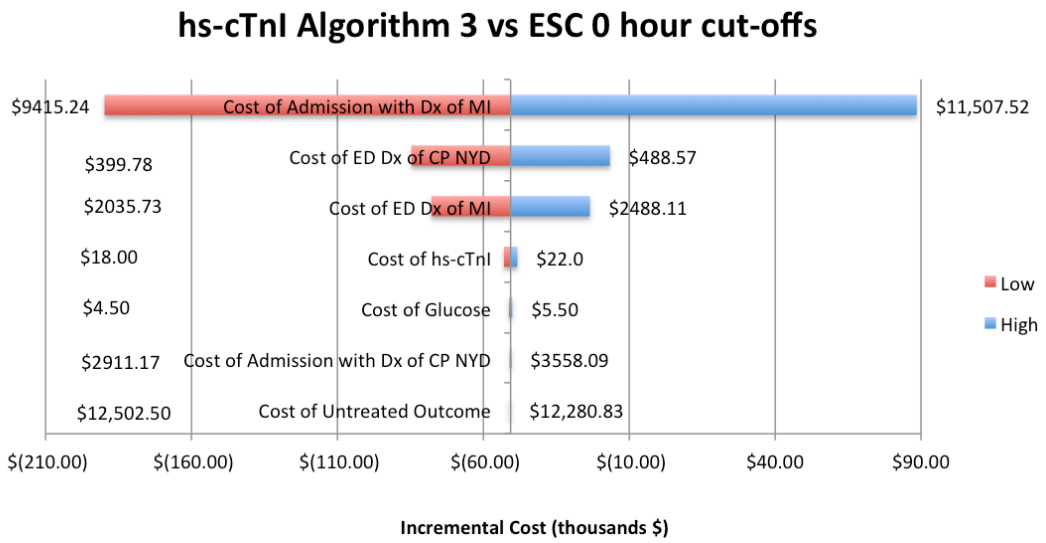


Figure 4. Tornado plot comparing the incremental cost of hs-cTnI algorithm 4 to the ESC 0 hour algorithm cutoffs when costs of algorithm 4 are varied +10% (high) and -10% (low). The Y axis indicates the base cost (ie. The cost of the ESC 0 hour algorithm). Cost labels indicate the cost per patient when varied and any costs in brackets indicate a negative number. Abbreviations: Dx=discharge diagnosis, CP NYD= final diagnosis of "Other CP" or "CP Unspecified"

