Ischemia modified-albumin as a biomarker of myocardial ischemia: Early diagnosis of acute coronary syndrome and cost effectiveness analysis

By

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Abstract

Ischemia modified-albumin (IMA®) is a useful early cardiac biomarker for the diagnosis of acute coronary syndrome. In this study the diagnostic efficiency and the cost effectiveness of the oxidative stress biomarker (ischemia modified-albumin), myocardial necrosis (high sensitivity cardiac troponin, heart fatty-acid binding protein), vascular stress (copeptin) and myocardial dysfunction and hemodynamic stress (B-type natriuretic peptide) were evaluated for the diagnosis of acute myocardial infarction and cost benefit in low risk patients presenting to the emergency department with chest pain.

This study was a retrospective observational study of a prospective randomised controlled trial. A surplus of well characterised blood samples were analysed for the above biomarkers. A meta-analysis study of the diagnostic performance of Ischemia modified-albumin assay in patients presenting with chest pain suggestive of acute coronary syndrome was conducted. The-cost-benefit analysis was based on doctor on demand scenario.

Four hundred and forty-four samples were made available for this study, of which 174 patients had samples taken on admission and at 90 min. Three patients had a final diagnosis of acute myocardial infarction and one patient died. The difference in ischemia modified-albumin concentration was statistically significant ($p = 0.002$) between patients presenting on admission and at 90 min after admission. NT-pro-BNP had the highest diagnostic efficiency on admission with an area under the receiver operator curve (AUC) of 93% (95% CI, 82-93%). IMA did not reach the desired diagnostic efficiency with an AUC ranging from 54%-58%. The combined diagnostic efficiency of IMA plus high sensitivity cardiac troponin had a sensitivity and specificity of 71% (95% CI, 56-82%) and 100% (95% CI, 98-100%) respectively. The remaining biomarkers were not diagnostically efficient when combined with IMA. All biomarkers demonstrated poor prognostic value in predicating major adverse cardiac events within 30 days. Meta-analysis (n = 4295)

demonstrated a sensitivity of 77.73% (95% CI, 72.21-83.24%) and specificity of 72.71% (95% CI, 64.09-81.34%) respectively. The negative predictive value and positive predictive was 80.13% (95% CI, 73.18-87.08%) and 67.91% (95% CI, 58.47-77.39%) respectively. The implementation of high sensitivity troponin plus IMA on admission would cost £638.00 per high-risk patient, compared to £464.00 for troponin alone as per current protocol.

In conclusion, IMA plus high sensitivity cardiac troponin is cost effective and could be used to rule-out acute myocardial infarction. High sensitivity cardiac troponin is the most diagnostically efficient biomarker for early diagnosis of acute myocardial infarction. IMA assay alone is not suitable for the diagnosis of acute myocardial infarction.

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Abbreviations

ABSU	Absorbance Unit
ACC	The American College of Cardiology
ACE	Angiotensin-converting enzyme
ACS	Acute coronary syndrome
ADHERE	The Acute Decompensated Heart Failure National Registry
AHA	American Heart Association
AHF	Acute heart failure
AMI	Acute myocardial infarction
ARR	Absolute risk reduction
AUC	Area under the curve
AVP	Arginine vasopressin
BNP	Brain natriuretic peptide
BNP	B-type natriuretic peptide
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CCBs	Calcium channel blockers
CE	Conformité Européene
CEA	Cost-effectiveness-analysis
CEBR	Centre for Economics and Business Research
CER	Controlled events rate
CHD	Coronary heart disease
CI	Confidence interval
CK	Creatine kinase
CK-MB	Creatine kinase isoform MB
cTn	Cardiac troponin
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CVD	Cardiovascular disease
DHP	Distinct types: dihydropyridine

DTT	Dithiothreitol
ECG	Electrocardiogram
ECLIA	Electro-chemiluminescence immunoassay
ECMR	Extracellular matrix remodeling
ECP	Oesophageal chest pain
ED	Emergency Department
EDTA	Disodium ethylene diamine tetra-acetic acid
EER	Experimental event rate
ER	Endoplasmic reticulum
ESC	European Association of Cardiology
FDA	The Food and Drug Administration
FN	False negative
FP	False positive
GERD	Gastroesophageal reflux disease
GRACE	Global Registry of Acute Coronary Events
GTN	Glyceryl trinitrate
HF	Heart failure
H-FABP	Heart fatty-acid binding protein
HIF	Hypoxia-induced transcription factors
Hs-cTnI	High sensitivity cardiac troponin I
Hs-cTnT	High sensitivity cardiac troponin T
IMA	Ischemia modified-albumin
IQR	Interquartile range
LV	Left ventricular
MACE	Major adverse coronary events
MeSH	Medical subject headings
MINAP	Myocardial Ischaemia National Audit Project
NCCLS	National Committee for Clinical Laboratory Standards
NCD	Non-communicable disease
NHS	National Health Service
NICE	National Institute of Health and Care Excellence
NNT	Number needed to treat

NO	Nitric oxide
NSTEMI	Non-ST-segment elevation myocardial infarction
OR	Odds ratio
PCI	Percutaneous coronary intervention
PETN	Pentaerythritol tetranitrate
POCT	Point-of-care testing
PTCA	Percutaneous transluminal coronary angiography
QALY	Quality adjusted life year
RATPAC	Randomised assessment of treatment using panel assay of cardiac biomarkers
RCT	Randomised control trial
ROC	Receiver operator characteristics curve
SA	Stable angina
SCA	Sudden cardiac arrest
STEMI	ST-segment elevation myocardial infarction
TIMI	Thrombosis in myocardial infarction
WBC	White blood cells
WHO	The World Health Organisation

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Dedication

I wish to dedicate this thesis to my wife Louisa and my son Noah for their support and encouragement. I also want to dedicate this thesis to my late father Ben Aissa, rest in peace, he would be very proud.

Declaration

I declare that whilst registered as a candidate for the award of Doctor of Biomedical Science, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and those that contributed to it and has not been submitted for any other academic award.

The work contained within this submission is my own work, and to the best of my knowledge and belief, it contains no material previously published or written by another person, except where due acknowledgment has been made in the text.

Abdel Hakim Zouita

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Chapter 1

Introduction

1.0 Introduction

According to the World Health Organisation (WHO), non-communicable diseases (NCDs), such as cardiovascular disease (CVD) include: acute coronary syndrome (ACS), coronary artery disease (CAD) and stroke which are responsible for approximately 17 million deaths globally per year. The 2014 Centre for Economics and Business Research (CEBR) report estimated that the UK's total annual cost of CVD, including health care systems, informal care and loss of productivity, accounts for £15.2 billion annually. The CEBR also estimated that the total cost would rise to £18.7 billion in 2020 (European Research C.f.E.a.B., 2014).

Since 2012, in the UK alone, CVD was responsible for an estimated 74,000 annual deaths in people less than 75 years old (Bhatnagar, 2015). Surprisingly, since the 1970s, the actual trend of CVD associated deaths in England has decreased among the same population by as much as 44%. Interestingly, for the first time, CVD is no longer the biggest killer in UK. In 2012, CVD reportedly caused 28% of all deaths, whereas cancer was attributable for 29% (Bhatnagar, 2015). The trend of CVD prevalence is changing; new evidence suggests that younger people between the ages of 35 to 54 years old are also affected (Ford, 2007; Gupta, Wang, Spertus *et al.*, 2014).

The long-term consequences of coronary artery disease (CAD), including ACS, are characterised by inflammation and slow growing lesions in the coronary arteries, known as atherosclerotic plaque. The plaque matures and, following destabilisation and rupture, forms a thrombotic embolus within the lumen (Lusis, 2000). This thrombotic embolus usually results in clinical complications such as acute myocardial infarction (AMI) and stroke. CAD involves two different processes: a fixed and rarely reversible process that gradually causes the narrowing of the arterial lumen and a dynamic process which is characterised by progressive and slow formation of atherosclerotic plaque with an unpredictable outcome such as thrombosis, vasospasm or both

(Fuster, 1992). The narrowing of the vessels, especially around the lumen, causes ischaemia. Chest pain is directly linked to the narrowing of the coronary arterial lumen, and inflammation. The combined effect can cause the thrombus to dislodge, leading to partial or complete coronary artery blockage and myocardial cell damage (Finn, Nakano, Narula, Kolodgie, & Virmani, 2010).

1.1 Brief overview of the mechanism of acute coronary syndrome

1.1.1 Aetiology of an acute coronary syndrome

Myocardial function requires a precise homeostatic balance; any changes due to insufficient oxygen supply, deprivation of nutrients, ischaemia reperfusion and decreased clearance of waste products can cause acute cellular changes including apoptosis (Saraste, 1997). At a cellular level, these changes can manifest as a malfunction of the sodium-potassium pump, DNA fragmentation and leakage of cardiovascular specific proteins such as cardiac troponin and heart fatty-acid binding protein (H-FABP) (Kloner, 1993; Kerr, Wyllie, & Currie, 1972). The myocardium can survive a short period (<1 h) of disruption to cellular homoeostasis; however, prolonged disruption results in irreversible damage and ultimately localised cell death (Fishbein, Wang, & Matijasevic et al., 2003).

ACS start with a slow development of atherosclerotic plaque in the coronary arteries and is characterised by a sudden manifestation of an acute ischaemic cardiac disease which can include unstable angina pectoris (UA), stable angina (SA), non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI). Thus, ACS includes a broad spectrum of diseases that share the same pathophysiological basis (Aroney, 2003). Cardiovascular ischaemic disease due to atherosclerosis is the most common cause of death and disability worldwide. In 2010, WHO

estimated that cardiovascular disease represents around 30% of global deaths (Lozano, Naghavi, Foreman et al., 2012).

Atherosclerosis is a long-term, silent chronic vascular pathology responsible for most patients admitted to emergency department (ED) with ACS. It is a chronic disease characterised by lipid deposition, plaque formation, immune cell infiltration and chronic inflammation (Fuster et al., 1992a). Atherosclerotic plaque formation is the result of a dynamic interaction between various components including modified lipid, monocyte-derived macrophages and activated vascular smooth muscle cells. The interaction of all these components results in the formation of a lesion in the arterial wall (Fuster, Badimon, Badimon, & Chesebro, 1992b).

The progression of vascular disease due to atherosclerotic plaque formation involves a combination of dysfunctional endothelial cells within the coronary arteries, lipid deposition such as low density lipoprotein (LDL), very low density Lipoprotein (VLDL) and apoE remnant in the intima, an exacerbated innate and adaptive immune system, the recruitment of other immune cells including mast cells, regulatory T-cells, T-helper cells, remodelling of the extracellular matrix, angiogenesis, free radicals and the proliferation of vascular smooth muscle (Linton, Yancey, Davies et al., 2000).

In addition, advance atherosclerotic plaque is characterised by a lipid core surrounded by a fibrous cap containing smooth muscle cells (Halvorsen et al., 2008). Over time, the plaque becomes vulnerable and at risk of causing a cardiovascular event, especially when its necrotic core encounters the blood and intrudes into the lumen. The interaction between exposed atherosclerotic plaque constituents, platelet receptors and coagulation factors finally cause platelet activation, aggregation and the formation of thrombus. Thus, the clinical consequences of thrombus formation are usually patient's presentation with an ACS.

High-risk plaques are generally characterised by their large lipid-rich necrotic core and a thin fibrous cap (< 65 μm) which is usually infiltrated by

inflammatory cells such as macrophages and natural killer cells. Cycles of plaque rupture and healing are frequent and well recognised; continued remodelling of these plaques usually contributes to coronary narrowing and changes of plaque morphology sometimes with or without clinical symptoms (Kubo, Maehara, Mintz *et al.*, 2010).

Individuals with stable atherosclerotic plaques can lead a normal life without an apparent cardiovascular complication such as SA or UA. However, disease progression can lead to the occurrence of acute cardiovascular events such as acute myocardial infarction (AMI), UA, SA and sudden cardiac death (Ross, 1999).

The exact composition of the lesion is normally unknown in individual patients; however, atherosclerosis is generally found in patients with stable angina, whereas a thrombotic lesion usually represents a life-threatening situation and is associated with AMI (Falk, 2006). The severity of findings on angioscopy and on coronary angiography reflects the clinical severity of ACS (Sherman, Litvack, Grundfest et al., 1986). Generally, the white clots are found in patients with NSTEMI and UA; in contrast, the red clots are found in STEMI patients. This difference requires different therapeutic approaches in STEMI/UA antithrombotic therapy as appropriate in order to prevent thrombosis and to facilitate endogenous fibrinolysis to dissolve the thrombus and hopefully reduce the degree of stenosis (DeWood, Spores, Notske *et al.*, 1980). On the other hand, in STEMI, the arteries affected are almost always occluded, and an urgent pharmacological or catheter-based reperfusion is required; the goal here is to restore blood flow to the affected area. Other therapies, such as lipid-lowering and anti-ischaemic therapies such as β-blockers are used in all cases to stabilise plaques over long term.

1.1.2 Molecular and cellular basis of atherosclerosis plaque formation

Atherogenesis refers to the development of atheromatous plaques in the inner lining of the arteries. Histopathological studies of human and animal experiments have demonstrated that atherogenesis is a progressive change in the monolayer of endothelial cells that line the inner arterial surface. Under normal physiological conditions, endothelial cells resist attachment of the white blood cells (WBC) passing near them; however, when the WBC are subjected to irritative stimuli such as hypertension, dyslipidaemia or pro-inflammatory mediators, smoking, chronic infection and genetic predisposition they tend to adhere and infiltrate between arterial layers (tunica intima and the tunica media), causing inflammation and initiating the process of atherosclerotic plaque formation (figure 1).

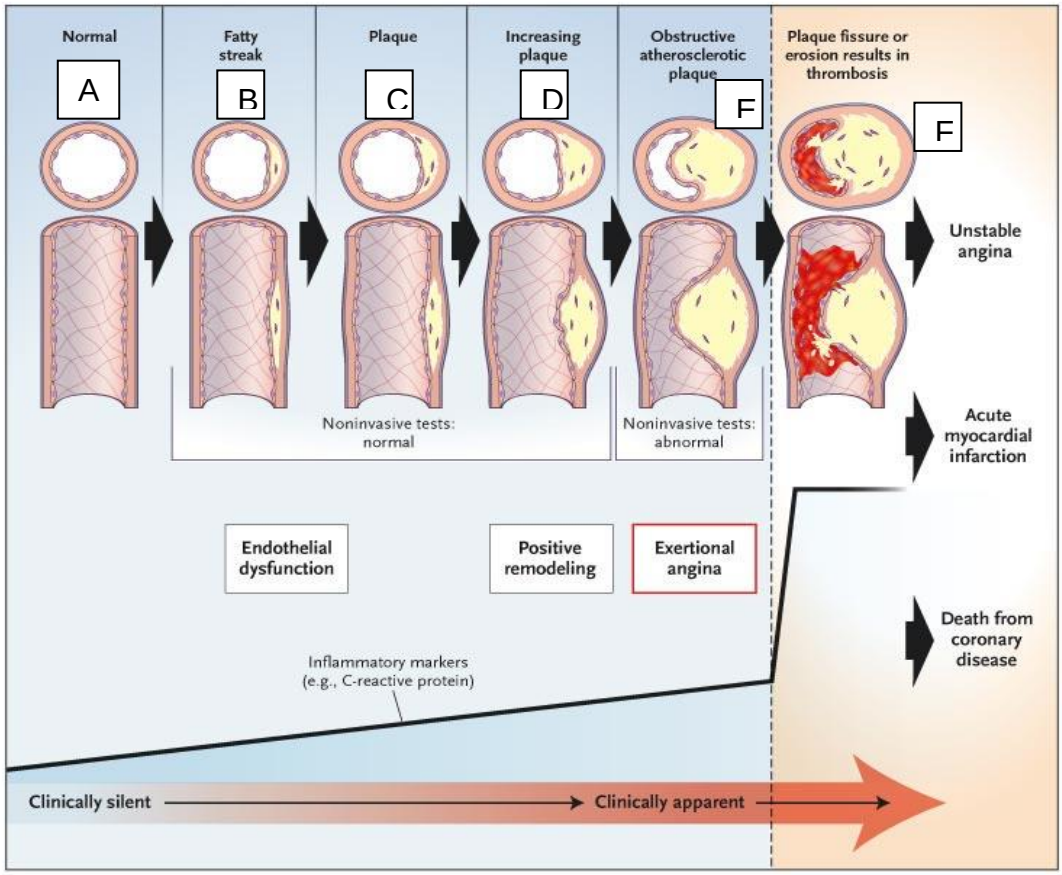

Figure 1: (A) Healthy coronary lumen, completely flat and undamaged, the main three vascular layers tunica intima and tunica media not compromised by fatty steak infiltration. (B) Infiltration of fatty streak between the tunica intima and the tunica media. (C) Recruitment of monocyte activation of smooth muscles and an exaggerated immune response characterised by cytokine release, pro-inflammatory biomarkers such as C-reactive protein and proteinases. (D) Fibrous cup rupture, explosion of the thrombogenic core and platelet aggregation. (E) Obstructive atherosclerosis plaque is present which may cause SA. (F) Life threatening atherosclerosis plaque rupture or erosion resulting in thrombus formation and the potential for causing UA or AMI and death. During the final stage, cardiac biomarkers such as Troponins and CK-MB may be present. (reproduced from healthline.com).

Although lipids have a central role in the pathogenesis of plaque formation, the mechanistic links between lipids and atherogenesis remain unclear (Linton, Yancey, Davies et al., 2000). Atherosclerosis is initiated at the site of endothelial injuries which can be caused by a variety of factors, including hypertension, high serum level of low-density lipoprotein (LDL), low serum level of low-density lipoprotein (HDL), apolipoprotein B 100 (apoB), impaired cholesterol transport, insulin resistance, free radical generation, hypoxia, a pre-existing condition such as diabetes mellitus and chemical toxins such as those from cigarette smoke (Zaman, Helft, Worthley *et al.*, 2000).

Once the structure of these cells is damaged, LDL can penetrate the coronary vessels' sub-endothelial space, i.e. the space between the tunica intima and the tunica media. Once the LDL is in the sub-endothelial space, it is modified by oxidation due to enzymes such as 5 lipo-oxygenase, phospholipase A2 and myeloperoxidase (Virmani, Kolodgie, Burke et al., 2005; Zaman, Helft,Worthley et al., 2000). The compromised and damaged endothelial cells express several adhesion molecules such as vascular cell adhesion molecule 1, intercellular adhesion molecule-1 and selectin. These molecules collectively promote the binding of circulating monocyte to the compromised and damaged vascular endothelial cell walls (Finn, 2010; Virmani,Kolodgie,Burke *et al.*, 2005).

Monocyte infiltration and localised production of stimulating factors cause the differentiation of monocyte to macrophages. Once these macrophages are exposed to LDL, they start to express scavenger receptors such as CD-36, that bind and promote the oxidation of LDL (Ridker, 2002; Rifai & Ridker, 2002). As the number of macrophages in the sub-endothelial space increases, more cholesterol, cytosolic lipid droplets form, and macrophages take on the appearance of a lipid-laden foam cell. At this stage, the T-lymphocyte and other inflammatory immune cells are recruited and start to infiltrate the developing lesion. T-lymphocyte infiltration is followed by the production of inflammatory cytokines such as interleukin-6 and interleukin-4, which in turn stimulate the production of the inflammatory biomarker C-reactive protein and

growth factors. The production of these proteins induces the smooth muscle cells to alter their cytoskeleton structure, and produce a matrix of metalloproteinase, and subsequently migrate from the media into the intimal space, where they proliferate and secrete extravascular matrix components that form a protective fibrous cap over the developing lesion (Ridker, 2002). Ongoing inflammatory process and the continuous recruitment of leukocyte infiltration will eventually lead to atherosclerotic lesion growth; at this stage, patient may experience SA symptoms. Over time, the atherosclerotic lesion continues to expand and becomes unstable. To make matters worse, activated macrophages exacerbate the stability of this lesion by the continued secretion of matrix metalloproteinase that degrade and weaken the matrix components of the lesion (fibrous cap). Moreover, activated macrophages continue to secrete various cytokines that inhibit smooth muscle proliferation and matrix production and subsequently promote smooth muscle cell apoptosis (Halvorsen et al. 2008). The continued build-up of macrophages eventually causes the plaque to rapture, usually from the base of the fibrous cap where it is weaker and contains few endothelial cells. When the plaque ruptures, platelets and coagulation factors in circulating blood are exposed to the thrombogenic component of the plaque. Thereafter, the dislodged plaque and the thrombogenic components act as a vehicle that allows platelet aggregation and coagulation. The formation of thrombin and the subsequent conversion of fibrinogen to fibrin, and release of Von Willebrand factors from the activated platelets create a cross-linked network that allows a thrombus to form. The thrombus size depends on the ruptured fibrous cap and the activity of the endogenous fibrinolytic pathway. The thrombus can both partially or completely occlude the coronary vessel lumen and initiate an acute coronary event such as an AMI.

Thus, the thrombus formation is the main cause of AMI. Histological and post-mortem studies have demonstrated that coronary luminal thrombus formation is the consequence of a ruptured or eroded atherosclerotic plaque. A study of 1847 fatal coronary thrombus concluded that plaque rupture is the major cause of coronary thrombosis irrespective of age (> 60 years old: 77%; < 60 years

old: 64%) or gender (women 55%; men 76%) (Sakakura,Nakano,Otsuka *et al.*, 2013). Conversely, thrombus over plaque erosion is frequently detected after coronary sudden death (Sakakura,Nakano,Otsuka *et al.*, 2013).

1.1.3 Brief overview of a vulnerable plaque

During the atherosclerotic plaque formation process, factors such as inflammation, lipid accumulation, free radical generation, apoptosis, proteolysis, thrombosis and angiogenesis are critical in determining the vulnerability of the plaque. Advanced atherosclerotic plaque is characterised by a lipid core surrounded by a fibrous cap containing smooth muscle cells (Halvorsen et al. 2008). Necrotic core expansion and the risk of plaque destabilisation and rupture is thought to involve the endoplasmic reticulum (ER) stress pathway (also known as unfold protein) as the main mechanism for smooth muscle cells and macrophage cell death in the core of the plaque (Gotoh, Endo, & Oike, 2011). The ER stress pathway is a cellular response mechanism to a dangerous build-up of misfolded proteins in the ER. Activation of this pathway down regulates protein translation. The halting of protein translation allows the endothelial cells a chance to recover. However, if the activation of the ER stress pathway is prolonged which is the case in CAD, the endothelial cells will ultimately initiate cell death usually through apoptosis (Guo,Ma,Liu *et al.*, 2017). The accumulation of cell debris and the constant recruitment of inflammatory cells will weaken the atherosclerotic plaque and eventually cause ACS.

1.1.3.1 The role of free radicals, hypoxia and vulnerable plaque

A cardiac ischaemic episode is characterised by a decreased blood flow, free radical such as superoxide and hydroxyl radicals freely circulating in the blood. Free radicals can be produced from a vast spectrum of process including the metabolism of thiols, flavins, catecholamine, oxidation of xanthine, and

cyclooxygenases-mediated oxidation of arachidonate. The nature of the free radicals high reactivity is thought to be responsible for the alteration of a variety of proteins including nucleic acids, lipids and the subsequent post-ischaemic dysfunction (Bolli 1988).