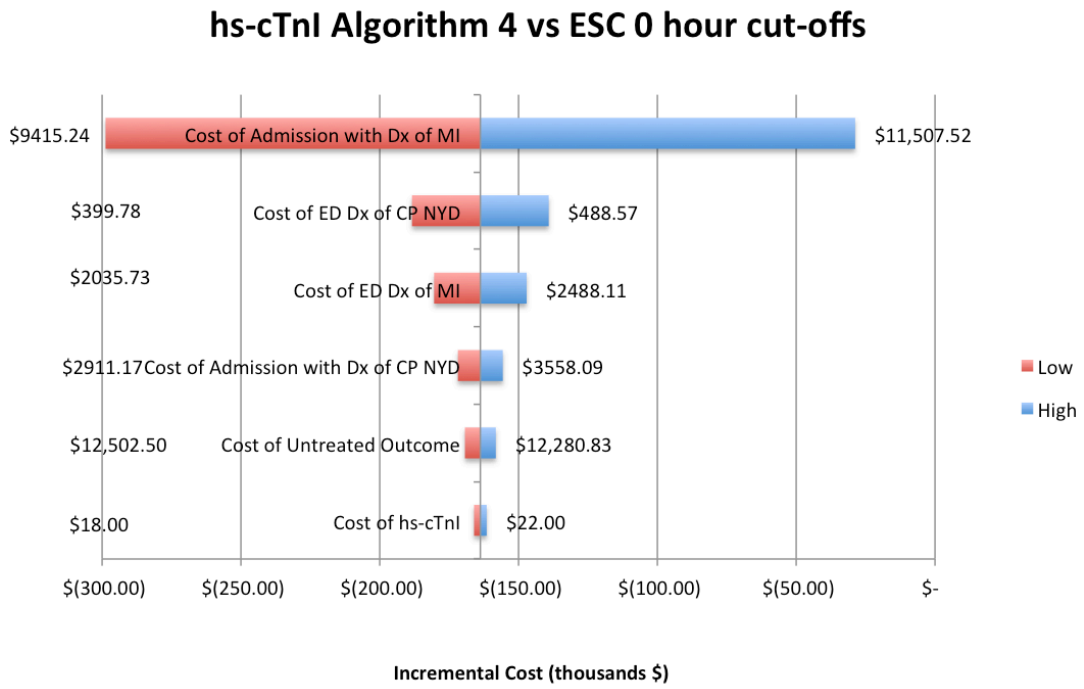


Figure 5. Tornado plot comparing the incremental cost of hs-cTnT algorithm 1 to the ESC 0 hour algorithm cutoffs when costs of algorithm 1 are varied +10% (high) and -10% (low). The Y axis indicates the base cost (ie. The cost of the ESC 0 hour algorithm). Cost labels indicate the cost per patient when varied and any costs in brackets indicate a negative number. Abbreviations: Dx=discharge diagnosis, CP NYD= final diagnosis of "Other CP" or "CP Unspecified"

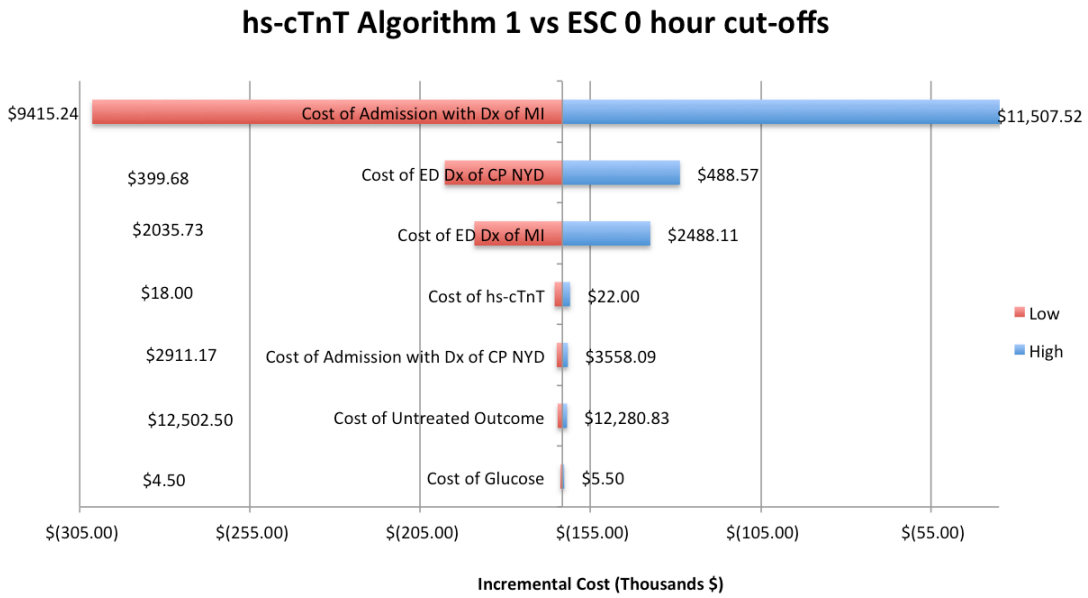


Figure 6. Tornado plot comparing the incremental cost of hs-cTnT algorithm 2 to the ESC 0 hour algorithm cutoffs when costs of algorithm 2 are varied +10% (high) and -10% (low). The Y axis indicates the base cost (ie. The cost of the ESC 0 hour algorithm). Cost labels indicate the cost per patient when varied and any costs in brackets indicate a negative number. Abbreviations: Dx=discharge diagnosis, CP NYD= final diagnosis of "Other CP" or "CP Unspecified"

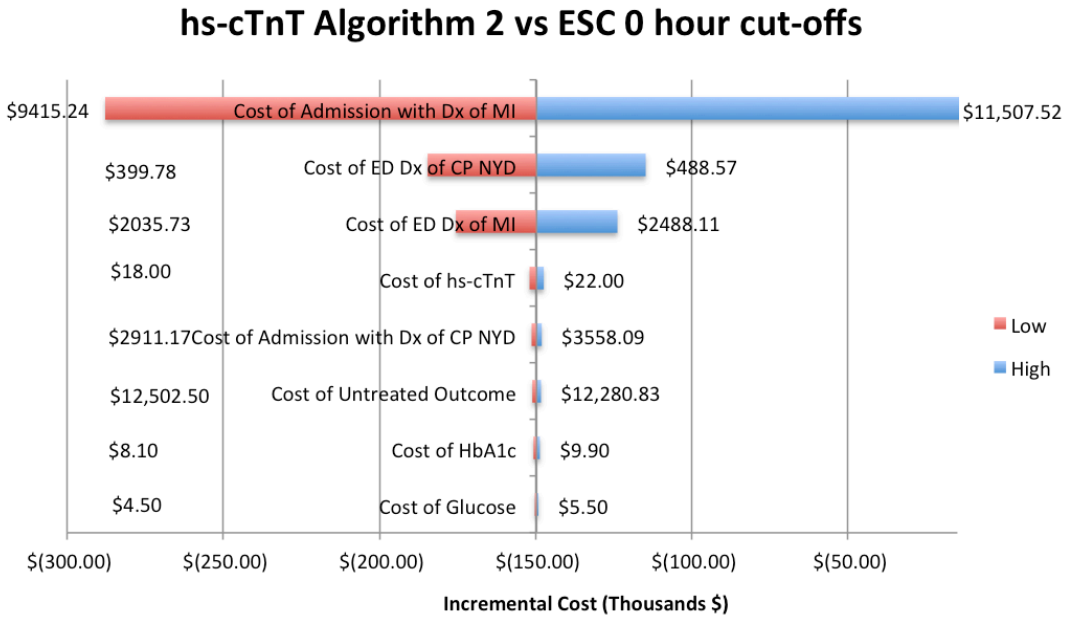


Figure 7. Tornado plot comparing the incremental cost of hs-cTnT algorithm 3 to the ESC 0 hour algorithm cutoffs when costs of algorithm 3 are varied +10% (high) and -10% (low). The Y axis indicates the base cost (ie. The cost of the ESC 0 hour algorithm). Cost labels indicate the cost per patient when varied and any costs in brackets indicate a negative number. Abbreviations: Dx=discharge diagnosis, CP NYD= final diagnosis of "Other CP" or "CP Unspecified"