Hypoxia develops because of oxygen increase or decrease. Under normal physiological conditions, oxygen diffuses across the vessel wall (thickness approximately 100 to 250 μm); however, when this thickness of the vessel wall is increased either due atherosclerosis or thrombosis, the oxygen is unable to diffuse freely and hypoxia occurs (Torres Filho et al. 1994). On the other hand, hypoxia may also arise from an increase in oxygen supply due to high demand of inflammatory cells (Murdoch et al. 2005). Hypoxia is not exclusively present around macrophage foam cells, it seems that hypoxia depend more on inflammatory micro environment (Sluimer et al. 2008). Hypoxic cells such as CD68 positive macrophages and other proteins such as lipopolysaccharide and angiotensin (II) are known to activate hypoxia-induced transcription factors (HIF 1 & 2) which is an oxygen sensor that is degraded in normoxia but remain unchanged during hypoxia. Hypoxia itself strives to restore oxygen supply by stimulating HIF-1 production and angiogenesis (Blouin et al. 2004a; Page et al., 2002). Atherosclerosis-induced hypoxia in humans correlates with HIF-1, Vascular endothelial growth factors and intraplaque angiogenesis (Sluimer, Gasc, van Wanroij, Kisters, Groeneweg, Sollewijn Gelpke, Cleutjens, van den Akker, Corvol, Wouters, Daemen, & Bijnens, 2008).

Thus , hypoxia is the precursor for the activation of many genes that play a major role in the cellular and tissue adaptation during low oxygen supply, especially during acute or chronic decreases such as CAD (Semenza, 2001). These genes induce erythropoietin, glucose transporters and vascular endothelial growth factor. Hypoxic conditions promote HIF-1 proteins which regulate genes that are activated and induced by low oxygen conditions (1%) (Blouin et al., 2004b). The ability of HIF-1 expression is thought to be part of a protective mechanism, especially against strokes and myocardial ischaemia. The basis of this protective function is not fully appreciated (Semenza 2001).

Hypoxia participates in the mechanism of the regulation of neo-angiogenesis in physiological and pathological conditions including atherosclerosis.

Hypoxia arises as a net result of calcification and thickened vessel wall around the atherosclerotic plaque; this physiological change to the arteries results in decreased oxygen diffusion capacity, while increasing oxygen consumption is a result of activated immune cells (Hermus et al., 2010). Although the exact mechanism between hypoxia and atherosclerotic plaque formation is unknown, Sluimer et al. (2008) demonstrated a correlation between hypoxia in carotid artery plaques and the presence of macrophages, angiogenesis influence and thrombus formation (Sluimer, Gasc, van Wanroij, Kisters, Groeneweg, Sollewijn Gelpke, Cleutjens, van den Akker, Corvol, Wouters, Daemen, & Bijnens, 2008). Sluimer et al. (2008) also found that hypoxia is significantly present in advanced atheroma with thrombus and inflammation.

1.1.3.2 Nitric oxide and vulnerable plaque

Nitric oxide (NO) has potent anti-atherosclerotic properties, and deficiency in NO promotes endothelial dysfunction (Gotoh & Mori, 2006). NO is released from the endothelial cells and reacts with prostacyclin to prevent platelet aggregation, the expression of adhesion molecules (ICAM and VCAM), neutrophil attachment to the endothelial wall and the proliferation of endothelial cells (Garg and Hassid 1989;Munzel, Sinning, Post, Warnholtz, & Schulz, 2008). During endothelial dysfunction and chronic inflammation, NO is consumed by ROS such as superoxide and forms a highly reactive intermediate peroxynitrite ($ONOO^-$). This reaction with superoxide and $ONOO^-$ severely depletes NO bioavailability and subsequently its protection (Beckman & Koppenol, 1996). Moreover, $ONOO^-$ is believed to act as a vasoconstrictor and a cytotoxic molecule capable of damaging proteins such as lipids and DNA (Beckman & Koppenol, 1996). The mechanism and the pathology of

endothelial dysfunction is multifactorial and remains unclear (Munzel, Sinning, Post, Warnholtz, & Schulz, 2008).

1.2 Overview of the pathological process that leads to the release of cardiac and non-cardiac specific biomarkers

Acute myocardial infarction is pathologically defined as a cardiomyocyte death due to prolonged ischaemia resulting from oxygen imbalance between oxygen supply and demand. Severe ischaemia, combined with significant reduction in oxygen supply to the myocardium, provokes a cascade of detrimental events including anaerobic glycolysis, inhibition of ATP-dependent cell transport process, electrolyte shift, cellular oedema, free radical generation, protein kinase activation such as ERK ½ sodium hydrogen and exchanger kinase and finally loss of myocardium membrane integrity (Nigam, 2007) . Once the membrane integrity is compromised, a steady release and the appearance of myocardium related biomarkers such as CK-MB, myoglobin, h-FABP, NT-pro-BNP and troponin occurs in the blood stream (Nigam, 2007). The release of cardiac specific proteins such as troponin, myoglobin and CK-MB is inevitable and expected; however, prolonged ischaemia or transient stress-induced ischaemia can also indirectly affect the release and the generation of other non-cardiac specific proteins such as copeptin and ischaemia modified-albumin. For a part of this thesis we hypothesised that cardiac ischaemia may result in quantifiable circulating levels of non-cardiac specific proteins such as IMA and copeptin in patients presenting to ED with chest pain suggestive of AMI. We also hypothesised that hs-cTn and other cardiac specific and non-specific biomarkers may collectively or individually improve the diagnosis of patients presenting with chest pain suggestive of ACS. Moreover, these biomarkers promise to offer a window into a complex disease such as ACS.

Blood biomarkers are often a by-product of the diseased state and may also directly participate in its pathogenesis. Among cardiovascular biomarkers, cTn, CK-MB and myoglobin are still considered as important diagnostic tools for detecting and predicting an onset of AMI in patients presenting to ED with

chest pain. A wealth of evidence and clinical trials exists to support the clinical and the diagnostic efficiencies of these cardiac specific biomarkers (Apple, 2011; Apple, Jesse, Newby *et al.*, 2007; Apple,Wu,Jaffe *et al.*, 2008; Collinson, 1998).

On the other hand, evaluation of novel and non-cardiac specific and cardiac specific biomarkers such as IMA, h-FABP and copeptin requires specialised assays that are not available routinely within the national health service (NHS) biochemistry laboratories. Novel cardiovascular biomarkers should be clinically useful, cost-effective and evaluated to the point of influencing patients' management and treatment. Hs-cTn, CK-MB and myoglobin have a large evidence for the diagnosis of AMI; BNP is also equally important in the diagnosis and the monitoring of heart failure (Di Marca,Rando,Cataudella *et al.*, 2018; Salama,El-Moniem,El-Hefney *et al.*, 2011a). In contrast, other novel biomarkers such as IMA, copeptin and h-FABP are still in research state, and further evaluation is required and pending. In conclusion, the purpose of this project is to contribute to the current knowledge associated with these novel biomarkers in the diagnosis of ACS.

1.2.1 An overview of the pathological processes that lead to the release of cardiac specific and non-specific biomarkers

1.2.1.2 Copeptin

The arginine vasopressin (AVP) released from the neurohypophysis into the circulation is primarily to induce water conservation by the kidney (figure 2). Although the main secretion of AVP is in response to hyperosmolarity, it can also be secreted in response to endogenous stress including hypoxia, acidosis and infection (Lukaszyk & Malyszko, 2015). Copeptin is an arterial stress biomarker associated with disease severity in other conditions such as sepsis, stroke, lower respiratory tract infection and AMI (Reinstadler, Klug, Feistritzer, Metzler, & Mair, 2015). Measurement of copeptin has been shown to have a very strong negative predictive value for AMI, especially when troponin is also negative (Reichlin,Hochholzer,Stelzig *et al.*, 2009).

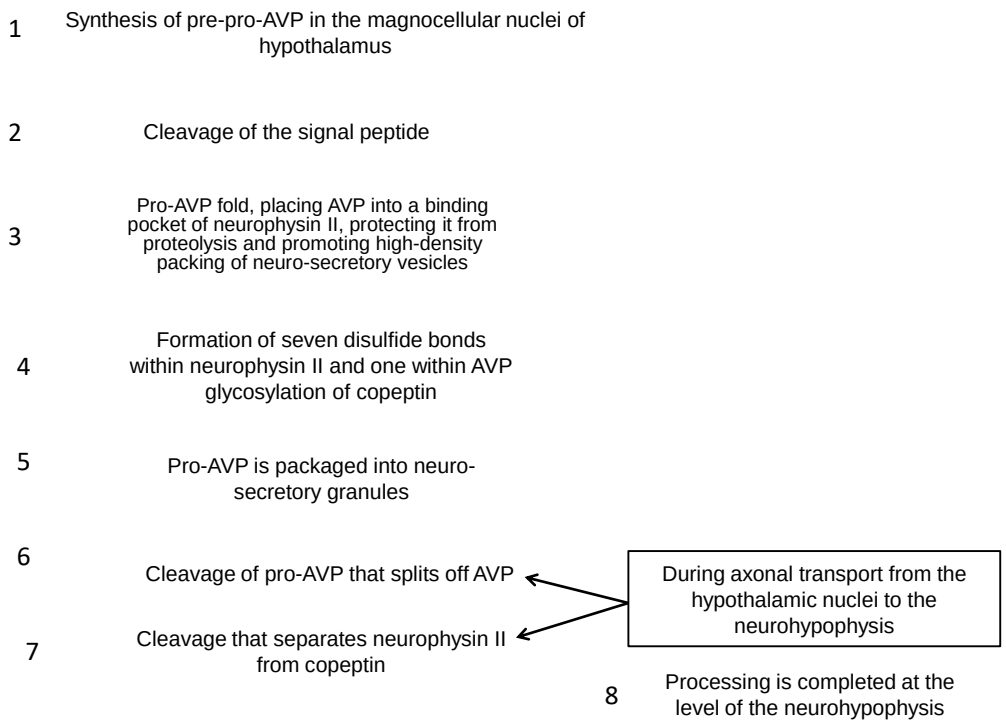

Figure 2: Schematic presentation of copeptin generation and maturation.

The diagnostic efficiency of copeptin as a standalone test is not promising for example measurement of copeptin concentration will be of little value for patient presenting late (> 3 h), as cTn will be already detectable (Bahrmann et al., 2013). However, copeptin may be used to assess re-infraction when cTn is already elevated (Keller,Tzikas,Zeller *et al.*, 2010). In conclusion, various studies suggest that copeptin is a biomarker of non-specific stress response and cannot safely be used in an ED setting for the diagnosis of AMI (Nemec,Koller,Nickel *et al.*, 2010; Nickel,Bingisser & Morgenthaler, 2012). In this thesis, I will explore the combined diagnostic efficiency of copeptin and IMA in the diagnosis of AMI.

1.2.1.3 Heart fatty acid binding protein

H-FABP participates in fatty acid metabolism by transporting fatty acid from the cell membrane to mitochondria for oxidation. H-FABP also protect against free radical accumulation during myocardial ischaemia (Jones, Prasad, & Das, 1990) and is the main source of energy in the heart accounting for 10% of the total body turnover of fatty acid (Fournier & Richard, 1990). The h-FABP has the advantage of an early release to the circulation as soon as the myocardial membrane is compromised. Thus h-FABP may be used as an early biomarker of cardiac ischaemia (Chan,Sanderson,Glatz *et al.*, 2004). Soluble cytosolic protein such as h-FABP is readily released to the circulation before structural proteins such as troponin, CK-MB and myoglobin (Figure 3). The main disadvantage of h-FABP is that it is not specific to the heart tissue and it is also present in skeletal muscle. A meta-analysis suggests that h-FABP testing as a standalone test did not fulfil the diagnostic criteria in term of sensitivity, specificity and the predictive values for safe rule-out protocol for patients suspected of AMI (Bruins Slot,Reitsma,Rutten *et al.*, 2010). The combination of h-FABP and troponin has been shown to increase the sensitivity and the NPV for diagnosing AMI (Lippi, Mattiuzzi, & Cervellin, 2013). However, the diagnostic efficiency of h-FABP and IMA is still to be established in patients with chest pain suggestive of AMI but not proven. Thus, the current study will examine the diagnostic relationship amongst h-FABP, IMA and other biomarkers.

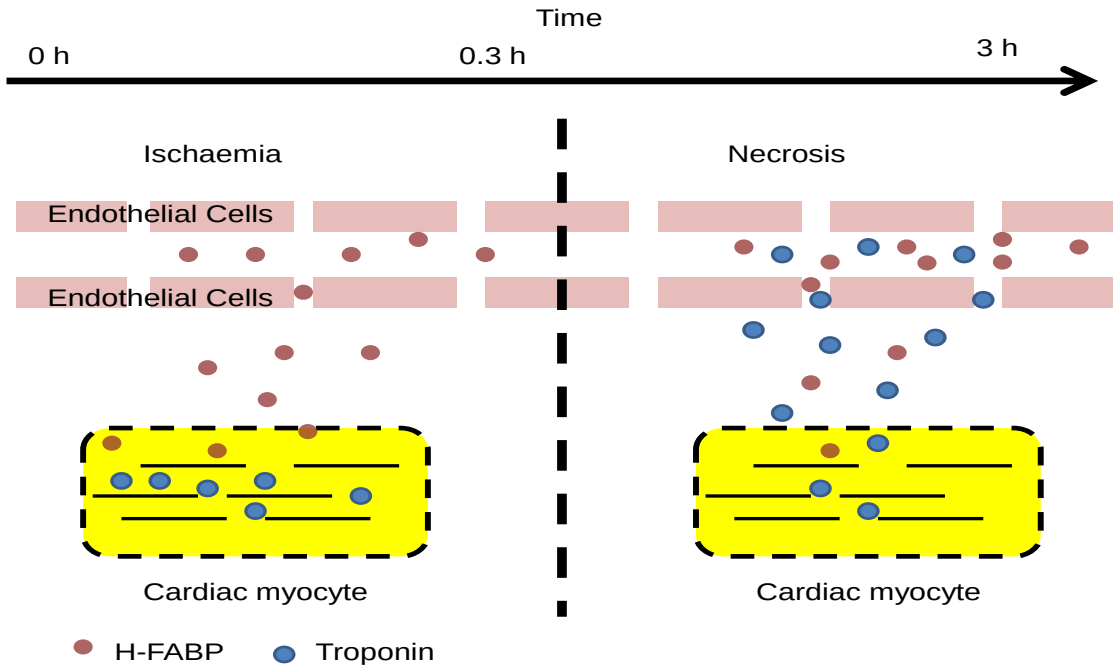

Figure 3 kinetic release of h-FABP during prolonged ischaemic cardiac events. H-FABP is a low molecular weight (15 KDa) cytoplasmic protein which occupies 5-15% of cytosolic protein of the cardiomyocyte; as such, it is the first molecule to appear in the blood stream following a cardiac myocyte membrane dysfunction. In contrast, cardiac troponin appears in the circulation only after cardiac myocyte destruction.

1.2.3.4 N-Terminal fragment of pro-BNP

BNP is produced predominantly in the left ventricular myocardium in response to myocyte distension caused by ventricular volume expansion or pressure overload. The exact mechanism of its release is unclear; however, it appears that BNP is regulated by post-translational processing; as such, its presence in the circulation increases very rapidly in response to stimulus.

Natriuretic hormones belong to the family of vasoactive peptides that regulate arterial and venous dilation. In the circulation, the pro-hormone is cleaved into BNP, the physiologically active form, and into the N-terminal fragment of the NT-pro-BNP. Under normal conditions, animal models have demonstrated that

BNP is produced primarily in the atria and is stored in secretory vesicles (Chopra,Cherian,Verghese *et al.*, 2013). BNP expression is increased in response to stretch in both atrial and ventricular tissues in heart failure. Natriuretic peptides physiologically play an important role in the regulation of blood pressure; they induce natriuresis and diuresis, act as a vasodilator and inhibit the renin-angiotensin-system. BNP also appears to be counter-regulatory molecule for the renin-angiotensin system. Thus, BNP increases glomerular filtration rate, increase sodium secretion by the kidneys, increase urine production while decreasing blood volume/blood pressure and have a diuretic and vasodilatory effect (Chopra,Cherian,Verghese *et al.*, 2013).

Natriuretic peptide exerts its effect by its ability to bind to three different types of membrane bound natriuretic peptide receptors (NPR) including NPR-A, NPR-B and NPR-C. It is important to mention that BNP mediates its biological actions by binding to NPR-A, as this type of receptor is the most abundant in the vascular endothelium system and is also present in the kidney, the adrenal gland and brain (Levin,Gardner & Samson, 1998). The mechanism of the degradation and the elimination of BNP are not fully understood (Chopra,Cherian,Verghese *et al.*, 2013).

Presently, BNP is used as a powerful sensitive, specific and prognostic biomarker for the onset of acute heart failure and as a screening tool for detecting left ventricular systolic and diagnostic tool for detecting left ventricular systolic and diastolic function.

BNP measurement is also used as a biological biomarker mainly in adult and elderly patients with heart failure. Clinically, the use of BNP and NT-pro-BNP is particularly useful in the diagnosis of heart failure in patients with acute dyspepsia and in ruling out heart failure. The diagnostic and the prognostic value of NT-pro-BNP are influenced by age, gender, obesity and renal function. There are only a few studies about BNP release in AMI; however; a study conducted by *Richards et al.*, who, while studying patients with AMI, found significantly higher BNP plasma levels as compared with healthy

controls (Durak-Nalbantic,Dzubur,Dilic *et al.*, 2012). The current study will examine the diagnostic efficiency of NT-pro-BNP, IMA and other biomarkers.

1.3 Human albumin metabolism

Human albumin (HA) (7×10^{-4} M), which occupies 50% of plasma proteins in a healthy individual, is the main protein that regulates the oncotic pressure and plays an important role in modulating the distribution of fluids between body compartments. Although the plasma concentration of HA is 7×10^{-4} M, it is still dependent on its biosynthesis, degradation rate and distribution between intravascular and extravascular compartments. HA is predominantly an interstitial protein (concentration 3×10^{-4}), and partially tissue-bound and therefore unavailable for circulation. The intravascular mass of HA is about 120 g and circulates from the blood into the capillary wall into the interstitial compartments including cerebrospinal fluid and returns to the blood through the lymphatic system. The trans capillary escape (movement of HA across capillary wall leaving intravascular space) of HA is 5% per hour (Margarson and Soni 1998;Mendez et al. 2005).

During its long life (18–21 days), an HA molecule makes approximately about 15,000 passes through the circulation transporting various kind of damaged proteins and accumulated ligands (Margarson & Soni 1998;Mendez, McClain, & Marsano 2005;Peters T 1996).

The liver is the main site for HA biosynthesis; however, traces of mRNA was detected in the kidney and pancreas with no evidence to suggest that this mRNA in this extra-hepatic tissue was translated. Moreover, the presence and the origin of HA in milk is still debatable (Peters, 1996). Interestingly, a small amount HA can also be produced in bone tissue and the microglial cells in brain (Ahn et al. 2008;Yamaguchi et al. 2010).

Several organs participate in the catabolism of HA at a rate of 14 g per day in a 70 kg healthy adult (Nicholson et al. 2000). HA in plasma often decreases during stress, trauma or sepsis; this drop can be the result of redistribution

from intravascular compartment into the extravascular compartments, decreased synthesis, and increased catabolism. Decreased HA during injury and infection can be as much as 2 x 10^{-4} M within 5 days, whereas, in healthy HA, it can also be excreted through the GI tract at the rate of 1 g per day and through the kidney at only a few milligrams (Peters, 1996).

Human albumin consists of non-glycosylated single chain polypeptide chain containing 585 amino acids. HA represents the most abundant protein in the human body (35 to 50 g/l) of all proteins in the plasma of healthy individuals and exerts a range of physiological and pharmacological functions including as antioxidant and transports small molecules such as drugs, fatty acids and bilirubin (Evans 2002;Rondeau and Bourdon 2011a). *In vivo* free circulating nitric oxide (NO) is readily taken by HA through its free thiol of Cys34 and releases NO to tissue exposed to low pH and hypoxia. However, during hypoxia, HA undergoes structural transition and releases NO in order to maintain the vascular tone (Minamiyama et al. 1996). Although the delivery mechanism of HA through a receptor is not clear, a receptor-mediated endocytosis has been postulated (Fasano et al. 2007;van der Vusse 2009).

The ability of HA to carry out these functions is believed to be due to its structure. HA is organised into three domains I, II and III from which each is further subdivided into subdomains A and B (Rondeau & Bourdon 2011a). The subdomains are linked with 17 intra-molecular disulphide bonds, which contribute to their rigidity without compromising on flexibility in shape and size of HA in response to pH changes or other biophysiological changes (Scatchard et al. 1944). HA is sensitive to glycation because of its long life (19 to 21 days) and high concentration (Charonis et al. 1990).

Under pH changes, HS could acquire a reversible conformational isomerisation. In physiological condition HA exert is buffering capability through its 16 histidine imidazole residues (Caironi and Gattinoni 2009;Colombo et al. 2012a;Pavone et al. 2010). Moreover, HS's tertiary dimensional structure enable albumin to transport small molecules; i.e., sites

in domain I and II are the major binding sites for drugs, whereas amino-terminus binding sites have a high affinity for metal ions including Co^{2+}, Cu^{2+}, Ni^{2+} (Rondeau and Bourdon 2011b).

1.3.1 Conclusion

Blood biomarkers are biological analytes that can be detected in the blood under defined and specific physiological conditions. As such, biomarkers are a rapid cost-effective way of peering through a patient's physiological window and differentiating disease states without invasive techniques. As mentioned above, cardiac biomarkers of necrosis such as myoglobin, troponin and CK-MB are well established; however, a reliable biomarker of cardiac ischaemia such as IMA remains to be demonstrated and is the focus of the current research project. In addition, cardiac non-specific biomarkers for the diagnosis of AMI such as copeptin, h-FABP and NT-pro-BNP are still in research state and require further investigation.

1.3.2 Ischaemia modified-albumin

Human albumin presents a substantial genetic polymorphism similar to that of haemoglobin; this genetic polymorphism which is usually heterozygous can leads to a rare asymptomatic inherited disorder known as allo-albuminemia (Amoresano et al., 1998). More than fifty structurally different variants of HA have been characterised by protein and/or DNA sequencing. Some of these variants also exhibit amino acid changes in the pro-peptide, the N-terminus and in subdomains IIB and IIIB (Kragh-Hansen, Brennan, Minchiotti, & Galliano, 1994).

More than 50 structurally different variants of HA have been characterised by protein and or DNA sequencing. These variants are categorised into three major type including single amino acid substitutions, glycosylated proteins and chain termination mutants (Madison et al. 1994). In addition, these variants

exhibit amino acid changes in the pro-peptide, the N-terminus and in subdomains IIB and IIIB (Kragh-Hansen, Brennan, Minchiotti, & Galliano 1994b).

Commercial HA is kwon to exhibit damage and degradation to its N-terminus (Chan et al. 1995a). N-terminal degradation *in vitro* is thought to be due pH and temperature; it is possible that this mechanism may also exist *in vivo* (Chan et al. 1995b). HA and bovine albumin protein contain an N-terminus sequence X-Y-His which play a considerable role as a binding site for endogenous Cu (II) and toxic Ni (II) (Bal et al. 1998). In contrast, other albumin from other species such as canine and porcine do not have the N-terminus of that of HA, instead they have His-3 substituted by Tyr which intern do not bind metal ions (Bal, Christodoulou, Sadler, & Tucker 1998).

HA Is the major carrier for Co^{2+}, Ni^{2+} and Zn^{2+} cations(Peters, Jr. 1985), the presence of variant significantly decrease the binding affinity HS to Ni^{2+} (pro-peptide variant), Co^{2+}(Blenheim and Varese variant) and Zn^{2+} (Christchurch and Redhill variant; Kragh-Hansen et al. 1994b). Although, the presence of these variants decreases the binding site for Ca^{2+}, Ni^{2+} and Zn^{2+} cations, it seems that Ca^{2+} concentration in the plasma is not effected, because Ca^{2+} concentration (2.1-2.6 mM) far exceed that of HA (Kragh-Hansen and Vorum 1993). On the other hand, Ni^{2+} and Zn^{2+} cations concentration is lower 0.044 µM and 20 µM respectively than that of HA. This difference between Ni^{2+}, Zn^{2+} cations and HA concentrations would suggest that bisalbuminaemia could have a greater biological and clinical significance (Kragh-Hansen, Brennan, Minchiotti, & Galliano 1994b).

Reactive species capable of causing damage *in vivo* including hydrogen peroxide, organic hydroperoxide and hypohalous acids with half-life of minutes, pyroxyl radical and nitric oxide half-life second, peroxynitrite half-life millisecond, superoxide anion, singlet oxygen, and the alkoxyl radicals half-life in millisecond (Kehrer 2000). The mechanism of action of these species is still unclear, because these species behave as initiators and product of cellular damage, making the determination of cause and effect challenging (Kehrer

2000). In addition, study of these species requires a high concentration; which *in vivo* is not achievable (Kehrer 2000).

Chronic disease and conditions such as cardiac ischaemia, chronic inflammation, cancer and aging, are known to trigger copper release (Cu II) and redox activity which can cause cellular damage due to the generation of reactive oxygen species (ROS) including hydroxyl radical ($OH^{\bullet}$) and superoxide ($O_2^{\bullet -}$) (Halliwell & Gutteridge, 1990a). *In vivo* hydroxyls radical is converted to hydrogen peroxide (H_2O_2) and superoxide. Superoxide is directly converted to either hydroxyl radical via the Haber-Weiss reaction or converted to H_2O_2 via Fenton reaction (see below) (Biaglow, Manevich, Uckun, & Held, 1997). Both reactions require transition metal such as copper and iron (Halliwell & Gutteridge, 1990b). While, iron concentration in human serum is greater than copper, the latter is 60 times faster in generating hydroxyl radical than iron (Chevion et al., 1993). Moreover, Cu II ions, when mixed with ascorbic acid or other reducing agents produce hydroxyl radicals (Biaglow et al., 1997).

Transitional metal can participate in injury is demonstrated via metal-mediated and site-specific Haber—Weiss reaction (Chevion 1988;Samuni et al. 1983).

- Haber—Weiss reaction (also known as iron catalysed reaction)

$$O_2^{\bullet} + H_2O \longrightarrow HO^{\bullet} + O_2 + HO^{-}$$

The chemical generation of the OH-radical via the classical Fenton reaction (Biaglow, Manevich, Uckun, & Held 1997).

$$Fe^{3+} + O_2^{\bullet -} \longrightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 + H^{+} \longrightarrow Fe^{3+} + H_2O + OH^{\bullet}$$

- Ascorbic acid or other reducing agents produce hydroxyl radicals

$2\ Cu^{+}$ Ascorbate $\longrightarrow$ $2\ Cu^{+}$ + dehydroascorbate $+2\ H^{+}$

$Cu^{+} + O_2 \longrightarrow Cu^{2+} + O_2^{\bullet -}$

$Cu^{+} + O_2^{\bullet -} + 2\ H^{+} \longrightarrow Cu^{2+} H_2O_2$

$Cu^{+} + H_2O_2 \longrightarrow OH^{-} + OH^{\bullet} + Cu^{2+}$

Copper is tightly regulated in both non-specific exchangeable and specific non-reversible by a range of carried plasma protein including caeruloplasmin witch bind approximately 65% of copper, and albumin (Bar-Or et al., 2001a). Once the copper is release to the circulation during ischaemia, it disrupts the cellular energy causing the conversion of pyruvate to lactate and subsequent local acidosis. Localized acidic conditions are known to cause caeruloplasmin to release more copper (Cu II) (Lamb & Leake, 1994). HA and specially its N-terminus tetrapeptide (Asp-Ala-His-Lys) sequence is a non-exchangeable binding site for Cu (II) and is capable of sequestering Cu (II) ions and inhibiting the formation of ROS. Moreover, by sequestering Cu (II), HA prevents the conversion of Cu (II) to Cu (I) in the presence of reducing agents such as ascorbate (Marx & Chevion, 1986). Free Cu (I) can react with oxygen to form Cu (II) and generate superoxide free radicals ($O_2^{\bullet -}$).

Although, the N-terminus of HA is the main site for irreversible binding of Cu (II), a “loose” non-specific binding site also exist and participate in the formation of ROS in the presence of reducing agents (Marx & Chevion, 1986). Glycated albumin binds 3 fold the amount of copper and ion compared to non-glycated (Eaton & Qian, 2002). The presence of non-specific binding site for Cu (II) reduces the benefit of using albumin as therapeutic agents for treating ROS related disease i.e. cardiac ischaemic diseases and copper toxicity (Quinlan, Coudray, Hubbard, & Gutteridge, 1992; Kreymann, Seige, Schweigart, Kopp, & Classen, 1999).

In vivo stability of alloalbumin is between 5% to 70% of the total serum albumin in heterozygote and may cause protein unbalance in circulation, thus abnormal alloalbumin may have a clinical and biological impact (Galliano et al. 1999). Two type of proalbumin shows high affinity to metal ions including Co^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+} (Kragh-Hansen et al. 1994a). Proalbumin of Sterling type shows abnormal fatty acid binding property (Kragh-Hansen et al. 1996) (Nielsen et al. 1997). It is therefore possible that IMA in some patients may be a genetic variant of albumin. Until IMA is well characterised and purified the nature of IMA is presently not clear.

1.4 The rational for using these novel biomarkers

There is currently a deficiency of biomarkers that can reliably describe indirectly the abnormal extracellular matrix remodelling (ECMR) which ultimately leads to cardiovascular related pathology. The lack of early biomarkers of atherosclerotic progression and lack of early biomarkers of atheromatic formation lead to the suggestion that some novel biomarkers such as biomarker of early myocardium necrosis (hear fatty-acid binding protein); oxidative stress (ischaemia modified-albumin) and vascular stress (copeptin) may help in the detection of ACS. Moreover, accurate monitoring of early cardiac ECMR could prompt early intervention and prevention of disease progression.

While current biomarkers of necrosis have greatly improved the diagnosis and treatments of AMI patients; there is still scope for improvement especially in the area of early detection of cardiac ischaemia due to atherosclorosis or unstable thrombus (Figure 4).

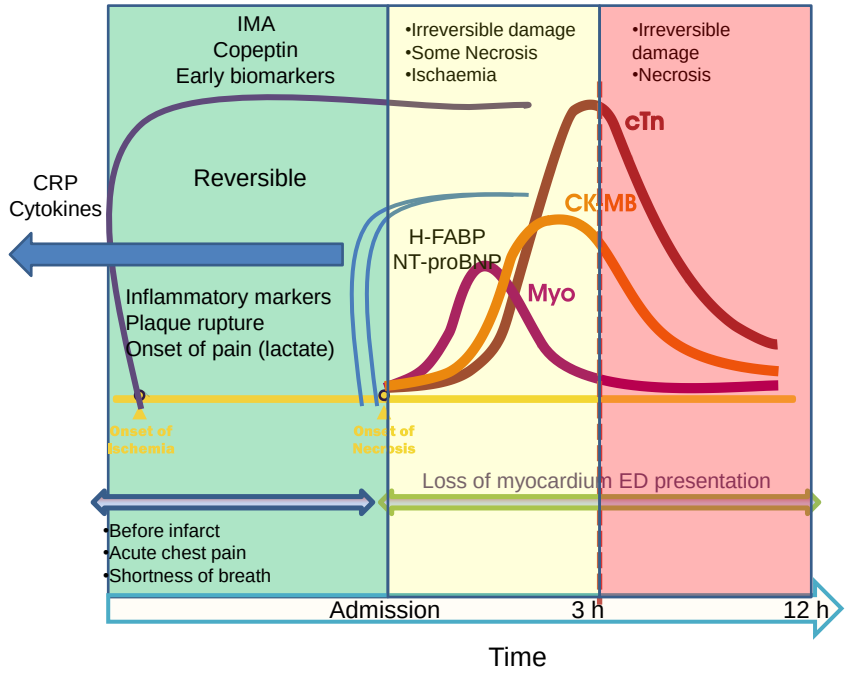

Figure 4: The green zone: Biomarkers of ischaemia such as IMA and copeptin among others are first released prior to myocardium damage. Yellow zone biomarkers of necrosis such as h-FABP, myoglobin and troponin early release myocardium can still be salvaged. Red zone irreversible myocardium damage at this stage AMI is unavoidable and biomarkers of necrosis predominantly troponins are detectable.

1.4.1 Clinical dilemma

Rapid identification of AMI is important in order to initiate treatment for better prognosis. Discriminating between genuine ACS and non-ACS patients is very challenging; especially in individuals without clear symptoms or history, ECG features and cardiac biomarkers. Routine diagnostic methods for risk stratification of patients presenting to ED with chest pain and ACS exist but none is adequately equipped to help clinicians to determine which patients can safely be discharged (Antman, 2000; Christenson, 2004). In order to speed up the diagnosis and to safely discharge the correct low risk patients it has been suggested that other early sensitive non-cardiac specific and cardiac specific biomarkers such as biomarkers of myocardium necrosis (hear fatty-acid binding protein), oxidative stress (ischaemia modified-albumin) and vascular stress (copeptin), might be used as stand alone or in combination with well-established cardiac biomarkers such as myoglobin, creatinine kinase isoenzyme MB (CK-MB) and hs-cTnI or hs-cTnT to rule-out ACS. The hypothesis is if some of these cardiac specific and non-specific biomarkers either alone or in combination can help discharge low risk patients safely. Early and safe discharge can spare patients from prolonged ED observation, expensive rule-out protocols for the NHS, crowding and inconvenience (Goodacre, 2003).

The rational for using IMA, H-FABP, copeptin and NT-pro-BNP is based on the promises that an early evaluation may predict the final diagnosis of AMI as defined by the elevation of hs-cTn at 0-3 h after the onset of chest pain.

Among these biomarkers mentioned above only the albumin cobalt binding (ACB®) assay, which measures ischaemia modified-albumin (IMA®), is cleared by CE marking in Europe and the Food and Drug Agency (FDA) in the USA to be used for the purpose of detecting myocardial ischaemia (Jaffe, 2006; Chan & Pronovost, 2004; Jaffe, Babuin, & Apple, 2006). The measurement of IMA concentration by the ACB assay was cleared by the American Food and Drug authority on the basis that it would help rule-out ACS, in low to moderate pre-

test probability conditions with negative necrotic cardiovascular biomarkers and normal ECG. ACB has a high negative predictive value (NPV) of 96% [95% CI 91-98%], and high sensitivity in predicting troponin negative or positive at 6 and 24 hours (Christenson et al. 2001b). Therefore, it's logical to study IMA and its contribution in the early diagnosis of AMI.

In addition IMA has also being suggested as a biomarker for monitoring transient myocardial ischaemia induced by vasospasm (Cho,Choi,Kim *et al.*, 2007). IMA is not a cardiac specific biomarker and can be found in various conditions including, renal disease, infection, inflammation, and liver disease (Abboud,Labreuche,Meseguer *et al.*, 2007; Apple & Jaffe, 2001; Bodi,Sanchis,Llacer *et al.*, 2005). Meta-analysis has shown that serum IMA could be used to monitor oxidative stress status in the development of diabetic retinopathy and thyroid pathology (Reddy,Bukke,Mahato *et al.*, 2017).

The importance of cardiac biomarker testing has been widely accepted as part of the initial assessment of patients presenting with chest pain suggestive of ACS. ACS pathophysiology is complex, thus presently it is unlikely that a single biomarker could be used to evaluate cardiovascular associated risks and for ACS diagnosis. Currently numerous evidence oppose to the single novel biomarkers use in an ED setting or in a chest clinic (Lin,Yokoyama,Rac *et al.*, 2012). Multi-biomarker score system incorporating current available biomarkers could potentially be used to assess ACS related risk and help patient's management (Bodi,Sanchis,Llacer *et al.*, 2005; Sabatine,Morrow,de Lemos *et al.*, 2002).

1.5 Conclusion

As describe previously ACS patients and NSTEMI/UA in particular present challenging dilemma to clinician in an ED setting. The current strategy deployed by the health professional is not entirely satisfactory in term of safely rule-in and rule-out patients presenting with chest pain suggestive of ACS (Christenson et al., 2004; Antman et al., 2000; Goldman et al., 1988; Tatum et

al., 1997). As mentioned before, normal ECG trace does necessary exclude AMI, moreover cardiac biomarkers of necrosis such as troponin I & T, CK-MB and myoglobin are not appropriate as these biomarkers are expressed only after cardiac cell death (Apple,Collinson & Biomarkers, 2012; Apple,Greenspan & Dietzler, 1982). NSTEMI/UA risk assessment and diagnosis should not be categorised into a binary fashion, in fact the risk evaluation of these cohort should be treated as a continuum as they are significantly running a high risk of AMI, recurrent AMI or death. It is clear that a rapid blood cardiac biomarker for ischaemia is required.

There is some evidence to suggest that multi-biomarker approach may be useful in ACS patient's management especially in an ED. The following biomarker, BNP (Brown,Sease,Robey *et al.*, 2007), copeptin (Reichlin,Hochholzer,Stelzig *et al.*, 2009), h-FABP (Haltern,Peiniger,Bufe *et al.*, 2010) and IMA (Collinson,Gaze,Bainbridge *et al.*, 2006; Peacock,Morris,Anwaruddin *et al.*, 2006) are currently under investigation. Collectively these biomarkers are known to increase during the inflammatory process or during cardiac ischaemia. The current guidelines proposed that clinician should tailor cardiac biomarkers for AMI patients based on prognostic biomarker such as cTn and IMA (Braunwald,Antman,Beasley *et al.*, 2002). The rationale for using these biomarkers is that if combined with h-cTn may improve the diagnosis of ACS or potentially contribute to rule-out protocol and help shortening patient's hospital stay thus saving resources.

1.6 Acute echocardiographic changes in ACS patients

The diagnosis of AMI is typically based upon patient's history, ECG finding and cardiac blood-based biomarkers. However, it has been demonstrated that these diagnostic tools alone may detected only approximately 30% of acute ischaemic events as a large majority of patients that present of ED with chest pain suggestive of AMI have a typical chest pain, a normal ECG and an early

normal level of cardiac biomarkers of necrosis (Lancellotti,Price,Edvardsen *et al.*, 2015).

Echocardiography diagnostic modality is a powerful tool for monitoring patients with acute cardiovascular disease. Echocardiography is the most versatile and cost-effective imaging techniques used to assess unexplained or unstable cardiovascular diseases. They are mobile and could be hand–held devices making them virtually useful every were.

Echocardiography when used and ED setting was shown to change therapy in 60 to 80% of patients presenting with acute cardiovascular disease (Neskovic,Hagendorff,Lancellotti *et al.*, 2013). Presently echocardiography is included in the universal definition of AMI and relevant international guidelines for the management of cardiac arrest (Douglas,Khandheria,Stainback *et al.*, 2007). In an ED or critical care setting echocardiography can be utilised to monitor cardiac output, to determine abnormalities of cardiac physiology and coronary perfusion (Garcia-Fernandez,Macchioli,Moreno *et al.*, 2001).

Echocardiography is one of the tools used in ACS patients with unexplained haemodynamic changes that require immediate evaluation (Romano,Dagianti,Penco *et al.*, 2000). The role of echocardiography utilisation in assessing AMI patients is twofold, first it helps establish the location and the extent of AMI; and secondly it provides prognostic information that help in the risk stratification. Echocardiography is an accurate non-invasive test capable of identifying myocardial ischaemia or necrosis (Shiran,Blondheim,Shimoni *et al.*, 2017).

The American College of Cardiology, the American Heart Association, and the American Society of Echocardiography (ACC/AHA/ASE) guidelines (2003) for echocardiography recommended the use of echocardiography in the diagnosis of suspected acute ischaemic patients, those with inconclusive ECG or AMI patients without detectable cardiac blood biomarkers (Cheitlin,Armstrong,Aurigemma *et al.*, 2003).

The 2011 appropriate use criteria for echocardiography allowed its use in the diagnostic of suspected AMI as appropriate (American College of Cardiology Foundation Appropriate Use Criteria Task,American Society of,American Heart *et al.*, 2011). During severe ischaemic episode cardiac regional wall motion abnormalities can be visualised echocardiographycally within seconds (12 ± 5 and 19 ± 8) of coronary artery occlusion (Lythall,Gibson,Kushwaha *et al.*, 1992). Moreover, cardiac wall motion abnormalities due to cardiac ischaemia can be detected echocardiographycally before any ECG detectable changes (Sabia,Afrookteh,Touchstone *et al.*, 1991). It is important to note that not all cardiac wall abnormalities detected echocardiographycally can be attributed to cardiac ischaemia as other conditions such as myocarditis and Tako-tsubo cardiomyopathy can mimic cardiac ischaemia finding. Thus, echocardiographical finding should be interpreted with caution and in relation to other clinical findings. Moreover, echocardiography utilisation of ACS patients in ED setting should only be allowed if trained specialist is available (Breitkreutz,Price,Steiger *et al.*, 2010). This could be a limitation in using echocardiography in rural or remote areas.

1.7 Cardiovascular risk factors

CVD is a chronic disease that can largely be prevented through lifestyle changes and reduction of behavioural risk factors such as unhealthy diet, physical inactivity, alcohol and tobacco smoking. Over time, these harmful lifestyle and behavioural risk factors individually or collectively lead to metabolic and physiological changes including dyslipidaemia, diabetes, hypertension and obesity. The long-term consequences of exposure to these risk factors from childhood to adult life can cause damage to the coronary arteries due to atherosclerosis formation. Strong evidence suggests that reducing these cardiovascular risk factors can prevent fatal and non-fatal AMI (Cohen,Assyag,Boyer-Chatenet *et al.*, 2014; Wald, 2003; Yusuf, 2004).

1.8 Clinical presentation of acute coronary syndrome

Patients presenting to the ED with suspected ACS exhibit a wide range of prodromal symptoms table 1 (McSweeney,O'Sullivan,Cody *et al.*, 2004). These include prolonged (> 20 min) anginal pain and retrosternal pressure or heaviness radiating to the left arm, neck or jaw. These symptoms can also be accompanied by nausea, abdominal pain, epigastric pain; recent-onset indigestion and chest pain (McSweeney,Cody,O'Sullivan *et al.*, 2003). Most of these complaints are frequently seen in younger adults (35-54 years), however atypical presenters are usually seen in older patients (> 75 years old), and those with existing co-morbidity such as diabetes, dementia and chronic renal failure (Canto,Fincher,Kiefe *et al.*, 2002; Culic,Eterovic,Miric *et al.*, 2002b). Patients presenting with prodromal symptoms have a good prognosis and rigorous cardiac investigations (Graham,Westerhout,Kaul *et al.*, 2008; Kloner, 1995). However, patients without prodromes are likely to be discharged with a high risk of ACS (Graham,Westerhout,Kaul *et al.*, 2008).

Table 1: The following 5 groups of potential atypical prodromal symptoms as determined by ICD-9-CM diagnostic coding; based on the data used by McSweeney et al, (2003).

	Prodromal symptoms
1	Pain (an aggregate of chest, arm, shoulder, neck, jaw, throat, or leg complaints)
2	Anxiety/fatigue (anxiety, sleep disturbances, weakness/fatigue)
3	GI disturbances (nausea/vomiting, loss of appetite, indigestion)
4	Head-related conditions (dizziness, headache, visual disturbances)
5	Other (sweating, shortness of breath, heart racing, cough, numbness).

The prevalence of chest pain and chest discomfort varies among the population in Western Europe. A British study of men (n = 7735) presenting with chest pain suggestive of AMI, found that in only 14% of cases, AMI was

responsible for the complaint and a further 24% experienced atypical chest pain (Lampe, 1998). As identified by National Clinical Guideline Centre for Acute and Chronic Conditions March 2010; when compared, the symptoms of ACS in ethnic populations are not different from the European population. The National Institute for Health and Care Excellence (NICE) guidelines requires clinicians to assess symptoms of ACS same way as in men and women regardless of ethnicity.

The circumstances leading to the misdiagnosis of ACS are multi-factorial including socioeconomic, gender, disease pathology, presentation and diagnostic limitations. Typically patients who are more likely to be inappropriately discharged from the ED are women bellow the age of 55, the odds ratio (OR) for discharge was (OR 6.7; 95% CI, 1.4-32.5), non-white OR for discharge was 2.2; (95% CI, 1.1-4.3) and women with atypical chest pain, normal cardiovascular biomarkers and a non-diagnostic ECG had an OR of 3.3, (95% CI, 1.7-6.3) (McSweeney,Cody,O'Sullivan *et al.*, 2003; Pope, 2000).

Approximately 25% of patients with non-classical symptoms or atypical presenters have suffered a silent AMI (Khafaji & Suwaidi, 2014). Atypical presenters contribute to under-recognition of the disease (Brieger,Eagle,Goodman *et al.*, 2004). The diagnostic and therapeutic evaluation of an atypical presenter is extremely difficult especially when the main diagnostic pointers such as troponin or ECG changes are masked or exacerbated by an existing condition (Table 2) or abnormal results (Nikolaou,Arntz,Bellou *et al.*, 2015). In addition, patients with acute chest pain but without persistent ST-segment elevation, NSTEMI or ECG changes are extremely difficult to identify and treat (Deshpande & Birnbaum, 2014). Delayed treatment is associated with a longer hospital stay and increased morbidity and mortality (Nikolaou,Arntz,Bellou *et al.*, 2015). Moreover, failure to identify NSTEMI patients adds cost to the healthcare system, delays treatment, potential for patients expose to unnecessary irradiation or exposure to contrast agents (Deshpande & Birnbaum, 2014).