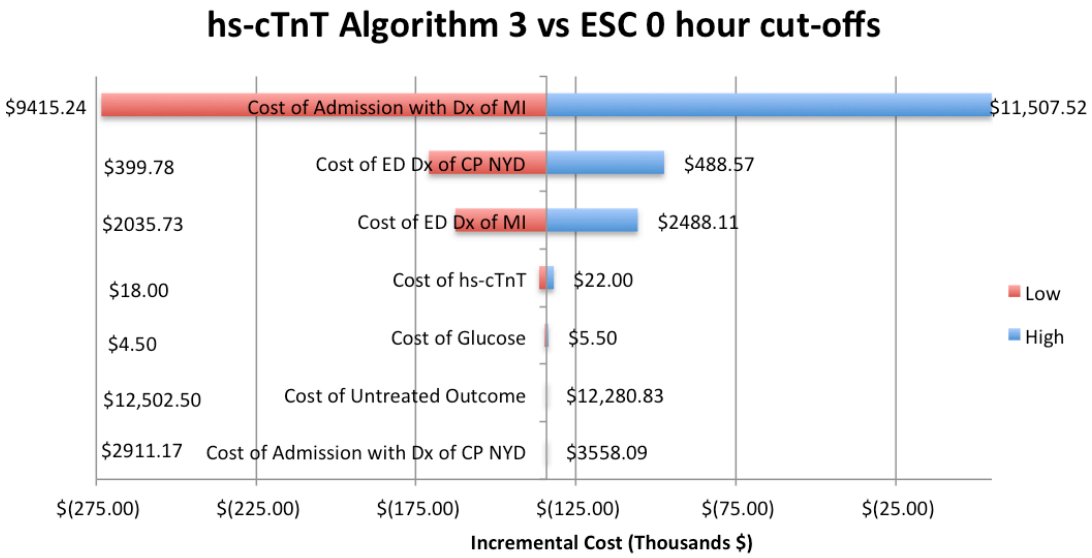
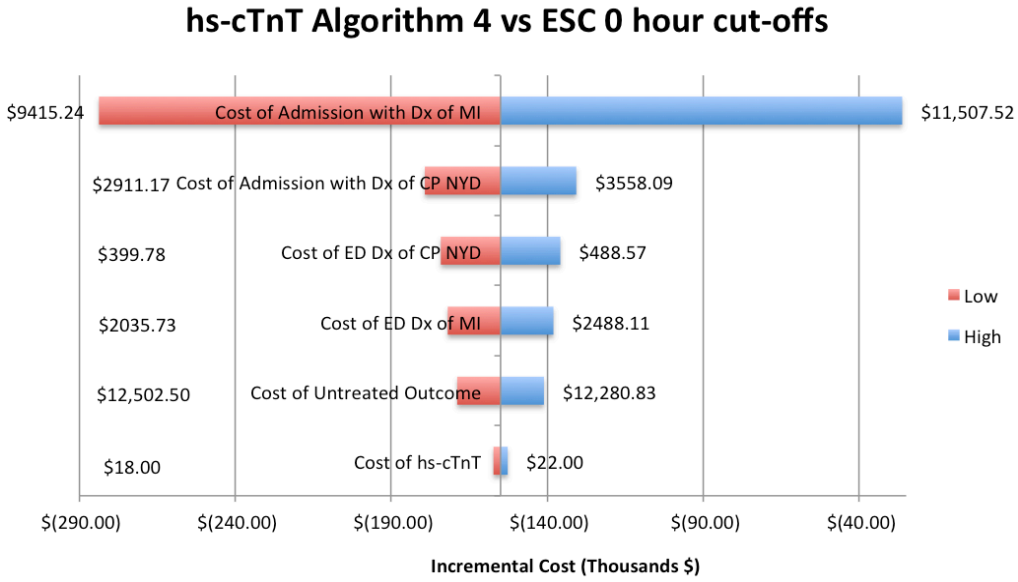


Figure 8. Tornado plot comparing the incremental cost of hs-cTnT algorithm 4 to the ESC 0 hour algorithm cutoffs when costs of algorithm 4 are varied +10% (high) and -10% (low). The Y axis indicates the base cost (ie., The cost of the ESC 0 hour algorithm). Cost labels indicate the cost per patient when varied and any costs in brackets indicate a negative number. Abbreviations: Dx=discharge diagnosis, CP NYD= final diagnosis of "Other CP" or "CP Unspecified"



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Permission to reprint chapter 5

Dear Erin Roberts,

I am completing a Ph.D. thesis at McMaster University entitled

"THE COMBINATION OF CARDIOVASCULAR AND GLYCEMIC BIOMARKERS FOR EARLY DECISION MAKING IN PATIENTS PRESENTING TO THE EMERGENCY DEPARTMENT WITH SYMPTOMS OF ACUTE CORONARY SYNDROME".

I would like your permission to reprint in full the following journal article in my thesis:

Shortt CM, Xie F, Whitlock R, Ma J, Clayton N, Sherbino J, et al. Economic considerations of early rule-in/rule-out algorithms for the diagnosis of myocardial infarction in the emergency department using cardiac troponin and glycemic biomarkers. [Epub ahead of print] Clin Chem as doi: 10.1373/clinchem.2016.261776

Please note that I am a co-author of this work.

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If these arrangements meet with your approval, please sign where indicated below and return this letter to me. Thank you very much.

Sincerely,

Colleen Shortt

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