Table 2: Cardiac and non-cardiac conditions that can mimic non-ST-elevation ACS (Reproduced from ESC Guidelines 2007).

Cardiac	**Pulmonary**	**Haematological**
Myocarditis	Pulmonary embolism	Sickle cell anaemia
Pericarditis	Pneumonia	-
Myopericarditis	Pleuritis	-
Cardiomyopathy	-	-
Tako-tsudo syndrome	-	-
Vascular	**Gastro-intestinal**	**Orthopaedic**
Aortic dissection	Oesophageal	Spam rib fraction
Aortic aneurysm	spasm oesophageal	Muscle injury
Aortic coarctation	Peptic ulcer	
Cerebrovascular disease	Pancreatitis	

1.9 Misdiagnosed Patients

In England and Wales chest pain is responsible for 700,000 admissions to the ED annually (Goodacre,Cross,Arnold *et al.*, 2005). Patients presenting with chest pain suggestive of ACS constitute the largest single cohort of patients in the UK emergency department's (Gavalova, 2012).

In the last four decades, the hospital mortality rate from AMI has seen a remarkable decline from 30-35% to 8-10% (Fox,Steg,Eagle *et al.*, 2007; Wenger,Hellerstein,Blackburn *et al.*, 1982). This decline, correlates with advances in patient management and the introduction of new therapies such as percutaneous coronary intervention (PCI), cutting edge analysers and the discovery of new cardiac biomarkers such as troponins and CK-MB (mass). The motivation for introducing these therapies was due to the realisation that thrombotic coronary occlusion progresses over a defined time frame of a few hours during which myocardial tissue can be salvaged. After the onset of myocardial ischaemia, there is a time frame of approximately of < 1 hour before

the myocardium starts to die and it will take at least 3 hours before the cardiovascular biomarkers (i.e. troponins, CK-MB and myoglobin) for cell death or necrosis start to be detectable in the circulation (Simoons, Boersma, Maas, & Deckers, 1997).

Before 1970s patients used to spend as much as 3 days in hospital for a definitive diagnosis of AMI; however, currently patients presenting to the ED from the onset of symptom to diagnosis is in the range of 1212 h in most cases (Kontos,Diercks & Kirk, 2010). Encouraging chest pain patients to present to ED during the early signs of ACS has paid off as early treatment of AMI within 1 h of onset of chest pain is associated with a mortality of 1-2% as opposed to that treated at 6 h and a mortality rate of 10-12% (Rawles, 1994).

The thrombolytic era of 1970s was concerned with therapeutic approach to restore the oxygen supply to the myocardial tissue; thus reducing tissue damage (Braunwald,Maroko & Libby, 1974). This strategy was validated in numerous trials and was proven to be valuable in the managements of ACS patients (Lomaestro, 1994). The successful reduction in mortality and morbidity associated with early therapy and national awareness initiatives encouraged patients with chest pain to present to the ED much earlier. To early identify and treat potential ACS patients; the healthcare system inadvertently finds itself under pressure in terms of resource allocation. Thus, effective use patient management and risk stratification, while maintaining acceptable cost to the health system is of importance (Roberts, 1998). Risk stratification starts with the first contact with the patients presenting to the ED with chest pain; generally those with < 5% probability of AMI can be identified from the presenting symptoms, past history and electrocardiogram (ECG) (Lee, 1985). The efficacy of this approach was supported by a study of 2271 low risk patients in which they found that 2.5% could be recognised (Farkouh, 2009).

Non-cardiac chest pain is very common in the general population (Fass & Achem, 2011). However, when dealing with patients presenting with chest pain suggestive of ACS, cardiologist's priority is to exclude any acute life-threatening cardiovascular condition; including AMI (STEMI), NSTEMI, UA,

SA, and pulmonary thromboembolism. ACS patients are clinically subdivided according to their family history, physical symptoms, ECG findings, cardiovascular biomarkers finding and risk-factor profile (Hamm, Bassand, Agewall, Bax, Boersma, Bueno, Caso, Dudek, Gielen, Huber, Ohman, Petrie, Sonntag, Uva, Storey, Wijns, & Zahger 2011).

Patients presenting the ED with chest pain suggestive of ACS without the classical ECG changes and with normal cardiovascular biomarker of necrosis (i.e. troponin, CK-MB) represent a challenge to clinicians. As these patients are at risk of higher morbidity and mortality (Xing,Pei,Tang *et al.*, 2018). The ECG has a developed criterion for risk stratification to allow clinicians to make timely decision on patient's management including pharmacological and coronary revascularization strategy if required. Currently, the leading precursor (symptom of chest pain) is the primary indicator that triggers the diagnostic and therapeutic intervention in the most ED. At presentation these patients are classified into two groups:

i. Patients with typical acute chest pain and persistent (>20 minute) ST-segment elevation ACS. These cohorts usually reflect an acute total coronary occlusion and subsequently develop an ST-elevation myocardial infarction (STEMI). This cohort of patients will; receive immediate treatment such as PCI
ii. Patients with acute chest pain but without persistent ST-segment elevation. These cohorts present with either persistent or transient ST-segment depression or T-wave inversion, flat T-wave, pseudo-normalisation of T-wave or no ECG changes at presentation. These patients are monitored with serial ECG and serial measurement of cardiovascular biomarkers of necrosis. Currently patients with no ECG changes (NSTEMI) are classified based on troponin measurement, and they will be further qualified into non-ST-elevation myocardial infarction (NSTEMI) or unstable angina (Figure 5).

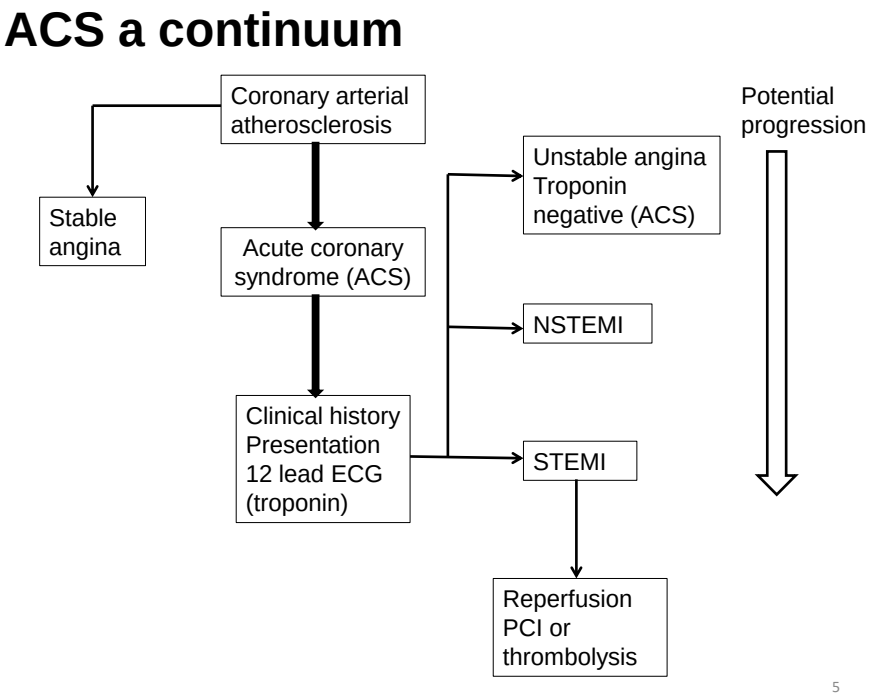

Figure 5: Schematic representation of possible outcome of patients admitted to ED with chest pain of cardiac origin.

Clinicians rely on physical examination, patients' history, ECG, blood biomarkers (i.e. troponin) and imaging techniques (echocardiography) to diagnose cardiac ischaemia (Pope et al., 2000). Despite the availability of these tools, diagnosis of ischaemic heart disease is still less specific or sensitive (Kaski & Garcia-Moll, 2002). Furthermore, these techniques often fail to discriminate between ischaemic heart disease and non-cardiac related disease. Collinson et al ., demonstrated that 7% of patients discharged from ED after being assessed for ACS have high levels of troponin level (Collinson, Premachandram, & Hashemi, 2000). A retrospective study of 10.689 patients admitted to ED with chest pain suggestive of ACS; found 2.1% were misdiagnosed among AMI cohort and 2.3% also misdiagnosed among UA patients (Pope et al., 2000). Moreover, between 2 and 8% of patients with AMI were mistakenly discharged (Cassidy et al., 2000).

Ideally, early biomarkers used in the ED should be highly sensitive, specific and should show a high NPV or PPV to rule-out or rule in AMI respectively. The C2010 International Liaison Committee on Resuscitation (ILCOR), support the use of biomarker of ACS only if they achieve sensitivity > 95% or specificity > 92% and a combined sensitivity of > 90% (Hazinski,Nolan,Billi *et al.*, 2010; Nolan,Hazinski,Billi *et al.*, 2010). However, in the majority of biomarkers utilised currently do not subscribe to this (Lippi & Guidi,2008). As result false positive and negative are common finding (Pour-Ghaz,Bob-Manuel,Marella *et al.*, 2018).

The conditions leading to the misdiagnosis of ACS patients is multifactorial including socioeconomic, disease pathology/presentation, inadequate training, patient's educational level and diagnostic limitation (Pourafkari,Tajlil,Ghaffari *et al.*, 2017). Women were more likely than men to have been discharged with the wrong diagnosis because women generally appear to have a higher rate of atypical symptoms or presentation such as shortness of breath, congestive heart failure and abdominal pain than men (George,Rapsomaniki,Pujades-Rodriguez *et al.*, 2015).

A study found that approximately 5.8% of black Americans with AMI were not admitted to cardiovascular department compared with 1.2 % of the white population; despite the evidence that suggest that black people have more risk factor for coronary disease than whites, this finding did not appear to have a strong influence on the diagnostic impression of clinicians (Cooper & Ford, 1992; Maynard,Fisher,Passamani *et al.*, 1986). A UK study found that elderly men and women were less likely to be referred for angiography despite clinical indication. The same study also highlighted the underuse of angiography across all patient groups (Bowling,Bond,McKee *et al.*, 2001).

The incidence of misdiagnosis of ischaemia in the ED may be reduced if clinicians interpret the ECG trace properly or address clinical factors or perception of unconscious or conscious bias that obscure the recognition of AMI in women and non-white population (George,Rapsomaniki,Pujades-Rodriguez *et al.*, 2015). Survey conducted by the European Society of

Cardiology (ESC) has shown that some clinicians are not fully aware of the best practice and the current guidelines for the management of ACS patients especially when the attending clinician is not a cardiology specialist (Rapezzi,Biagini & Branzi, 2008; Taghaddosi,Dianati,Fath Gharib Bidgoli *et al.*, 2010).

Occasionally some patients will not present with the classical symptoms in fact, 1 in 20 patients with an AMI is an atypical presenter (Puleo, 1994; Ting, 1991). Atypical presenters contribute to under-recognition of the disease (Brieger,Eagle,Goodman *et al.*, 2004). The diagnostic and therapeutic evaluation of an atypical presenter is extremely difficult especially when the main diagnostic pointers are masked or exacerbated by existing conditions such as rib fraction, renal disease or abnormal results i.e. inconclusive ECG trace and normal troponin concentration (Apple,Murakami,Quist *et al.*, 2003). Once cardiac source of chest pain is excluded other conditions should be considered, for example an individual who is taking part a vigorous exercise after a period of physical inactivity may experience heaviness, chest pain discomfort and even damage or trauma. Muscular skeletal causes are common source of chest pain. Chest pain can also be triggered by pulmonary conditions such as infection of the lungs. It is also known that anxiety or an underlying psychiatric disorder can present as non-cardiac chest pain in some patients (Katz & Castell, 2000).

The majority of patients presenting to ED with chest pain suggestive of ACS are found to have an oesophageal related chest pain (Hong, 2010). The three main causes of oesophageal chest pain results from an underlying disturbed nerve sensation, mucosal dysfunction and muscle. Gastroesophageal reflux disease in particular when reflux of acid is involved can present with chest pain, swallowing difficulties or heartburn. Oesophageal related chest pain can also be attributed to an oesophagus spasm or caused by a mobility disorder of the oesophagus. Chest pain induced by oesophagus can also be caused by an abnormal sensory function of the oesophagus called hypersensitivity. In

this condition the muscle nerve and receptors of the oesophageal wall are extremely sensitive (Hong, 2010; Rao, 2011).

Some patients with CAD have chest pain that is unresponsive to conventional medical therapy and are considered to have a refractory chest pain. This refractory chest pain is usually wrongly perceived to be of cardiac origin and poses a common clinical problem that induces anxiety in both patients and clinicians (Liu,He,Chen *et al.*, 2013).

Condition like pulmonary embolism (PE) is potentially life-threatening condition and is manifested due to blockage in a blood vessel that supplier the lungs. PE can cause chest pain and breathlessness similar to that seen in patients with ACS.

To minimise the misdiagnosis of AMI, the European Society of Cardiology (ESC) guidelines for the management of ACS patients; requires that all patients admitted to ED with chest pain suggestive of ACS should have a resting 12-lead ECG carried out and interpreted by a qualified person within 10 minutes of arrival. The problem with using ECG is that a completely normal ECG does not exclude AMI (50% sensitivity). Several studies found that approximately 5% of patients, who were sent home because of normal ECG, were subsequently readmitted with UA or AMI (Goodacre,Cross,Arnold *et al.*, 2005). In addition, up to 50% of patients presenting to ED with chest pain do not show STEMI in their ECG assessment (Apple & Murakami, 2005). The current study is in agreement with previous ECG finding as approximately 75% of patients admitted to ED with chest pain suggestive of ACS had a normal ECG, only 3% had bundle branch block (BBB) and 8% had an inversion T-wave. To complicate the diagnosis; cardiac Ischaemia is present in the territory of the circumflex artery thus not frequently detected by 12-lead ECG but may be recorded by the lead V_3R, V_4R and V_7-V_9 (Bassand, Hamm, Ardissino, Boersma, Budaj, Fernandez-Aviles, Fox, Hasdai, Ohman, Wallentin, & Wijns 2007).

The ESC guidelines for the management of ACS and in particular patients with suspected NSTEMI requires the measurement of a biomarker of cardiomyocyte injury such as hs-cTnI or hs-cTnT. Cardiovascular biomarkers of cardiomyocyte injury are highly cardiac specific and are the “gold standard” biochemical test for the diagnosis of AMI. However, hs-cTnI and hs-cTnT are only released to the circulation after cardiomyocyte injury and it takes 6 to 12 hours to reach the desired sensitivity and specificity. The introduction of hs-cTn technology has led to the quantification of myocardial injury; these assays increased the diagnostic efficiency of AMI patients on admission and reduced “troponin blind spot” e.g. the time a takes cTn to be detected in the circulation (Slawson, 2018). To complicate the matter further, hs-cTn can also be detected in healthy population. Thus, the presence of hs-cTn is always associated with AMI; equally the absence of hs-cTn does not exclude ACS. Thus, hs-cTn needed to be interpreted with caution. Generally, any troponin is always worse than no troponin, and more troponin is always worse than less troponin. In the mean-time patients are required to stay in hospital for observation in order to confirm or exclude AMI and if no change is observed patients are discharged and sent home (Taghaddosi,Dianati,Fath Gharib Bidgoli *et al.*, 2010).

It is apparent that the clinician should not rely on clinical judgment alone to determine whether an individual patient who presents to ED with chest pain suggestive of ACS has indeed an ACS. Effort to reduce the number of misdiagnoses is urgently required and warranted.

1.10 Summary of current treatment of stable angina

Coronary artery disease (CAD) is major cause of death globally and is predicted to remain a leading cause of death in the next 20 years. CAD is responsible for approximately 3.8 million and 3.4 million men and women respectively worldwide (Mathers & Loncar, 2006).

CAD develops when the main blood vessels that supply the heart with blood, nutrients and oxygen become diseased or damaged by the presence of an atherosclerotic plaque and inflammation. CAD is type of heart disease characterised and manifested by SA pectoris also known as angina of effect is a direct result of ischaemia caused by an imbalance between oxygen supply and demand to the myocardium (Figure 6). SA patients may also present with atypical symptoms such as breathlessness, discomfort and pain to the front of the chest (this pain is believed to be due to lactic acid build up), nausea and gastrointestinal discomfort. SA is the main symptom of myocardial ischaemia and is usually caused by atherogenesis and plaque formation in the epicardial arteries, spasm or normal or plaque containing arteries restricting blood flow and therefore oxygen to the myocardium. SA is a chronic condition with a low incidence of adverse acute coronary events (Task Force,Montalescot,Sechtem *et al.*, 2013).

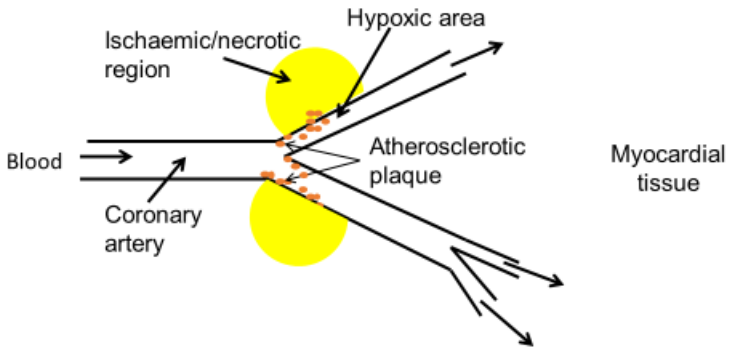

Figure 6: Ischaemia as being the condition where the supply of oxygen to muscle is insufficient to meet the demand. Atherosclerosis plaque formation reduces blood supply to the myocardium and if prolonged anginal pain is believed to be due to the lactic acid build-up.

In addition, SA is a type of ischaemic heart disease characterised by a predictive chest pain during physical activity that resolves with rest or pharmacological intervention like the administration of sublingual nitro-glycerine. In severe cases other drug classes such as calcium antagonists and angiotensin-converting enzyme (ACE) may be used (Depre,Wijns,Robert *et al.*, 1997).

The diagnosis of SA is based on a detailed clinical history which includes assessing the location, severity, duration, magnitude and the precipitating factors of angina. In most cases the diagnosis can be made based on the history and physical examination (Pryor,Shaw,McCants *et al.*, 1993). Although, clinical history is useful in diagnosis of SA; other modalities such as coronary angiogram, cardiac magnetic resonance, and ECG can also be used. Non-invasive procedures such as the evaluation of haemoglobin levels can be of value; it has been demonstrated that low haemoglobin is associated with increased mortality in SA patients (Mishra,Ray,Dalal *et al.*, 2016). Moreover, features such elevated heart beat at rest (≥ 70 beat per minute) in SA patients is associated with a poor prognosis (Mishra,Ray,Dalal *et al.*, 2016). The treatment of SA patients is for long term and requires supervision (Task Force,Montalescot,Sechtem *et al.*, 2013).

1.10.1 Possible treatments for stable angina

Generally, the treatment of SA includes medication to modify atherosclerosis, anti-anginal drugs and aggressive treatment of causative risk factors such as lowering lipid. The overall managements of SA patients consist of promoting life style changes coupled with pharmacological interventions; however, in severe cases especially in the elderly, renal dysfunction and diabetes patients' revascularisation procedures may be warranted.

NICE guidelines require newly diagnosed SA patients to be put on Beta-blocker and calcium channel blocker as the first line of treatment. The

treatment strategy of SA is to improve the quality of life, by reducing the number and the severity of the anginal attacks and to protect against future adverse cardiac events. Moreover, SA long term management strategy consists of practical measures to reduce the burden of risk factors such as cholesterol and lifestyle risks in order to slow disease progression (Kones & Rumana, 2016). Thus, management of these risk factors is crucial for the secondary prevention of ischaemic cardiac events.

1.11 Pharmacological interventions

1.11.1 Beta-blockers

The benefit of beta-blockers in SA patients is extrapolated from studies aimed at AMI patients, thus, to my knowledge there is no confirmatory trials of the merit of managing SA patients with this class of drugs (Andersson,Shilane,Go *et al.*, 2014).

Beta-blocker is a class of drugs designed to work by blocking the effect of the hormone epinephrine. The effect of blocking epinephrine causes the heart to contract slowly with less force; thus, reducing blood pressure. Beta-blockers also help blood vessels to relax and to increase blood flow and oxygen to the myocardium. Reduction in heart rate allows greater diastolic period consequently an increase coronary perfusion that also improves myocardial oxygen supply. Beta-blockers were also found to help control both asymptomatic and symptomatic ischaemic attacks (Heidenreich,McDonald,Hastie *et al.*, 1999a).

1.11.2 Nitrate

In 1867, Lauder Brunton described the anti-anginal property of nitrate and subsequently first used as a pharmacological agent in 1879; since then the agent is used worldwide until today (Boden,Padala,Cabral *et al.*, 2015). Nitrate is readily absorbed, and it is available in various formulation including, tablet, sprays and patches.

Anti-ischaemic agents act by decreasing myocardial oxygen consumption, heart rate, blood pressure, or depressing the left ventricular contractibility and/or inducing vasodilatation. Suspected SA patients with ongoing ischaemic discomfort are given sublingual nitro-glycerin (NTG) (0.4 mg every 5 min for a total of 3 dose) if not contraindicated. They are also given supplemental oxygen if their arterial saturation is less than 90% or if the show sign of respiratory distress or possible hypoxemia. Oxygen therapy is only administered if arterial saturation is less than 90%, to minimise free radical generation.

Glycerine tri-nitrate (GTN) and pentaerythritol tetranitrate (PETN) are converted to nitrate by the enzyme mitochondrial aldehyde dehydrogenase and also by a non-enzymatic pathway involving the reaction with thiol derivative (Munzel,Daiber & Gori, 2011). Nitric oxide is a potent coronary vasodilator. The major therapeutic benefit of nitrate is its effect as a vasodilator. Nitro-glycerin act by decreasing the myocardial pre-load and LV end diastolic volume, subsequently resulting in a decrease in myocardial oxygen consumption. Moreover, nitrates also dilate normal as well as atherosclerotic coronary arteries and increase coronary flow.
In the absence of any clinical contraindications nitrate might be administered intravenously to patients presenting with SA. The dose is usually titrated upwards until symptoms are relieved, or side effects such as hypotension occur.

When symptoms are controlled or a side effect is noted, intravenous nitrates may be substituted by nitrate-like drugs such as sydnonimines or potassium channel activators (vasodilator) (Tamargo,Caballero,Gomez *et al.*, 2004). However, the use of this class of drugs is not recommended for patients taking phosphodiesterase-5 inhibitors (sildenafil, vardenafil, tadalafil) because of the risk of profound vasodilatation and low blood pressure (Tamargo,Caballero,Gomez *et al.*, 2004).

1.11.3 Calcium antagonist

Calcium antagonist (CAs) decrease angina discomfort by inhibiting inward calcium current through the cell membrane not only in the myocardium but also in various type of tissue including: vascular smooth muscle in both coronary arteries, cardiac conduction tissue and peripheral vessels. Reduced intracellular calcium causes smooth muscle cell relaxation, vasodilatation and subsequently increases coronary blood flow. All CAs subclasses are potent vasodilators (table 3).

Table 3: Subclasses of calcium antagonist (adapted from the \European society of cardiology)

Type	Properties	Drugs
Dihydropyridines (DHPs) Non-Dihydropyridines (DHPs)	Peripheral and coronary vasodilators, negative inotropic action	Amlodipine, nifedipine, felodipine, isradipine, nicardipine, nisoldipine
Phenylalkylamines	Additional negative chronotropic and inotropic actions	Verapamil
Benzothiazepine	Additional negative chronotropic and inotropic actions	Diltiazem
Mixed sodium and CA	Non-selective blocking delayed rectifier K^+ current and fast Na^+ current. Also, inhomogeneous electrical effects.	Bepridil
Antihistamine	Used for migraine prophylaxis, peripheral vascular disease, vertigo, but not for angina	Funarizine

1.11.4 Brief description of the mode of action of various calcium antagonist subclasses.

1.11.4.1 Calcium channel blockers

Calcium channel blockers (CCBs) are class of drugs that non-competitively inhibit calcium movement through voltage-dependent L-type calcium channels, thus resulting in chronotropic, slower conduction, negative inotropic effects and smooth muscle relaxation. CCBs are available in two distinct types: dihydropyridine (DHP) and non-DHP. The non-DHP CCBs which include verapamil and diltiazem and is characterised by its ability to inhibit sinus node activity; thus, reducing heart rate and act as an anti-anginal drug. A meta-analysis found that CCBs when compared with placebo reduces the risk of heart failure in SA patients by 82% (95% CI, 73-92%) (Bangalore,Parkar & Messerli, 2009). This meta-analysis also highlighted an association between CCBs and decreased risk of stroke and SA.

1.11.4.2 Dihydropyridines (DHPs)

This class of drugs reduced blood pressure (BP), myocardial oxygen consumption and myocardial wall tension. As a consequence, DHPs action patients' experience fewer anginal attacks, reduces the need for nitrate and improve ischaemic ST-segment changes (Heidenreich,McDonald,Hastie *et al.*, 1999b).

CCBs therapy has been proven to be an effective anti-anginal drug and it is widely used for the management of hypertension. However, the prognostic efficacy of CCBs for SA patients is limited. High dose of nifedipine was associated with increased mortality (Furberg,Psaty & Meyer, 1995). In contrast the ACTION study which conducted a long-term efficacy of nifedipine concluded that the latter safely relieves anginal pain and was not linked to adverse cardiac events in patients with SA and hypertension (Poole-Wilson,Lubsen,Kirwan *et al.*, 2004). In addition, the ESC guidelines on stable coronary artery disease for the management of SA; concluded that both CCBs and beta-blocker are equally appropriate for SA management; the usual dose of CCBs and side effect are summarised in table 4.

Table 4: Common dose and side effect associated with calcium antagonist

Drug	Duration action	Usual dose	Common side effect
Dihydropyridines (DHPs)			
Nifedipine slow release	Long	30-180 mg/d	Hypotension, oedema, flashing, constipation, nausea
Amlodipine	Long	5-20 mg (daily)	Headache, oedema
Felodipine sustained release	Long	5-10 mg (daily)	Headache, oedema
Isradipine sustained release	Medium	2.5-10 mg (twice a day)	Headache, fatigue
Nicardipine	Short	20-40 mg (3 times a day)	Headache, oedema, dizziness, flushing
Non-dihydropyridines (non-DHPs)			
Diltiazem immediate release	short	30-80 mg (4 times a day)	Hypotension, flashing, oedema, dizziness, bradycardia
Diltiazem slow release	Long	120-320 mg (daily)	Hypotension, flashing, oedema, dizziness, bradycardia
Verapamil immediate release	short	80-160 mg (3 times a day)	Hypotension, negative inotropic, oedema, HF, bradycardia
Verapamil slow release	Long	120-480 mg (daily)	Hypotension, negative inotropic, oedema, HF, bradycardia

1.11.4.3 Angiotensin converting enzyme inhibitors

The maintenance of the haemodynamic stability and the regulation of arterial blood pressure, water and electrolyte balance are controlled by the renin-angiotensin-aldosterone system. Angiotensin-converting enzyme inhibitors act by inhibiting the conversion of angiotensin I to angiotensin II in the lung, thus preventing the potent action (vasoconstrictor) of angiotensin II and consequently prevents a narrowing of blood vessels and an increase of blood pressure. ACE inhibitor also promotes vasodilatation and correct endothelial dysfunction by inhibiting the degradation of bradykinin (Landmesser & Drexler, 2006). Bradykinin is the active peptide for the kallikrein-kinin system and they are potent endothelium-dependent vasodilators that contribute to hypotension in the systemic circulation (Golias,Charalabopoulos,Stagikas *et al.*, 2007). ACE also acts by lowering the total peripheral resistance. This is achieved by blocking the enzyme that convert angiotensin I to angiotensin II. Currently there are three sub-classes of ACS inhibitors are available:

1. Sulfhydryl group; e.g. captopril
2. Decarboxylase group; e.g. Enalapril, lisinopril
3. Phosphonate group; e.g. Fosinopril

Based on animal models the ACE inhibitors are further classified according to their ability to cross the brain blood barrier, the two most common are summarised in table 5.

Table 5: Common ACE inhibitors based on their ability to cross the blood brain barrier

ACE inhibitors that cross blood brain barrier	**ACE inhibitors that not cross blood brain barrier**
• Fosinopril • Lisinopri • Perindopril • Ramipril • Trandolapril	• Benazepril • Enalapril • Moexapril • Quinapril

Current evidence strongly suggests that patients with stable vascular disease such as SA are more likely to benefit from these class of drugs (Heart Outcomes Prevention Evaluation Study,Yusuf,Sleight *et al.*, 2000; Latini,Tognoni,Maggioni *et al.*, 2000).

1.11.4.4 Chelation therapy

Chelation therapy involves a series of intravenous infusion of disodium ethylene diamine tetra-acetic acid (EDTA) in combination with other substances. Disappointing clinical studies could not confirm the efficacy of EDTA's infusion. However, a reduction of 18% in risk of cardiovascular events was observed (HR – 0.82; 95% CI, 0.69-0.99; p = 0.035) (Escolar,Lamas,Mark *et al.*, 2014; Lamas,Goertz,Boineau *et al.*, 2012; Lamas,Goertz,Boineau *et al.*, 2013). To date chelation therapy is not offered to SA patients and additional studies are still pending regarding the utility of chelation therapy in SA patients.

1.11.4.5 Statins

The efficacy of statin in reducing and preventing cardiovascular events is well documented. Several meta-analyses have showed a significant reduction in cardiovascular related death and can safely reduce the 5-year incidence of major adverse cardiac event (MACE) (Baigent,Keech,Kearney *et al.*, 2005; Mills,Rachlis,Wu *et al.*, 2008). These meta-analyses also concluded that statin should be used as a primary therapy for cardiovascular related diseases. Moreover, a Cochrane meta-analysis assessing the potential harm and benefit of statin used by patients with no history of CVD; found that it's use causes a reduction of all-cause of mortality (OR 0.86, 95% CI 0.79-0.94%) as well as in revascularization rates (RR 62, 95% CI, 54-72%) (Taylor,Huffman & Ebrahim, 2013). A meta-analysis looking at intensive vs. moderate use of statin therapy found a significant (16%) reduction of cardiovascular death or AMI; they also concluded statin high or moderate intensity should be prescribe to SA as a routine regardless of lipid levels (Cannon,Steinberg,Murphy *et al.*, 2006).

1.11.4.6 Revascularisation

Coronary revascularisation involves restoring perfusion to ischaemic myocardium. In SA patients' revascularisation is only carried out when all possible treatments fail to manage SA crisis, or a significant stenosis is detected (Task Force,Montalescot,Sechtem *et al.*, 2013).

Numerous meta-analyses showed that revascularisation may help control myocardial ischaemia more efficiently than medication alone in some patients (Stergiopoulos,Boden,Hartigan *et al.*, 2014; Windecker,Stortecky,Stefanini *et al.*, 2014). Coronary revascularisation involves two modalities one is coronary artery bypass grafting (CABG) and the other is PCI.

1.11.4.7 Coronary artery bypass grafting

There were 17,600 coronary CABG operations in UK in 2013 compared with 23,100 in 2004 (Farooq,Serruys,Zhang *et al.*, 2013). Patients with significant and severe complex (SYNTAX >22) multivessel disease or left ventricular dysfunction may benefit from CABG surgery due to lower cardiac events associated with this procedure. However, certain severe SA cases with existing comorbidity such as diabetes may require CABG. Meta-analysis showed that over 5 and 10 year's period the CABG group showed significantly lower cardiovascular related mortality compared to medical treatment group (10.2 vs. 15.8%; OR 61 [95% CI, 48-77%], p = 0.0001) and (26.4 vs. 30.5%; OR 83% [95% CI, 70-98%], p = 0.03) (Trikalinos,Alsheikh-Ali,Tatsioni *et al.*, 2009). A meta-analysis showed that in high and medium risk patients, CABG was associated with a good survival benefit (Mohr,Morice,Kappetein *et al.*, 2013).

1.11.4.8 Percutaneous coronary intervention

Percutaneous coronary intervention (PCI) coupled with medical treatment in SA patients is the common outcome for most SA patients (Mack,Head,Holmes *et al.*, 2013). However, in terms of superiority of treatment, a recent meta-analysis reviewed PCI vs. medical treatment found that PCI did not show significant improvement in all-causes of cardiovascular mortality (RR 0.85; 95% CI 0.71-1.01), cardiac death (RR 0.71; 95% CI 0.47-1.06), AMI (RR 0.93; 95% CI 0.7001.24) , or repeat revascularisation (RR 0.93; 95%CI 0.76-1.14) during short or long term follow-up (Pursnani,Korley,Gopaul *et al.*, 2012). However, PCI demonstrated a better angina relief compared to medical therapy. These finding are consistent with numerous studies (Stergiopoulos,Boden,Hartigan *et al.*, 2014; Task Force,Montalescot,Sechtem *et al.*, 2013; Windecker,Stortecky,Stefanini *et al.*, 2014).

1.11.4.9 Conclusion

The medicine, angioplasty, or surgery study (MASS II) compared the efficacy of PCI, CABG and medical treatment in patients with SA; multi-vessel and preserved ventricular function found that CABG, PCI and medical therapy demonstrated a significantly higher incidence of AMI, additional revascularisation and cardiac death. However, CABG was better than medical therapy at eliminating anginal symptoms (Windecker,Stortecky,Stefanini *et al.*, 2014). In addition, studies compared CABG and PCI concluded neither of these procedures could demonstrate effectiveness for the full spectrum of SA patients who requires revascularisation. Even so, CABG demonstrated a better revascularisation than PCI (Mishra, 2016). The incidence of revascularisation after PCI is higher that after CABG; in contrast, the incidence of strokes is higher after CABG surgery than after PCI. Moreover, a study noted a mild reversible cognitive decline was associated with CABG surgery. However, CABG remains essential for the treatments of patients with significant stenosis of 50% or more, triple-vessel disease, diabetes mellitus and impaired LV function. Detailed evaluation CABG is beyond the scope of this thesis and will not be discussed in detail.

1.12 Life-style changes and risk factors

Consensus and evidence have clearly demonstrated the importance of modifiable lifestyle factors such as diet, physical activities, obesity and smoking in the development of CVD (Micha,Kalantarian,Wirojratana *et al.*, 2012). Thus, intervention targeting these behaviours may improve outcomes for CVD and non-CVD patients.

1.12.1 Diet and weight loss

A study of 31,600 high-risk patients conduct in 40 countries showed that higher-quality diet such as that of the Mediterranean countries reduces diet-induced CVD (Smyth,Dehghan,O'Donnell *et al.*, 2015). Dietary approach to stop hypertension i.e. diet rich in fruit, omega-3, vegetable and diet low in saturated fat has demonstrated a reduction in blood pressure and is effective nutrition-based strategy to prevent CVD (Lavie,Milani,Mehra *et al.*, 2009).

Obesity and overweight and related consequences such as high-density lipoprotein (HDL) hyper-triglyceridaemia, increased number of small low-density lipoprotein (HDL) and dysglycaemia are known as a risk factors for CAD (Galper,Wang & Einstein, 2015; Wang,Zhou,Galper *et al.*, 2015).

1.12.2 Physical activity

Sedimentary life style is a major risk factor of CVD (Warren,Barry,Hooker *et al.*, 2010). Regular physical activities and aerobic exercises have been linked with decreased CVD events (Terada,Johnson,Norris *et al.*, 2016). A meta-analysis demonstrated that high occupational physical activity offer a protection against ischaemic stroke compared with inactive and moderate occupational levels (RR = 57%, 95% CI, 43-77%) and (RR = 77%, 95% CI, 60-98%) respectively (Lollgen,Bockenhoff & Knapp, 2009).

1.12.3 Smoking

Tobacco smoking and second hand smoking increases the risk of CAD by 2.8 to 3 fold and is associated with high mortality (Ramakrishnan,Bhatt,Dubey *et al.*, 2013). Abstaining for smoking reduces the risk of future cardiovascular events. Providing Nicotine replacements and sustained release of bupropion SR are used to aid patients stopping smoking.

1.13 Conclusion

Changing patient's habits and lifestyle have been proven effective in reducing long-term cardiovascular related risks. Smoking cessation, active counselling, in addition to drug intervention is necessary. Weight reduction, regular physical exercise, such as aerobic activities if possible every day, low salt diet and reduced saturated fat intake is recommended. Regular intake of fruit and vegetable, and moderate alcohol intake may be beneficial (Mozaffarian,Appel & Van Horn, 2011; Terada,Johnson,Norris *et al.*, 2016). Management of low-density lipoproteins (LDL) and high-density lipoprotein (HDL) cholesterol as well as triglycerides are essential in long term SA management (European Association for Cardiovascular,Rehabilitation,Reiner *et al.*, 2011). Although, the long-term impact of lipid lowering agents is less well established; the best evidence of LDL reduction is achieved by using statins (Dorresteijn,Boekholdt,van der Graaf *et al.*, 2013).

The current data indicates that long-term statin therapy improves outcome for all forms of CAD. This beneficial effect was shown in all subgroups, including men, women, and elderly (European Association for Cardiovascular,Rehabilitation,Reiner *et al.*, 2011). Recent meta-analysis including 9 clinical trials concluded that early administration of statin decreases overall mortality (odds ratio 0.89, 95% confidence limits 0.82-0.96) when compared to the control group (Nunes, 2017).

1.14 The health economics of acute coronary syndrome

Healthcare systems in the Western world have seen an increase in the introduction of new diagnostic laboratory tests designed to meet the need for individual patients (Caliendo,Gilbert,Ginocchio *et al.*, 2013). The aim of the introduction of these new diagnostic techniques is to increase the sensitivity and specificity for the presence or the absence of a disease, prognostic information, patient's managements and monitoring of treatment. However, clinicians have raised concern about the inappropriate utilisation of these new and traditional diagnostic tests; for example, creatine kinase isoform MB (CK-MB) and myoglobin is no longer justified as an aid for the diagnosis of AMI, because the measurement of hs-cTn is diagnostically efficient and is the most cost-effective diagnostic strategy for AMI (Kim & Hashim, 2016; Price, 2008). A survey conducted in 28 countries and 303 laboratories looking at practices of using outdated cardiac biomarkers against current guideline found that 11% offer myoglobin 59% CK, 8.3% CK-MB. 30% lactate dehydrogenase isoenzyme 1 and 34% AST (Collinson,van Dieijen-Visser,Pulkki *et al.*, 2012).

Health commissioners are concerned about healthcare cost and question whether the benefit of introducing new diagnostic tests is cost effective and necessary (Appleby, 2013; Price, 2008). In order to provide good value for money when introducing a new laboratory diagnostic test, rigorous and challenging new questions are being asked, including whether testing directly improves patient outcomes and whether the benefits of testing outweighs the risks? (Fang et al., 2011). Unfortunately, the UK does not have in place a formal system for the evaluation and the implementation of new diagnostic tests; however, it relies on the European Directive, Conformite Europeenne marking (CE) and Food and Drug Agency (FDA), the diagnostic industry, the National Institute for Health, Care Excellence (NICE) and various specialised organisations to appraise the clinical utility of new diagnostic tests (Price, 2008).

Health authorities and healthcare commissioners have called for a ’value based‘ health care system, the latter requires healthcare suppliers to focus on care pathways, with the view of improving patient outcome and increasing value for money; therefore the payment will be based on value rather than cost (Lee, Neumann, & Rizzo 2010a). Although, the usefulness of this system in not fully appreciated available evidence suggests that this system could reduce the overall US health spending by 30% without compromising quality (Garber et al. 2007). The Healthcare providers generally react to medical conditions and injury only when they occur and do not spend resources on preventing them. This has contributed to a population health status that has not changed despite increasing spending (Trogdon,Finkelstein,Nwaise *et al.*, 2007).

Since the 1980s the hospital mortality rate from AMI has seen a remarkable decline from 30-35% to 8-10% (Wenger,Hellerstein,Blackburn *et al.*, 1982). This decline correlates with advances in patient management and the introduction of new therapies such as PCI, improvement in automation and the discovery of new cardiac biomarkers.

The successful reduction of mortality and morbidity associated with early thrombolytic therapy encouraged patients with chest pain to present to emergency department (ED) much earlier. Laboratory medicine groups and cardiologists recommend a turnaround time of 60 min for cardiac biomarker analysis (Wu,Apple,Gibler *et al.*, 1999). The American College of Cardiology (ACC) and the American Heart Association (AHA) go further to recommend a turnaround time of 30 min. Encouraging chest pain patients to present early to ED has proven to be the right strategy; as early treatment of AMI within 1 h of onset of chest pain is associated with a mortality of 1-2% as opposed to that treated at 6 h and a mortality rate of 10-12% (Amsterdam,Kirk,Bluemke *et al.*, 2010; Rawles, 1994).

1.14.1 Welfare/Pareto economics

Welfare economics is described as “achieving a social maximum derived from individual desires” it is also concerned with improving human welfare and social condition. There are different points of view from which to perform economic evaluations including patient’s individual preference, society and tax payers. Preference is presented by utility functions and subsequently the overall welfare is the product of all the individual preferences. Moreover, utility is considered the only outcome of interest and social welfare is the sum of all individual’s welfare. Preferences of individuals are typically determined by how those individuals’ value and prioritise health benefit over other services.

Pareto economics is a branch of welfare economics which considers preferences and is a method that groups utilities among individuals in order to determine whether a particular resource allocation could improve social welfare. It is of course when aggregating utility which represent individuals, there some numerous states that can be manifested, this can result in a society gaining or loosing utility.

Pareto optimality effectively exists when *“no further changes in the economy can make one person better off without at the same time making another worse off”*. In contrast, Pareto efficiency is when *“there is improvement in utilities for some; however, no one is worse off”*. The status of how “well-off” individual is can be measured in either concrete good or in natural units such as quality-adjusted-life-year (QALY). Pareto non-comparable states manifest when individuals gain utilities and the expenses of re-allocation for example funding for hs-cTnT while losing CK-MB test for the diagnosis of AMI.

1.14.2 Extra-welfarism

Extra-welfarism also considers utilities and preferences, however, it also allows for consideration and inclusion of other health indicators such as patients satisfaction and the burden of caregivers. As a result, healthcare providers and care personal can be a source included in how outcome will be valued by policy makers. It is of course different countries prefer to allocate their resources based on their population preference. Generally, health authorities worldwide and health economic expert use cost-effectiveness analysis (CEA) as a standard method for evaluating whether a new health intervention provides value for money for the tax payer and society as a whole (Neumann et al. 2005).

1.14.3 Theoretical basis of economic evaluation of healthcare

An increasing number of health providers worldwide are adopting results from cost-effectiveness analysis (CEA) as one of the criteria to inform decisions on allocation of healthcare recourses. Identifying a fair and optimal allocation of continuously hard to come buy recourses to maximize health benefit will be key challenge to healthcare systems such NHS, government agencies and managed-care organisations. Medical innovation and research is expected to continue to produce new medical interventions and treatments of diseases. However, budgetary constraints will not allow healthcare systems to make all these interventions available to everybody. It has been argued that the use of CEA threshold might lead to uncontrolled growth in health care expenditures (Gafni A, 1993). Although, most other countries do not include health economic analysis input into their decision making process; Australia, Canada, Sweden and UK have implemented guidelines for resources-allocation decision based on formal health-economic analysis; currently the most popular approach being the CEA (NICE, 2008).

The implementation of CEA during decision making and recourses-allocation will enable transparency, consistency and positively enhances healthcare policies. Inevitably this will clarify what policy-makers regard as an “acceptable threshold” of CEA below which they will make a medical intervention available to their citizens.

Health economic evaluation is based on the recognition of the effectiveness of an intervention but not the limiting factor as decision-making also requires considering the cost to the tax payers. The implementation of CEA in healthcare decision making reduces the burden of responsibility upon those who previously made decision based on instinct and professional opinion (Devlin N, 2004).

1.14.3.1 Cost-effectiveness analysis

A cost-effectiveness analysis itemises the additional recourses consumed for an improvement in the effects for example survival or quality-adjusted life year (QALY) associated with a health intervention compared to another. It’s also involves the estimation of the cost and effect of an intervention and one or more alternative; once the cost and deference in effect is calculated it is then presented as ratio .i.e. the cost per unit of health outcome (Weinstein & Stason, 1977). Because this format is concerned with difference between two or more treatments or options, analyst refers to these evaluations as incremental costs, incremental effect, and the incremental cost-effectiveness ratio (ICER); for example, for treatment (a) and (b), we calculate their respective cost and effects, and we calculate the difference in cost and difference in effects. The effects of each event can be calculated using many different types of units for example IMA diagnostic test could be evaluated in term of cost and number of case detected per hundred patients screened. This format of analysis is designed to help decision–makers allocate resources across the health system. ICER is effectively a measure of the additional cost per unit of health gained. The underlying calculation for the ICER comparing IMA assay versus

IMA plus hs-cTnT for the diagnosis of AMI could be calculated using the following equation:

$$\text{ICER} = \frac{Average\ Cost\ (£) IMA - Average\ Cost (£)\ hs-cTnT}{Average\ Effect\ IMA - Average\ Effect\ \ hs-cTnT}$$

Patients and health commissioners may value a new diagnostic test despite the fact it does not contribute to clinical decision i.e. does not change treatment (Lee et al. 2010a). Schwartz et.al., found that 87% of adults favoured cancer screening even in the absence of effective treatment option (Schwartz et al. 2004). Policymakers may even consider adopting a "Soft threshold" which allows room for consideration of other preferences; it is problematic to deny a minority group with a rare disease new treatment based on cost.

Healthcare economic based on CEA is in my view is a balancing act between the need of the community and the available recourses after all tax payers' views should be considered.

1.14.3.2 The concept of threshold

The concept of "threshold" refers to the level of costs and effects that an intervention must achieve to be acceptable and implemented in a healthcare system. This is usually means ratio between monetary cost and measures of health gain in the denominator (Weinstein M, 1973). The recommended measure of health gain is usually expressed as Quality Adjusted Life-Years (QALY) which represent years of healthy life (Raisch, 2000). QALY is usually involves the cost utility analysis. A QALY considers longevity and quality-of-life. The number of QALYs accrued by a patient is estimated by multiplying the years of survival by quality of life measured on a scale from zero e.g. zero being dead to 1 been in good health.

Disability Adjusted Life-Year (DALY) was developed by the World Bank and WHO to estimate the burden of disease on society. DALY is a health outcome with two components the duration of life time lost due to premature death (year of life lost (YLL)) and the reduction in quality of life due a disability (years of life with disability (YLD)) (Murray 1994). However, WHO base their CEA assessment on cost per DALY, though the method used to calculate the values for DALY has been questioned (Melse JM, 2000).

DALY's primary scale is 0-1, with 0 perfect heath and no disability and 1 death. The duration of life lost due to premature death is calculated by comparing actual age at death with standard life expectancy i.e. 82.9 for women 79.2 for men in the UK (Applied method of CEA in health-Gray, page 108-109).

Calculation in reduction of quality of life due to disability is based on six-level classification system, with weight allocated by expert for example class 2 those with limited ability to perform most activities in two of the following areas: recreation, education, procreation or occupation were given a weight of 0.40 so each year in the state will lose 0.40 DALY. The objective of DALY is to minimize the number of year lost were QALY to maximize the number of years a person accumulates (Applied method of CEA in health-Gray, page 108-109).

1.14.3.3 Cost-consequence analysis (CCA)

Involves the estimation of the cost and the consequences of two interventions, and the final decision on the right method is left to the decision-maker. This model assumes that the decision-maker known what they are doing i.e. interpret and synthesis in some way. In addition, this model also assume that the decision-makers are the right people to decide how to prioritise different outcomes of an intervention i.e. lower cost but poorer health and long hospital admission, or better long-term quality of life but more frequent short-term complications. Because of the complex and knowledge required to make the right decision this model is rarely used.

1.14.3.4 Cost-minimisation analysis (CMA)

Cost-minimisation analysis model assumes that the health outcomes and the interventions are equal, and the objective is to evaluate the cost, to choose the lowest. Moreover, this format is usually used in parallel with a clinical trial that failed to demonstrate a clear outcome between two treatments. The problem with using CMA is that it assumes that failure to prove the hypothesis in a trial applies that the two treatments are equal. Moreover, CMA do not consider the statistical power used in that trial; it is possible to find some trials who may not have the right statistical power; as a result, the use of this type of format is usually infrequent and not appropriate for the current study as IMA assay detect ischaemia whereas, troponin is used to assess AMI.

1.15.1 Decision analytic modelling: Markov models

1.15.1.1 Markov models: what are they?

Markov models (MM) analyse uncertain process over time. There are used when decision and timing of events is important and when events may happen more than ones for example in SA attacks in ACS. MM also is suited for modelling long term disease outcome, where cost and effect are spread over long period of time i.e. chronic disease or situation where event are likely to occur i.e. AMI. MM also suited in studies assessing the cost effectiveness of disease management including the diagnostic efficiency of the introduction of IMA plus hs-cTnT in an ED setting.

MM comprises a set of health states in which an individual can be found i.e. SA. These states are framed in a way that at any given time patients can only be in one health state; thus, all individuals for example with undifferentiated chest pain can be categorised into one health state (Figure 7). In this project the health state of interest is concerned with patients presenting with chest pain suggestive of AMI i.e. NSTEMI. Thus, there is no need to examine actual AMI patients or UA patients as these patients will be considered an emergency and treated accordingly. MM is concerned with transition during a series of

cycles consisting of short time intervals. Moreover, individuals within given state are assumed to be identical this is the limitation of MM, because the transition probability only depend on the current health state and not changing health state- this is known as the Markovian assumption (Athanassoulis, 2015). It must be noted that the exact transition times between disease states are generally difficult to observed, and only a proportion of individual disease histories is usually observed for example in renal patients or in certain cancer. Thus, the transition of disease states in this project is based on blood biomarkers changes, because if patient's history and ECG changes are consistent with AMI patients will start treatment immediately irrespective of blood biomarkers results.

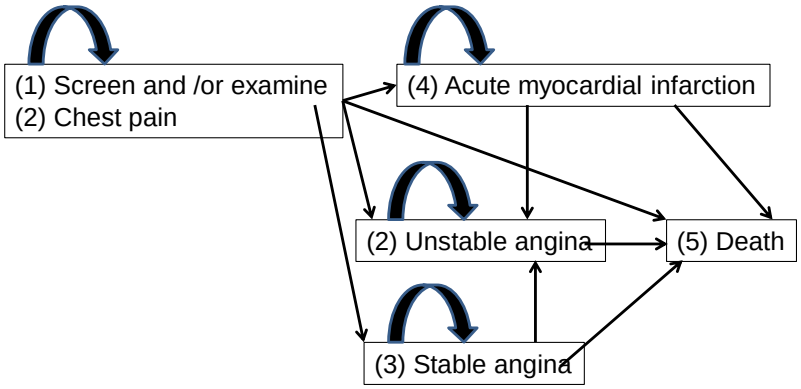

Figure 7: Markov model (b) transition between sate are indicated by the arrows. Adapted from Kondo et al. Clin Exp Nephrol 2012.

1.16 Summary

CEA according the World Health Organisation (WHO) recommend that the CEA should be tree time the gross domestic product (GDP) per capita (Kondo et al. 2012). However, the application of CEA to the concept of threshold is controversial because of the type of cost that should be included; it has been argued that CEA should not include just the direct cost of the disease but also the total resources including the consequences resulting from mortality. Thus, CEA ratio should include the measures that increase life expectancy rather than improve the quality of life (Johannesson M, 1996). Currently there is no global consensus on the definition outlining all cost that should be included in CEA. The adoption of a threshold by healthcare system usually adopts an explicit threshold where by a group of decision-makers formally adopt and make public in advance any threshold by which their decisions on recourse allocation would be framed. On the other hand, policymakers may adopt and implicit threshold which are not official or public. In the UK, implicit threshold are often challenged and undermine public confidence especially were a community is denied a given treatment (George B, 2001). It reasonable to say that explicit threshold is not immune from challenges; thus policymakers need to meet the need of their community in a fair way (Hans-Georg, 2004).

The research presented in this thesis is an attempt to provide an empirical-based quantification of the costs the NHS faces when considering whether health benefit associated with new technologies are expected to offset health care cost that may be utilised elsewhere. Early identification and correct diagnosis of patients presenting with chest pain suggestive of AMI may save recourses (table.6) including hospitalisation e.g. bed occupancy, expensive treatments e.g. PCI, lifelong medication, lost revenue, social issues if the patient is the main “bread winner” and general wellbeing.

Table 6: Unit cost (adapted from BMJ)

Unit cost	Unit	Base-case value (£)
PCI	Procedure	1,410
CABG	Procedure	4,900
Repeat PCI	Per –Item	3,000
Angiogram	Procedure	750
Non- cardiac ward	Day	160
CCU	Day	240
Outpatients	Day	460
Cardiac day case	Visit	60
Non-cardiac day case	Visit	109
Guildwire	Visit	182
Stent	Item	62
Guilding catheter	Item	600
Blood	Item	37
Full blood account	Unit	85
Blood	Unit	4
Endoscopy	12.5 mg vial	246
Tirofiban	20 mg vial	146
Eptifibatide	75 mg vial	49
Clopidogrel	28 tablet pack 75 mg	35

NICE has been reluctant to specify a single cost effectiveness threshold used in its decision. It has also argued that factor other than CEA are taken into consideration by various advisory committees. Since 2004, the NICE guidelines use a threshold range of £20,000 to £30,000 QALY gained. The latest guideline indicates that a threshold of less than £20,000 is likely to lead to recommendation unless the evidence for it adoption is not robust or uncertain (Devlin N, 2004). Of course, mortality is a more relevant outcome indicator for some interventions e.g. ACS than for others for example epilepsy

and for this reason NICE would expect a better evidence before a new intervention is applied. It is apparent that if IMA plus hs-cTnT assay could identify AMI patients and reduce the mortality, a rate threshold greater than £20,000 may be justified.

1.17 Summary review of cardiac biomarkers

In modern medicine cardiac biomarkers play an important role in the diagnosis, risk assessment, management and treatment of patients. The introduction of new cardiac biomarkers has been increasing and evolving since 1950s (Figure.8). One of the earliest cardiac biomarkers was alanine amino-transferase (AST) followed by myoglobin and creatine kinase (CK); since the introduction and the development of cardiac troponin (cTn) these biomarkers has fallen out of use.

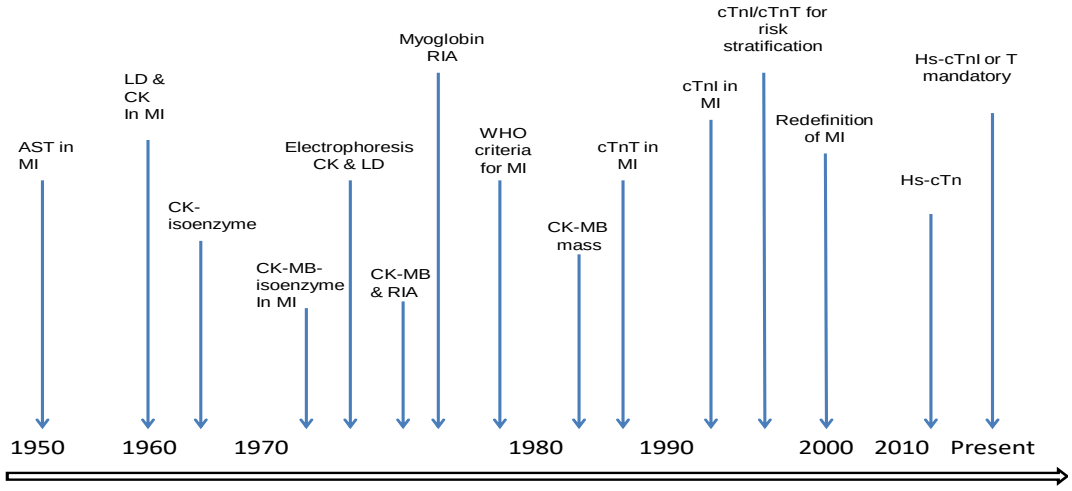

Figure 8: Historical progressions of cardiac biomarkers used to assess acute coronary syndrome, myocardial infarction (MI), Aspartate transaminase (AST); creatine kinase (CK); creatine kinase-MB isoform (CK-MB); radioimmunoassay (RIA); cardiac troponin I (cTnI), cardiac troponin T; (cTnT); lactate dehydrogenase (Ld); WHO, World Health Organisation. (Courtesy of David Gaze. St George's NHS Trust. London).

The importance of cardiac biomarker testing has been widely accepted as part of the initial assessment of patients presenting with an unexplained chest pain suggestive of ACS. ACS pathophysiology is complex, thus presently it is unlikely that a single biomarker could be used to evaluate cardiovascular associated risks and for ACS diagnosis (Bali,Cuisset,Giorgi *et al.*, 2008). Numerous evidences oppose to the single biomarkers use in an ED setting or in a chest clinic (Bali,Cuisset,Giorgi *et al.*, 2008; Lin,Yokoyama,Rac *et al.*, 2012). The current guidelines propose that clinician should tailor cardiac biomarkers for NSTEMI patients based on prognostic ability such as that with cTn (Braunwald,Antman,Beasley *et al.*, 2002).

1.18 Conventional cardiac biomarkers

1.18.1 Myoglobin

Myoglobin is a 17.8 kDa cytoplasmic haem protein found in striated muscles, it constitutes 2% of the total muscle protein. Myoglobin facilitates oxygen storage for the oxidative phosphorylation in the muscle fiber. Myoglobin evaluation is considered as a primary biomarker for monitoring rhabdomyolysis patients, accidental trauma and until decade ago for AMI (Lindsay,Carr,Draper *et al.*, 2015). Myoglobin is a non-specific cardiac biomarker and can be present in a range of conditions including severe renal insufficiency, alcohol abuse and skeletal muscle trauma (Gilkeson et al., 1978) (Mair et al., 1992). The relationship between myoglobin release to the circulation and AMI was first observed by Kagen et al., in 1975 (Kagen,Scheidt,Roberts *et al.*, 1975). Following an AMI myoglobin is elevated in the circulatory system within 1-2 h and peaks between 6 and 9 h before returning to normal within 24 h. Although, myoglobin is useful in the early detection of AMI, it still has a major limitation including its rapid clearance, thus potentially misdiagnosing patients presenting > 24 h after AMI. Patients presenting late > 24 h after their acute phase may have a falsely negative results within the normal range (Grenadier,Keidar,Kahana *et al.*, 1983).

A meta-analysis has shown that the clinical sensitivity of myoglobin for the diagnosis of AMI has reached 90% with serial sampling (Balk,Ioannidis,Salem *et al.*, 2001). However, the poor specificity is considered to limit myoglobin clinical use as biomarker in the ED setting to rule-in or rule-out AMI. Despite poor specificity the ESC, the National Academy for Clinical Biochemistry and the ACC, has recommended the used of myoglobin as an early cardiac biomarker when troponin is not available (Alpert,Thygesen,Antman *et al.*, 2000).

1.18.2. Creatine kinase

Creatine kinase (CK, E.C number 2.7.3.2) has a molecular weight of 86 kDa and exists as a dimer of two subunits, B and M. CK isoforms molecular weights varies between 39 to 42 kDa and are synthesized in various tissues and cell types such as the brain and skeletal muscle. Their role is to catalyze the transfer of high-energy phosphate from ATP to creatine. Three different dimeric CK isoforms exist including CK-MB, CK-MM and CK-BB. The CK-MM is mainly found in striated muscle including the heart. The CK-MB isoform is predominantly found in the myocardium with only 1-3% located in skeletal muscle. The CK-BB isoform is found only in the brain (Tsung, 1976). Each subunit has a dedicated gene that encode for either the M or B subunit (Villarreal-Levy,Ma,Kerner *et al.*, 1987). Total creatine kinase (CK) activity refers to the cumulative activity of all three isoforms in a given sample.

The discovery of substantial amount of CK-MB in the myocardium made it the most used cardiac biomarker in 1980s (Saenger & Jaffe, 2008). The relative specificity of CK-MB refers to the ratio of CK-MB/CK-MM isoforms. Relative increase of CK-MB concentration to values > 3-5% of total CK may indicate cardiac origin. Until the last decades CK-MB isoform was the primary biomarker for the diagnosis of AMI and was considered the “Gold Standard” (Lee & Goldman, 1986).

The advantage of small molecules such as CK-MB is that they are rapidly cleared from the circulation by renal system, and usually detected in the circulation in a small and predictable concentration; as a such a rapid increase of concentration of this analyte during AMI is clinically useful and noticeably detectable. During AMI, serum CK and CK-MB begins to increase within 4-8 h and reaches peak concentration around 24 h and it return to baseline within 2 to 3 days (Ellis, 1991). Increased CK-MB concentrations does not necessarily translate into AMI; in fact it could indicate potential myocardial injury (Saenger & Jaffe, 2008).

After the introduction of a very sensitive (level of detection (LoD) < 1 µg/L) automated immunoassays known as mass assays. The measurement of CK-MB using highly specific monoclonal antibody against MB dimer including M and B subunit; this has improved assay sensitivity to 50% at 3 h and 80% at 6 h and 90-100% at 10-12 h after the onset of chest pain suggestive of AMI (Lee,Rouan,Weisberg *et al.*, 1987). However, highly sensitive CK-MB (mass) assay comes at the expense of an increased detection and frequency of CK-MB concentration due to skeletal muscle injury and or renal failure (Mair,Artner-Dworzak,Dienstl *et al.*, 1991). As a result an estimated 20% of false negative due skeletal muscle injury or renal failure is recorded (Robbins,Epstein & Shah, 1997). In addition, CK-MB estimation significantly varies among platforms due to lack of CK-MB standardization (Christenson,Vaidya,Landt *et al.*, 1999).

After the introduction of the high sensitivity cardiac troponin (hs-cTn) and the revision of the guidelines for diagnosing AMI in 2000 and the subsequent update in 2007; CK-MB as the biomarkers for AMI has been replaced with hs-cTn for AMI patients' management (Alpert,Thygesen,Antman *et al.*, 2000; Thygesen,Alpert,White *et al.*, 2007). Recent guidelines published in 2014 by AHA and ACC clearly stated that CK-MB do not provide additional value for the diagnosis of ACS (Amsterdam,Wenger,Brindis *et al.*, 2014). In contrast, the ESC and the ACC state that any elevation above the reference range of troponin or CK-MB isoform is evidence of myocardial necrosis and that the patient should be classified as having AMI (Anderson,Adams,Antman *et al.*, 2007). The reason for this is that cardiac troponin remains high for days whereas CK-MB return to base line within 24 h (Saenger & Jaffe, 2008). . CK-MB is still offered across the UK, as I can aid in the diagnosis of AMI.

1.18.3. Cardiac troponin

Troponin (80 kDa) is a complex molecule comprising of three protein isoforms including troponin I, troponin T and troponin C. These proteins are housed in the sarcomeric filaments and are essential for contraction and relaxation in

both cardiac and skeletal muscle (Figure 9). The cardiac and skeletal isoforms of troponin T and troponin I; are encoded by separate genes (Anderson,Greig,Mark *et al.*, 1995). Interestingly, despite the difference in opinion an estimated 5-10% of free form of the total cardiac troponin I (cTnI) and cardiac troponin T (cTnT) is believed to be present in the cytosol (Katus,Remppis,Scheffold *et al.*, 1991; Thygesen,Mair,Katus *et al.*, 2010). The implication of free cTn detection revolutionizes our understanding of the presence of cTn in healthy population and this can be utilised as a biomarker of cardiac ischaemia.

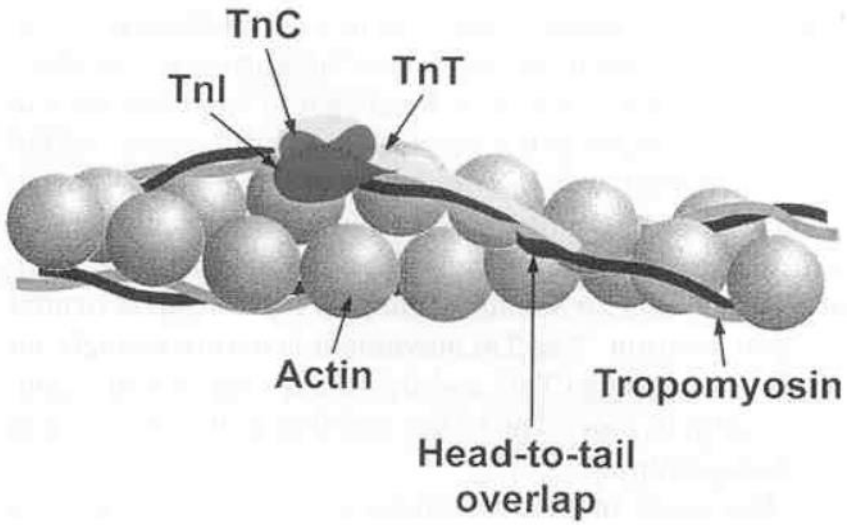

Figure 9: Regulatory cardiac troponin complex; the three subunits are troponin T, I and C. Troponin T binds and attach troponin complex to tropomyosin; whereas troponin I modulate the interaction of acting and myosin by inhibiting actomyosin adenosine triphosphate activity, and finally troponin C act as a calcium binding subunit of the troponin complex (courtesy of Clinical Biochemistry).

Cardiac troponin T and I molecule is highly specific for the myocardium and contains a unique amino acid sequence that are translated by a dedicated

genes (Sheng & Jin, 2016). This, unique amino acid sequence of cardiac isoform cTnT and cTnI allows the generation of highly specific monoclonal antibodies and a very reliable mode of detection of these isoforms after myocardial injury (Jaffe, 2001; Smith et al., 2013; Regwan et al., 2010).

False-positives are known to exist in a condition where heterophilic antibodies are present, this antibodies effect only non-standardized cTnI immunoassays (Panteghini, 2009). Using the current generation high sensitivity cardiac troponin (hs-cTnT) immunoassay in patients with skeletal muscle disorders has raised concerns regarding cardiac-specificity. A controversial study found that some patients with skeletal myopathies but with no evidence of myocardial injury or raised CK-MB appear to exhibit a potential re-expression of cTnT isoforms or immunoreactivity to cTnT immunoassay (Jaffe et al., 2011). Generally, cTnT or cTnI are considered the biomarkers of choice for the diagnosis of AMI worldwide. Utilisation of cTn evaluation in clinical settings complement clinical assessment and ECG in the diagnosis, risk stratification and treatment of low risk patients and those with suspected NSTEMI (Thygesen et al., 2010; Newby et al., 2012). A series of meta-analysis and various studies confirmed that the estimation of cTnI or cTnT in patients with ACS has a superior diagnostic and a prognostic value than myoglobin and CK (Balk, Ioannidis, Salem, Chew, & Lau, 2001; Olatidoye, Wu, Feng, & Waters, 1998; Thygesen,Mair,Giannitsis *et al.*, 2012).

The kinetic release of cTnT and cTnI concentrations after myocardial injury begin to rise between 3-6 h after the onset of AMI and reach a maximum peak concentration after 12-24 h (Collinson, 1998). The cTnI or T in AMI patients usually shows a peak concentration within the first day after the onset of symptom and persists for approximately a week (Katus,Remppis,Scheffold *et al.*, 1991; Michielsen,Diris,Kleijnen *et al.*, 2007).

The Universal Definition of Myocardial Infarction evaluated the use of cardiac troponin in patients presenting with chest pain suggestive AMI as a mandatory. The 99th percentile upper reference limit (URL) as established in apparently

healthy individuals, has been recommended as the clinical decision threshold for the diagnosis of AMI. It must be noted that the definition and standarisation of healthy individuals has not been properly defined and is still debated (Chenevier-Gobeaux,Bonnefoy-Cudraz,Charpentier *et al.*, 2015; Keller,Ojeda,Zeller *et al.*, 2013; Panteghini, 2009).

In recent years the sensitivity of cTn immunoassays has improved considerably and a new generation of hs-cTn immunoassay has been introduced to the market. The introduction of these new improved hs-cTn immunoassays has the potential to speed up triage and treatment of patients presenting to the ED with chest pain suggestive of AMI (Apple, 2011; Reichlin et al., 2009; Keller et al., 2009).

This new generation of hs-cTn can only be described as such if they comply with the 2007 guidelines of the universal definition of myocardial infarction (Thygesen,Alpert,White *et al.*, 2007) and can demonstrate the following:

1) < 10% total imprecision at the 99th percentiles of a reference population
2) Able to quantitate (limit of detection (LoD)) at least 90% of healthy individuals (Apple, 2009; Thygesen,Alpert,Jaffe *et al.*, 2012).

The value of using the 99th percentile as a diagnostic cut-off of AMI was confirmed by several studies (Keller,Zeller,Peetz *et al.*, 2009; Reichlin,Hochholzer,Bassetti *et al.*, 2009). Currently most laboratories in the UK offer hs-cTn as the first diagnostic cardiac biomarker for patients admitted to the ED with chest pain suggestive of AMI. Commercially available hs-cTn immunoassays now achieve an analytical sensitivity of up to 100 times greater than conventional troponin assays (1 ng/L versus 100 ng/L) (Apple, 2009; Chenevier-Gobeaux,Bonnefoy-Cudraz,Charpentier *et al.*, 2015). In addition, measuring hs-cTn allows early detection of cardiac injury as soon as the cardiomyocyte membrane permeability occurs (de Lemos, 2013).

Since the introduction of hs-cTn immunoassay the sensitivity has increased from 83% (95% CI, 76-90%) to 95% (95% CI, 90-98%), while the specificity

decreased from 93% (95% CI, 91-95%) to 80% (95% CI, 77-83%) (Reichlin et al., 2009). These assays are now capable of detecting myocardial damage in healthy populations (Saenger et al., 2011; Todd et al., 2007). The significance of detectable hs-cTn in healthy population is unclear (Carlton,Gamble & Greaves, 2013). However, this increased sensitivity of hs-cTn immunoassay comes at the cost of decreased specificity and the power to rule-in AMI (Wu, Lu, Todd, Moecks, & Wians, 2009). Other studies supported this observed general decrease in hs-cTn specificity (Januzzi, Jr. et al., 2010; Kavsak, Wang, Ko, MacRae, & Jaffe, 2009).

To improve the specificity of hs-cTn and its diagnostic utility the current guidelines require serial blood sampling; ideally blood should be measured for hs-cTn concentration at the first sign of chest symptom and 3 h after admission (Hamm et al., 2011). Repeated hs-cTn measurement in the second time maximizes the safety and certainty to rule-in or rule-out an AMI. The measurement of the early absolute changes (second) of hs-cTn within 1 h can be used as a surrogate for 3 or 6 h to rule-in or rule-out diagnosis of AMI in NSTEMI patients (Thygesen et al., 2012c).

The ESC guidelines recommend a single hs-cTn measurement where NSTEMI patients present with post 6 h chest pain and a GRACE score of < 140 (Hamm et al., 2011). The ESC also recommends that if the patient still shows signs suggestive of ACS a third hs-cTn measurements may be required. The reason for this is that the presence of hs-cTn is not specific enough for diagnosing AMI, and especially patients with excising cardiovascular co-morbidities such as heart failure and renal failure (Reiter et al., 2011).

Although, serial measurements of hs-cTn at admission and 3 h is useful; some evidence suggests these guidelines are not fully followed by professionals for various reasons. Notably, the lack of clear definition of rise and a fall (delta changes) of hs-cTn concentration has led many clinicians to adopt a change of ≥ 20% as a practical cut-off. Conversely, another study has showed that cut-off value of ≥ 20% needed to be reassessed and increased in patients with

hs-cTn in a lower range, older patient, and in-patients with existing co-morbidities such as renal failure (Apple, 2009; Giannitsis,Becker,Kurz *et al.*, 2010). The integration of ≥ 20% delta rules over 2 h period has increased specificity to 92.4% (95% CI, 90-94%) but reduced sensitivity to 56% (95% CI, 48-63.2%) (Aldous,Pemberton,Richards *et al.*, 2012).

The full integration of hs-cTn has yet to be demonstrated in terms of its diagnostic utility, risk stratification and above all the implication of the presence of hs-cTn for healthy population. The implementation and the introduction of hs-cTn have multiple advantages provided that there is a revised algorithm outlining the management of the ACS patients. Most AMI patients will show substantial cTn concentration changes; however, cTn concentration change is not a diagnostic of AMI but an indication of myocardial injury (Hamm et al., 2011; Thygesen et al., 2012a). It is clear, that clinicians interpretation of hs-cTn differ when considering clinical context (Newby,Jesse,Babb *et al.*, 2012).

1.19 Novel biomarkers in the assessment of acute coronary syndrome

1.19.1 B-type natriuretic peptide

Natriuretic hormones belong to the family of vasoactive peptides that regulate arterial and venous dilation. B-type natriuretic peptide (BNP) is produced by the myocyte as a pro-hormone (Figure 10). BNP consist of 32 amino acids (AA) which are release to the circulation in response to ventricular dilatation and hemodynamic stress (Christenson & Tang, 2006; Levin,Gardner & Samson, 1998).

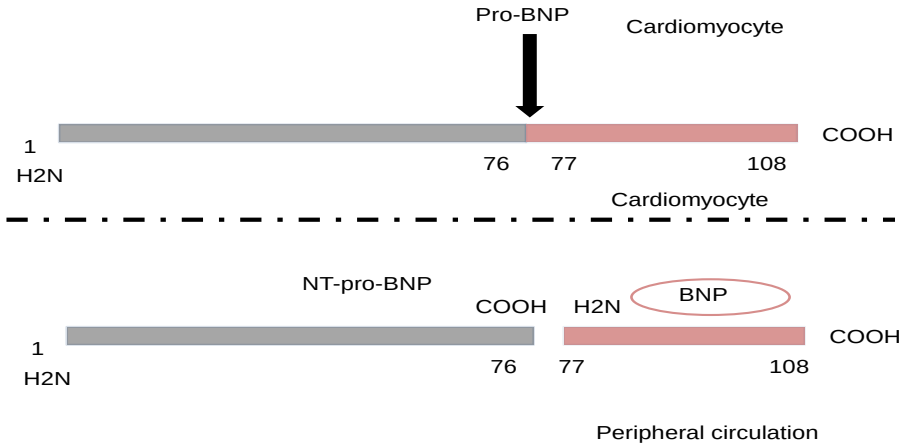

Figure 10: B-type natriuretic peptide molecular processing. NT-pro-BNP the stable portion of B-type natriuretic peptide is cleaved at amino acid position 76.

In the circulation the pro-hormone is cleaved into BNP the physiologically active form and into the N-terminal fragment of the NT-pro-BNP. BNP exert its vaso-relaxant effect through its interaction with the rennin-angiotensin-aldosterone system. The clearance of NT-pro-BNP from the circulation is three times lower than BNP, making it ideal for monitoring congestive heart failure (CHF) and acute heart failure (AHF) patients. These patients will typically show a minor variation in concentration which can be readily detectable (Buckley,Marcus,Yacoub *et al.*, 1998). Currently assay for both BNP and NT-pro-BNP are commercially available.

The measurement of BNP concentration is mainly used to assess left ventricular pressure and act as an aid in the diagnosis of CHF (Qi,Mathisen,Kjekshus *et al.*, 2001; Roberts,Ludman,Dworzynski *et al.*, 2015). However, elevated BNP concentration is also present in ACS and is associated with high risk of adverse cardiac events (Arakawa,Nakamura,Aoki *et al.*, 1996). The mechanism of BNP release in ACS patients is not fully understood. One explanation is that ischaemic attack during or pre-AMI may

contribute to the heart wall stretch inducing the neurohormonal activation (Marumoto,Hamada & Hiwada, 1995). This hypothesis is supported by the detection of BNP gene expression in ischaemic and infarct region of the heart (Ndrepepa,Braun,Schomig *et al.*, 2007). During artery bypass grafting cardiac biopsies tissue show that BNP is over-expressed in ischaemic myocardium in comparison to non-ischaemic regions in STEMI (Ruck,Gustafsson,Norrbom *et al.*, 2004).

AHF reflects the cardiomyocyte integrity and it is usually associated with myocardial injury and elevated cardiac troponin. TACTICS-TIMI 18 trial (Treat Angina with Aggrastat and Determine the Cost of Therapy with Invasive or Conservative Strategy - Thrombolysis in Myocardial) found that NSTEMI patients with elevated BNP concentration had more severe stenosis (Sadanandan,Cannon,Chekuri *et al.*, 2004). BNP release involving undamaged myocytes may have an advantage in assessing the physiological consequences of ischaemia and AMI (Wiviott,de Lemos & Morrow, 2004). Increased concentrations of BNP are associated with larger infarct size and poor prognosis in patients with STEMI (Arakawa,Nakamura,Aoki *et al.*, 1996). Elevated BNP concentration was also found in older patients and patients with renal dysfunction; the exact mechanism of this is unclear (McCullough,Duc,Omland *et al.*, 2003; Redfield,Rodeheffer,Jacobsen *et al.*, 2002). NT-pro-BNP and hs-cTn evaluation for patients presenting to the ED with chest pain suggestive of ACS may add an incremental value in term of patients risk stratification, clinician informed decision regarding treatments and diseases monitoring (Ketchum & Levy, 2011). However, how to estimate the risk utilising NT-pro-BNP and hs-cTn evaluation is unclear (Ketchum & Levy, 2011).

Several studies concluded the measurement of BNP or NT-pro-BNP, day 2 to 5 after AMI is strongly associated with long and short-term mortality (Omland,Aakvaag,Bonarjee *et al.*, 1996; Richards,Nicholls,Yandle *et al.*, 1998). The Acute Decompensated Heart Failure National Registry (ADHERE) which includes information about 84,872 patients with AHF and

cardiac troponin (cTn) concentration on admission; found that 6% of this cohort had an elevated cTn (Fonarow,Peacock,Horwich *et al.*, 2008). However, no correlation exists between elevated BNP concentration and cTnT (Yamamoto,Sato,Yasutake *et al.*, 2006). Currently NT-pro-BNP is not recommended for diagnosis of AMI across the UK.

1.19.2 Copeptin

Copeptin was first described by Holwerda in 1972 (Holwerda, 1972). Arginine vasopressin (AVP) is a vasoactive neuropituitary hormone secreted in the hypothalamus. AVP is one of the main hormones involved in the hypothalamic-pituitary-adrenal axis; it is derived from a 164 amino-acid precursor, pre-pro-vasopressin that comprises AVP, signal peptide, copeptin and neurophysin 2 (Figure 11) (Brownstein, Russell, & Gainer, 1980). Copeptin separation from the AVP protein is a result of a multi-step enzymatic processing, reshaping of the precursor molecule, the cleavage of the signal molecule and finally the separation of the neurophysin II from copeptin (Acher,Chauvet & Rouille, 2002; Morgenthaler,Struck,Jochberger *et al.*, 2008).

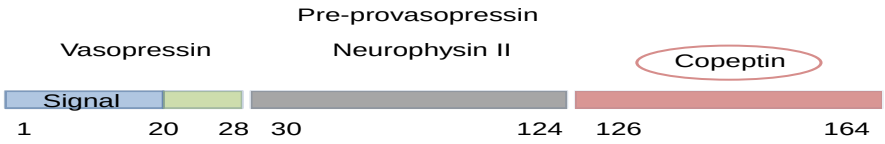

Figure 11: Structure of the vasopressin pre-pro-hormone consisting of the signal peptide, the vasopressin hormone, the carrier protein neurophysin II and the glycoprotein copeptin. The numbers indicate the amino-acid position where the cleavages occur. Copeptin consists of 39 amino-acid.

The AVP released from the neurohypophysis into the circulation is primarily to induce water conservation by the kidney. Although, the main secretion of AVP is in response to hyperosmolarity, it can also be secreted in response to endogenous stress including hypoxia, acidosis, infection and AMI (Reinstadler, Klug, Feistritzer, Metzler, & Mair, 2015, Lukaszyk & Malyszko, 2015).

In vivo, 90 % of AVP is bound to circulating platelets, AVP has a short life of 10 to 30 min. the rate of degradation means that AVP cannot be clinically measured (Balanescu,Kopp,Gaskill *et al.*, 2011). Copeptin is a stable glycol-peptides consisting of 39 AA located in the C-terminal portion of the precursor pre-pro-vasopressin (pre-pro AVP) and can easily be measured. Copeptin secretion is directly stimulated by the activation of AVP system. Therefore, its measurement directly reflects AVP status and acts as a surrogate biomarker for AVP.

Establishing the exact time of the onset of myocardial ischaemia is very difficult; because of patient related delay prior to hospital admission (Liebetrau

et al., 2013b). Copeptin concentration is significantly increased after 30 min and 90 min from 16.0 pmol/L; IQR, 13.4-20.2 pmol/L and 31.9 pmol/L; IQR, 16.4-117.1 pmol/L respectively and return to baseline after 24 h (Liebetrau,Nef,Szardien *et al.*, 2013).

NICE has recommended the use of copeptin in conjunction with cTn and ECG in the diagnosis of AMI. NICE suggested that patients presenting to ED with chest pain suggestive of AMI could be discharged if copeptin, cTn and ECG are all negative. Two recent controlled trials have showed that low risk patients can be safely discharged if both copeptin and troponin were negative (Maisel,Mueller,Neath *et al.*, 2013; Mockel,Searle,Hamm *et al.*, 2015). The combined use of copeptin and hs-cTn may exclude the need for serial blood sampling, prolonged monitoring of patients and could be cost effective strategy in diagnosis of AMI (Reichlin,Hochholzer,Bassetti *et al.*, 2009). However, due to copeptin low specificity; its measurement cannot replace serial measurement of cTnT to rule in or to rule-out AMI (Nemec,Koller,Nickel *et al.*, 2010; Nickel,Bingisser & Morgenthaler, 2012). Thus, despite the NICE recommendation copeptin is not routinely offered as a part of the diagnosis of AMI in NHS hospitals across the UK. The current ACS/AMI management protocol is unlikely change until prospective studies such as RCT have been conducted and demonstrated that the addition of copeptin and hs-cTn can safely rule-out this cohort of patients (Lipinski,Baker,Escarcega *et al.*, 2015).

1.19.3. Heart fatty acid binding protein

Fatty-acid binding proteins (FABP) also known as mammary-derived growth inhibitors are intra-cellular proteins expressed in tissue with active fatty acid metabolism such as in the heart and liver (Viswanathan, Hall, & Barth, 2012). Nine phenotypes of the FABP family have being identified; currently each FABP is referred to according to the origin of it synthesis for example adipose tissue (A-FABP), liver (L-FABP), intestine (I-FABP) and heart tissue (H-FABP). The H-FABP content is approximately 80%-90% lower in skeletal muscle than

that in heart muscle; and the amounts in the liver, brain and small intestine are insignificant (Ishii,Ozaki,Lu *et al.*, 2005).

H-FABP is a low molecular weight (15 kDa) cytoplasmic protein; that occupies 5-15% of cytosolic protein of the cardiomyocyte (Charpentier et al., 2010). H-FABP participates in fatty acid metabolism by transporting fatty acid from the cell membrane to mitochondria for oxidation. H-FABP also protect against free radical accumulation during myocardial ischaemia (Jones,Prasad & Das, 1990) and is the main source of energy in the heart accounting for 10% of the total body turnover of fatty acid (Fournier & Richard, 1990).

The first evidence to suggest that H-FABP can be used as cardiac biomarkers for the diagnosis of ischaemic heart disease was made in the 1990s when two independent groups showed that H-FABP concentration was significantly increased after AMI (Kleine,Glatz,Van Nieuwenhoven *et al.*, 1992; Tanaka,Hirota,Sohmiya *et al.*, 1991). Despite this discovery initial studies performed using a non-specific polyclonal antibody assay were disappointing (Kilcullen,Viswanathan,Das *et al.*, 2007). As a result, only few studies were conducted, examining the role of H-FABP as an early biomarker of myocardial injury. Since then most studies have focused on comparing the diagnostic efficiency of H-FABP and that of cardiac troponin (Bruins Slot,Reitsma,Rutten *et al.*, 2010).

H-FABP low molecular weight allows an early release of this protein into the circulatory system before larger molecules such as troponin. Therefore, H-FABP is used as an early biomarker of ischaemia (Chan,Sanderson,Glatz *et al.*, 2004). Conversely, small molecules such as H-FABP and myoglobin readily pass through the glomerular membrane, reabsorbed and metabolized in tabular epithelial cells, thus patients with renal disease may present with a falsely elevated H-FABP concentration (Tanaka,Hirota,Sohmiya *et al.*, 1991). Older patients with normal renal function are also known to have higher concentration of H-FABP compared to younger adults (Bathia,Carless,Viswanathan *et al.*, 2009).

During myocardial injury H-FABP is detected in the circulation within 30 min to 1 h, reaches peak concentration within 6 to 8 h; and return to baseline around 24 to 36 h (Kleine,Glatz,Van Nieuwenhoven *et al.*, 1992; Pelsers & Glatz, 2005).

Chest pain patients must be assessed for other disease in order to establish the sources of H-FABP including skeletal muscle, brain, testis and ovaries (Servonnet,Delacour,Dehan *et al.*, 2006). The ratio of plasma concentration of myoglobin and H-FABP appears to be useful in differentiating H-FABP due to myocardial injury from that of skeletal muscle injury (Van Nieuwenhoven,Kleine,Wodzig *et al.*, 1995; Yoshimoto,Tanaka,Somiya *et al.*, 1995). Since myoglobin is no longer offered in the most UK laboratories, utilisation of the ratio calculation is less likely to be adopted in an ED setting. Myoglobin to H-FABP ratio may be subject to interference by the patient's renal clearance capacity and is therefore unreliable.

Experimental studies demonstrated that H-FABP is down regulated during ischaemic stress; the heart substitute its energy supply from fatty acid to glucose metabolism (Arumugam,Sreedhar,Thandavarayan *et al.*, 2016). The ability of the heart tissue to switch energy utilisation from H-FABP to glucose during ischaemia may lower the diagnostic efficiency of H-FABP determination especially in cardiac ischaemic patients.

Meta-analysis suggest that H-FABP testing as a standalone test did not fulfill the diagnostic criteria in terms of sensitivity, specificity and the predictive values for a safe rule-out protocol in patients suspected of AMI (Bruins Slot,Reitsma,Rutten *et al.*, 2010). The combination of H-FABP and cTn is shown to increase the sensitivity and the negative predictive value (NPV) for diagnosing AMI (Lippi,Mattiuzzi & Cervellin, 2013). In support of the meta-analysis finding, Carroll colleagues, reported that combining H-FABP and cTn increases the sensitivity from 42%-75% to 76%-97%, but at a price of reduced specificity from 94%-100% to 65%-93% (Carroll,Al Khalaf,Stevens *et al.*, 2013).

Published studies showed that the diagnostic efficiency of H-FABP lacks the require methodological design including the inappropriate use of study population; one study used STEMI cohort and other did not use hs-cTn (Bhardwaj,Truong,Peacock *et al.*, 2011; Body,McDowell,Carley *et al.*, 2011; Chan,Sanderson,Glatz *et al.*, 2004; Gururajan,Gurumurthy,Nayar *et al.*, 2010; McCann,Glover,Menown *et al.*, 2008). However, H-FABP testing may be useful as a risk stratification tool. The magnitude of the increase of plasma H-FABP concentration correlate with the size of the infarct (Bajaj,Rathor,Sehgal *et al.*, 2015).

H-FABP evaluation as a routine test in and ED setting is unlikely; as its estimation does not have an incremental value superior to that of hs-cTn. Thus, currently H-FABP is not offered by NHS as a test for the diagnosis of AMI.

1.19.4 Ischemia modified-albumin

Human albumin (66.7 kDa) is a non-glycosylated single chain polypeptide containing 585 AA. It is the most abundant protein in the human body (35-50 g/L) and exerts a range of physiological and pharmacological functions including transport of small molecules such as drugs, fatty acids, and bilirubin and acts as an antioxidant (Evans, 2002; Rondeau & Bourdon, 2011). Human albumins (HA) occupy 50% of total plasma proteins and serve to regulate oncotic pressure. In physiological conditions HA exerts its buffering capability through its 16 histidine imidazole residues (Caironi and Gattinoni 2009;Colombo et al. 2012a;Pavone et al. 2010).

The liver is the main site of albumin biosynthesis. Traces of albumin mRNA have been detected in the kidney and pancreas, however, there is no evidence of protein translation. The presence and the origin of albumin in milk is still debatable (Peters T, 1996). On the other hand, some evidence demonstrates that a small amount albumin can also be produced in bone tissue and the microglia cells in brain (Yamaguchi et al., 2010; Ahn et al., 2008).

The circulation of albumin between the interstitial spaces the intravascular environment, the lymph drainage and the blood stream is a dynamic and continuous process which eventually causes albumin to degrade (10-12 g/24 h) ubiquitously at a rate equal to that of synthesis (Gattinoni, Carlesso, & Caironi, 2005). However, during cardiac ischaemia, albumin is subject to modification by free radicals and reactive oxygen species (ROS); inhibiting and altering its function to sequester Cu (II) ions through its N-terminus and inhibition of ROS formation. Modification of the N-terminus through acetylation or deletion of one or more residues makes cobalt or copper binding impossible. This damaged albumin is known as ischemia modified-albumin (IMA®).

IMA can be indirectly measured using the cobalt-binding assay (Bar-Or, Lau, & Winkler, 2000a). The IMA assay was cleared by FDA on the basis that it would help rule-out ACS, in low to moderate pre-test probability conditions with negative necrotic cardiovascular biomarkers such as cTn and a normal ECG.

The use of IMA is based on the premise that an early evaluation of this biomarker may predict the final diagnosis of AMI. IMA has being suggested as a biomarker for monitoring transient myocardial ischaemia induced by vasospasm (Cho,Choi,Kim *et al.*, 2007). IMA is not a cardiac specific biomarker and can be found in various conditions including, renal disease, infection, inflammation, and liver disease (Abboud,Labreuche,Meseguer *et al.*, 2007).

Diseases and conditions such as cardiac ischaemia, chronic inflammation, cancer and aging, are known to trigger copper release (Cu II) and redox activity which can cause cellular damage due to the generation of reactive oxygen species (ROS) including hydroxyl radical ($OH^{\bullet}$) and superoxide ($O_2^{\bullet -}$) (Halliwell & Gutteridge, 1990a). *In vivo,* a hydroxyl radical is converted to hydrogen peroxide (H_2O_2) and superoxide. Superoxide is directly converted to either hydroxyl radical via the Haber-Weiss reaction or converted to H_2O_2 via the Fenton reaction (Biaglow, Manevich, Uckun, & Held, 1997). Both reactions require transition metal such as copper and iron (Halliwell & Gutteridge, 1990b). While, iron concentration in human serum is greater than

copper, the latter is 60 times faster in generating hydroxyl radical than iron (Chevion et al., 1993). Moreover, Cu II ions, when mixed with ascorbic acid or other reducing agents produce hydroxyl radicals (Biaglow et al., 1997).

Copper is regulated in both non-specific exchangeable and specific non-reversible by a range of carrier plasma protein. This, includes caeruloplasmin that bind approximately 65% of copper, and albumin (Bar-Or et al., 2001a). Once the copper is release to the circulation during ischaemia, it disrupts the cellular energy causing the conversion of pyruvate to lactate and subsequent local acidosis (Figure 12). Localised acidic conditions causes caeruloplasmin to release more copper (Cu II) (Lamb & Leake, 1994).

HA and specially its N-terminus tetra-peptide (Asp-Ala-His-Lys) sequence is a non-exchangeable binding site for Cu (II). It is capable of sequestering Cu (II) ions and inhibiting the formation of ROS. HA prevents the conversion of sequestered Cu (II) to Cu (I) in the presence of reducing agents such as ascorbate (Marx & Chevion, 1986). Free Cu (I) can react with oxygen to form Cu (II) and generates superoxide free radicals ($O_2^{\bullet -}$). It is also possible that IMA also act as an antioxidant to reduce injury during reperfusion (Sinha,Gaze,Tippins *et al.*, 2003).

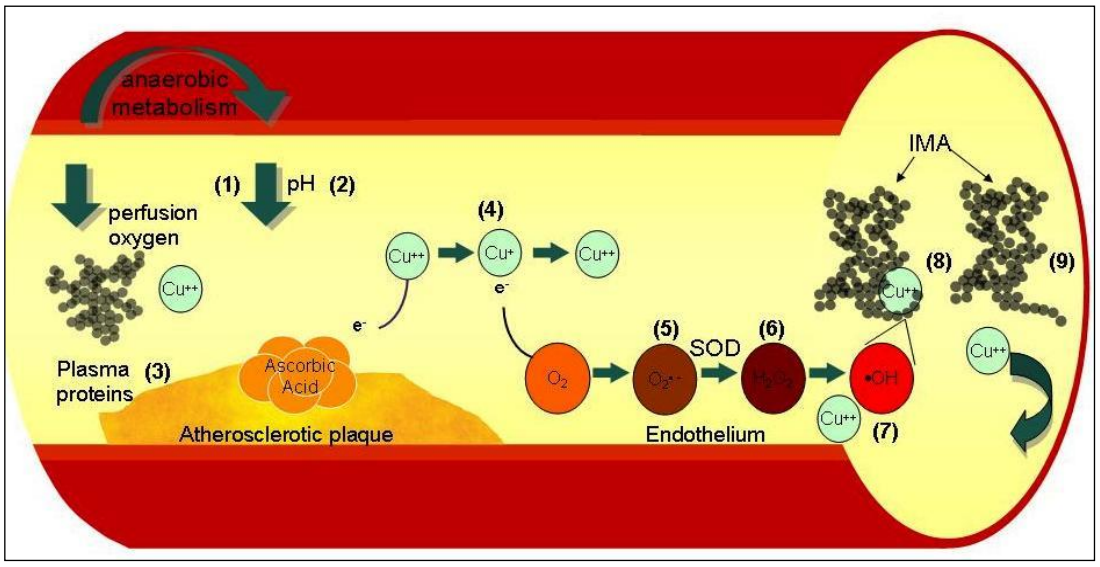

Figure 12: Mechanism of Ischaemia Modified Albumin generation. 1&2) Tissue hypoxia from anaerobic metabolism reduces ATP and causes a lower localized pH inducing acidosis. 3) Cu^{++} ions are released from plasma proteins such as caeruloplasmin. 4) In the presence of ascorbic acid, Cu^{++} is converted to Cu^{+}. Cu^{+} reacts with O_2 to form $O_2^{\bullet -}$. 5&6) Superoxide dismutase dismutates the $O_2^{\bullet -}$ to H_2O_2, which in presence of Cu^{++} or Fe^{++}, undergoes the Fenton reaction forming $OH^{\bullet}$ hydroxyl radicals. 7) Free Cu^{++} is scavenged by HA, where it binds tightly to the N-terminus. 8) $OH^{\bullet}$ radicals alter the amino acid N-terminus of HA rendering it incapable of binding Cu^{++}. 9). This altered form is known as IMA. (From: Human serum albumin. Japan. Otagiri (2013) p 357).

The generation of IMA through free radicals is widely accepted as the preferred theory; however, the exact mechanism is unclear. Albumin from other species such as canine and porcine have a different N-terminus configuration and cannot bind metal ions such as copper (Bal et al., 1998). A study found that 8 patients with increased IMA had 11 amino-acid residues at the NH_2 terminus when cleaved using rapid liquid-phase Edman degradation. They also found that 6 out 7 patients had a normal NH_2 terminal, except one non-ischaemic patient who appeared to lack two amino acid DA (Asp (D) or Asp-Ala (DA)) residues (table 7) (Bhagavan et al., 2003). This study concluded that the missing AA may due to the presence of an albumin variants. A group of an

independent researchers also found that some of these variants also exhibit amino acid changes in the pro-peptide, and the N-terminus (Kragh-Hansen, Brennan, Minchiotti, & Galliano, 1994). The N-terminal degradation *in vitro* is thought to occur because of pH and temperature changes. It is possible that this mechanism may also exist *in vivo* (Chan, Dodsworth, Woodrow, Tucker, & Harris, 1995b).

Table 7: Human albumin N-terminal amino acid sequences from ischaemic and non-ischemic patients. (Adapted from (Bhagavan et al., 2003).

Subject	**IMA Concentration (ABSU 470 nm)**	**NH_2-terminal HA Sequence of Human albumin**
Control (normal wild type)	0.35	DAHKSEVAHRF
Non-ischaemic patient	0.80	…..HKSEVAHRF
Ischaemic patient	0.76	DAHKSEVAHRF
Ischaemic patient	0.76	DAHKSEVAHRF
Ischaemic patient	0.74	DAHKSEVAHRF
Ischaemic patient	0.70	DAHKSEVAHRF
Ischaemic patient	0.93	DAHKSEVAHRF
Ischaemic patient	0.73	DAHKSEVAHRF

1.19.5 Kinetic and clinical utility of ischemia modified-albumin

Bar-or et al., (2001b) found that IMA concentration was significantly ($p < 0.001$) higher immediately after PTCA and return to base line after 6 h. A further study found that during PTCA procedure IMA rose within minutes, remained elevated at 6 h and returned to base line at 12 h (Quiles,Roy,Gaze *et al.*, 2003;

Sinha,Vazquez,Calvino *et al.*, 2006). IMA concentration was not significantly different from base line at 6 h and 24 h; this, suggests that IMA occur in the first few minutes of transient coronary occlusion (Bar-Or et al., 2001b).

The fluctuation of IMA concentrations has been found to correlate with the number of transluminal balloon inflations during PCI procedure (Quiles,Roy,Gaze *et al.*, 2003). Patients who undergo PCI procedures have a higher concentration of IMA following one hour of induced coronary ischaemia, and the concentration of IMA fall after 24 h to normal (Bar-Or et al., 2001b)(Hjortshoj, Dethlefsen, Kristensen, & Ravkilde, 2009). The rapid normalisation of IMA after PCI suggests a limited diagnostic window. Thus patients presenting to the ED after 3 h may not benefit from the NPV of IMA (Hjortshoj et al., 2009). No correlation was found between patients with post-PCI elevated IMA and other cardiovascular biomarkers such as CK-MB, myoglobin and conventional cTn (Bar-Or et al., 2001b).

Several studies demonstrated that exercise may induce fluctuation in IMA concentrations (Apple,Quist,Otto *et al.*, 2002; Falkensammer,Stojakovic,Huber *et al.*, 2007; Middleton,Shave,George *et al.*, 2006; Shave,George & Gaze, 2007; Zapico-Muniz,Santalo-Bel,Merce-Muntanola *et al.*, 2004). The effect of skeletal muscle on IMA concentration may increase the rate of false positives and subsequently limit is utilisation for the diagnosis of AMI.

Meta-analysis has shown that IMA has a 91% NPV for excluding AMI in an ED setting and 97% sensitivity when combined with cTnT and with normal ECG (Peacock et al., 2006). Various studies also supported that IMA has superior NPV of 96%, (95% CI, 91-98%) and could be safely used to rule out chest patients in an ED setting (Christenson,Duh,Sanhai *et al.*, 2001; Keating,Benger,Beetham *et al.*, 2006; Roy,Quiles,Aldama *et al.*, 2004), Govender, De, Delport, Becker, & Vermaak 2008). Although, IMA has a good NPV this does not necessary translate into practical triage tools in an ED setting, it NPV merely indicate the disease prevalence in the studied population (Sbarouni,Georgiadou,Panagiotakos *et al.*, 2008).

Bali and colleagues recruited 79 patients with NSTEMI who presented with chest pain within 3 h. The mean age was 68.8 ± 14 years and 73.4% were men. Fifty-three (67%) patients have significant ECG changes, sixteen (20%) presented at admission with detectable cTn of cut-off value 0.03 ng/L. They found that the median IMA concentrations of 115 U/mL and predicted major adverse cardiovascular events (MACE) with 1 year (Bali,Cuisset,Giorgi *et al.*, 2008). Conversely, Worster et, al., (2005) found that IMA is a poor predictor of cardiac events within 72 h of admission after chest pain (Worster et al., 2005).

Due to the non-specific nature of IMA some clinicians do not support the use of IMA evaluation as an effective risk stratification tool in patients presenting to the ED with chest pain suggestive of ACS (Keating,Benger,Beetham *et al.*, 2006). IMA is a biomarker of ischaemia with rapid clearance and narrow diagnostic window; thus not useful as a diagnostic test for AMI (Hjortshoj,Dethlefsen,Kristensen *et al.*, 2009). IMA evaluation and its clinical utility studies have shown controversial results and produces a significant results when used as part of a panel of tests (Abadie et al., 2005).

1.20 The challenge: is to correctly identify low risk patients

In England, chest pain is annually responsible for 238,000 admissions to the ED (Bidmead, 2015). Patients with chest pain suggestive of ACS constitute one of the largest single cohorts of patients presenting to ED in the UK (Bidmead, 2015; Gavalova, 2012). Managing this cohort of patients is complicated and can lead to medical errors (Deshpande & Birnbaum, 2014).

Discriminating between ACS and non-ACS patients is very challenging; especially in individuals without clear symptoms, ECG features and cardiac biomarkers. Routine diagnostic methods for risk stratification of patients presenting to ED with chest pain and ACS exist but none is adequately equipped to help clinicians to determine which patients can safely be discharged (Antman, 2000; Christenson, 2004). To expedite the diagnosis and to safely discharge the correct low risk patient's various strategies have been

employed including continuous automated ECG recording, imaging, risk scoring systems and the introduction of hs-cTn (Collinson, 2006; Hascoet,Bongard,Chabbert *et al.*, 2012). Despite, the presence of a range of diagnostic and prognostic tests with improved sensitivity and specificity; the number of patients discharged with the wrong diagnosis is still unacceptably high (Collinson, 2000b; Hascoet,Bongard,Chabbert *et al.*, 2012). However, despite these attempts at improving the triage protocol, inappropriate discharge and the misdiagnosis of low risk patients across the UK are estimated to be between 2% and 7% (Cassidy,Carroll,Cote *et al.*, 2000; Wildi,Zellweger,Twerenbold *et al.*, 2015). This, inappropriate discharged can be due to a various reasons including misinterpretation of ECG finding, atypical presentation, limited cardiac biomarkers sensitivity and specificity and inexperienced clinicians (Kontos,Diercks & Kirk, 2010). Presently the correct diagnosis of NSTEMI patients is still challenging and requires considerable resources to manage.

The aim of this study was to investigate the diagnostic potential of IMA in conjunction with other various biomarkers in assessing low risk patients with probable AMI. Prior to this study and to the best of my knowledge, there is no other studies looking at the combined diagnostic efficiencies of IMA, hs-cTnI or hs-cTnT in low risk patients presenting with chest pain suggestive of AMI. There are limited studies targeting the combined diagnostic efficiency of IMA, copeptin, NT-pro-BNP and H-FABP.

1.21 Aim of the research project

The overall aim of the present study was to investigate the diagnostic potential of IMA assay in conjunction with other biomarkers, in assessing AMI.

I. To test the diagnostic efficiency of IMA assay alone and in combination with the hs-cTn.

II. To test the diagnostic efficiency of IMA assay in combination with H-FABP, copeptin and NT-pro-BNP.

III. To test the predictive values of IMA and various biomarkers for major adverse cardiac events (MACE) at 30 days.

IV. To consider the cost benefit of introducing IMA assay alone or in combination with a novel biomarker or hs-cTn in an ED setting.

V. To explore the potential implication and future use of these new biomarkers at a professional level.

Chapter 2
Meta-analysis

Title: Performance of Ischemia modified-albumin assay in the diagnosis of acute myocardial infarction in patients presenting with chest pain suggestive of acute coronary syndrome

Abstract

Background: This is an update of an early Cochrane meta-analysis review first published 2006. Ischaemia modified-albumin (IMA®) was first thought to be useful biomarker for assessing cardiac ischaemia. Patients with ischaemic heart disease are at risk from a future coronary event. Clinicians therefore find it difficult to safely discharge patients who present to accident and emergency department with chest pain suggestive of ACS; but without evidence of cardiac necrosis or ECG changes. We though to conduct a meta-analysis to evaluate IMA assay in the diagnosis of AMI in patients presenting with chest pain suggestive of ACS.

Methods: The searched MEDLINE, EMBASE and Cochrane database up to March 2018 and used bivariable random-effect modeling to obtain summary parameters for diagnostic accuracy.

Results: We identified 26 studies (n = 4295) that assessed the use of Ischaemia modified-albumin assay. The sensitivity and specificity of IMA assay in diagnosing AMI at presentation was 77.73% (95% CI, 72.21-83.24%) and 72.71% (95% CI, 64.09-81.34%) respectively. The negative predictive value and positive predictive value for IMA assay in diagnosing AMI at presentation was 80.13 (95% CI, 73.18-87.08%) and 67.91 (95% CI, 58.47-77.39%) respectively.

Conclusion: IMA assay used at presentation has a good sensitivity and negative predictive value. However, IMA assay has a reduced specificity. IMA assay optimisation is required before prime-time use.

2.0 Introduction

In England, chest pain suggestive of acute coronary syndrome (ACS) represent the largest single cohort and is responsible for approximately 237,832 admissions to the emergency department (ED) per-year (Bidmead, 2015).

ACS is a sudden manifestation of an acute ischaemic cardiac disease which can include stable angina (SA), unstable angina pectoris (UA), non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI). All these diseases have a common pathophysiological origin in that they all manifest as a consequence of a sudden rupture or erosion of atherosclerotic plaque (Libby, 2001; Toutouzas & Stefanadis, 2006).

Patients presenting to the ED with suspected ACS exhibit a wide range of prodromal symptoms these include prolonged (>20 min) anginal pain and reterosternal pressure or heaviness radiating to the left arm, neck or jaw (McSweeney,O'Sullivan,Cody *et al.*, 2004). These symptoms can also be accompanied by nausea, abdominal pain, epigastric pain; recent-onset indigestion and chest pain (McSweeney,Cody,O'Sullivan et al., 2003). Patients presenting with prodromal symptoms have a good prognosis and rigorous cardiac investigations (Graham,Westerhout,Kaul *et al.*, 2008; Kloner, 1995). However, patients without classical prodromes or with NSTEMI are likely to be discharged with a high risk of developing AMI (Graham,Westerhout,Kaul *et al.*, 2008). AMI is associated with poor prognosis; so immediate and accurate diagnosis is important in order to allow appropriate treatment and care as soon as possible (Bhardwaj,Truong,Peacock *et al.*, 2011).

Currently there are a number of cardiovascular biomarkers such as troponin, CK-MB and myoglobin that will detect myocardial necrosis. However, a reliable ischaemic biomarker is not available. Patients with ischaemic heart disease are at risk from a future coronary event. Clinicians therefore find it

difficult to safely discharge those patients who present to ED with chest pain suggestive of ACS but without clear evidence of ECG changes and/or the presence of cardiovascular biomarkers such as cardiac troponins. Diagnosis of these cohort of patients is further complicated; because chest pain experienced by these patients is not always due to a cardiovascular disease; it can also be caused by other conditions and diseases such as pulmonary embolism or myocarditis. This is not ideal because if these vulnerable patients are positively identified, a proven and beneficial treatment is available; these treatments could prevent further complications such as AMI and expensive procedures such as percutaneous coronary intervention (Braunwald et al., 2002).

Inappropriate discharge of misdiagnosed AMI patients across the UK is estimated to be between 2% and 7% (Cassidy, 2000; Collinson, 2000a). The challenge for clinicians is to correctly identify genuine high risk from low risk ACS patients. Although, a number of potential ischaemic cardiovascular biomarkers such as heart fatty-acid binding protein have been identified, only ischaemia modified-albumin (IMA) has received approval by the US Food and Drug Administration (Bar-Or, Lau, & Winkler, 2000) and European Directive, Conformite Europeenne marking (CE). IMA can be indirectly measured using Cobalt-binding assay (Bar-Or, Lau, & Winkler, 2000; Brown,Sease,Robey *et al.*, 2007; Reichlin,Hochholzer,Stelzig *et al.*, 2000; Collinson,Gaze,Bainbridge *et al.*, 2006; Peacock,Morris,Anwaruddin *et al.*, 2006). The albumin-cobalt binding assay (IMA assay) was cleared by the American Food and Drug Authority (AFDA) on the basis that it would help rule-out ACS, in low to moderate pre-test probability conditions with negative necrotic cardiovascular biomarkers such as cardiac troponin and normal ECG. The mechanism of albumin-cobalt binding assay methodology and characteristics has been described previously (Bar-or D, 2001; Sinha,Roy,Gaze *et al.*, 2004).

2.1 Ischaemia modified-albumin

Human albumin (66.7 KDa) is a non-glycosylated single polypeptide chain containing 585 amino acids (AA). The most abundant protein in the human body (35 to 50 g/l) and exert a range of physiological and pharmacological functions including transport of small molecules such drugs, fatty acids, and bilirubin and act as an antioxidant (Evans, 2002; Rondeau & Bourdon, 2011). Human albumin (HA) occupies 50 % of plasma protein and regulates oncotic pressure. In physiological condition HA exert is buffering capability through its 16 histidine imidazole residues (Caironi and Gattinoni 2009;Colombo et al. 2012a;Pavone et al. 2010).

The circulation of albumin between the interstitial spaces the intravascular environment, the lymph drainage and the blood stream is a dynamic and continuous process which ends up causing albumin to degraded (10-12 g/24 h) ubiquitously with rate equal to that of it synthesis (Gattinoni, Carlesso, & Caironi, 2005). However, in disease state for example during cardiac ischaemia albumin is subject to free radicals and reactive oxygen species (ROS) attack; which in turn damages its integrity to carry certain function such as sequestering Cu (II) ions through its N-terminus and inhibiting the formation of ROS. Modification of the N-terminal through acetylation or deletion of one or more residues makes cobalt or copper binding impossible. This damaged albumin is known as ischaemia modified-albumin.

Bar-or et al. observed that IMA concentration is elevated in patients admitted to ED with ACS compared to normal healthy individual. Subsequently a pilot study of 139 patients attending ED department with acute chest pain, found that 99 patients with evidence of myocardial ischaemia had significantly elevated levels of IMA (mean ABSU ± SD; 0.52 ± 0.09) compared to 40 patients with no evidence of ischaemia (mean ABSU ± SD; 0.31 ± 0.09, $p < 0.0001$)(Bar-or D, 2001).

2.3 Methods

2.3.1 Search strategy

Medline (PubMed and Ovid), EMBASE and Cochrane database searches were conducted using combinations of the key trigger words and medical subject headings (MeSH) terms in the title only and title and/or abstract search limit fields. Both English and American-English spellings were adopted to broaden the search strategy. The search included all years between 2000 and 2018; paper excluded if used in previous meta-analysis in order to allow direct study comparison. All titles and abstracts were screened to determine relevance to the research question 'what is the diagnostic efficiency of ischaemia modified-albumin levels in patients with AMI'. Research letters, editorials and reviews were automatically excluded. Other potentially relevant studies not highlighted by the Medline and EMBASE searches were investigated by hand searching the reference lists of the papers and published review articles identified.

Medline and PubMed were used to acquire all IMA data; all the data were reviewed according to the Cochrane Collaboration Handbook for meta-analysis. Included studies were required to satisfy the inclusion criteria table 8.

Titles and abstracts were screened by me and independently by cardiovascular research scientist to ensure that they meet the inclusion criteria. Agreement was estimated using kappa statistic (K = 92 CI%, 86 to 98%) p = 0.001 indicating significant agreement according to Koch and Landis (Liberati,Altman,Tetzlaff *et al.*, 2009). Full texts of the selected articles were appraised by me.

Table 8: Meta-analysis inclusion criteria

	Meta-analysis inclusion criteria
1	IMA must be assessed for low risk patients presenting to ED with chest pain suggestive of ACS
2	IMA must be measured on admission
3	Study entry criteria must clearly be defined
4	Ischemia modified-albumin must be measured by the automated Roche Mira Cobas plus analyzer or ACB test
5	Ischemia modified-albumin must be measured according to the manufacturers recommendation and using albumin-cobalt binding assay (Ischemia technologies, Denver, CO)
7	Study end points were clearly defined
8	Adverse cardiac events were well documented
9	Ethical approval was obtained

2.3.2 Data analysis and meta-analysis statistics

The diagnostic sensitivity, specificity, negative predictive value (NPV) and positive predictive values (PPV) where reported in the papers, were abstracted. Sensitivity and specificity In the majority of cases, the dOR was calculated by construction of two-by-two contingency tables (Appendix 4). Patients were first dichotomised according to outcome (presence or absence of all cause of ACS during follow-up). Each group was then dichotomised into those above (IMA positive) or below (IMA negative) the clinical cut-off quoted in the paper by the authors. The sensitivity and specificity were calculated with construction of the 95% confidence interval (95% CI).

All data was transferred from the data collection forms to the Comprehensive meta-analysis statistical package (version 2.2.057; Biostat, New Jersey, USA). Using the sort facility, studies were listed by year of publication then alphabetically by first authors surname for each analysis and sub-group

analysis. The meta-analysis was performed to pool the data and calculate the overall weighted of sensitivity, specificity, NPV and PPV from the individual study.

The random effects model calculated according to the methods of DerSimonian and Laird to incorporate between-study variability (DerSimonian & Laird, 1986)(8) was used. This allows for more balanced study weights where large studies are assigned less relative weight and small studies are assigned more relative weight. Forest plots were constructed for each meta-analysis to display individual study and overall sensitivity, specificity, NPV, PPV and 95% confidence intervals.

The presence of publication bias was formally tested by construction of Funnel plots of log ratio versus standard error. Quantitative analysis was performed formally using Egger's regression asymmetry test (Irwig,Tosteson,Gatsonis *et al.*, 1994) and Begg & Mazumdar test for Kendall's tau (Kendall, 1938). Assessment of heterogeneity was examined by the Q-test and I^2 statistic (Christeinsen, 2005). A value of Q was deemed significant if p = < 0.10 and I^2 values of 25, 50 and 75% were deemed small medium and large proportions respectively.

2.3.3 Results literature search

The total searched yield 89 potentially relevant articles from which Medline produced 48 and EMBASE 41. Unable to acquire 4 articles, 17 articles has data missing e.g. 95% confidence interval for NPV and PPV which is essential for meta-analysis. One article used inappropriate STEMI patients. The final articles included in the meta-analysis were 26 with complete data (Figure 13). Full list of articles considered are in appendix 4.

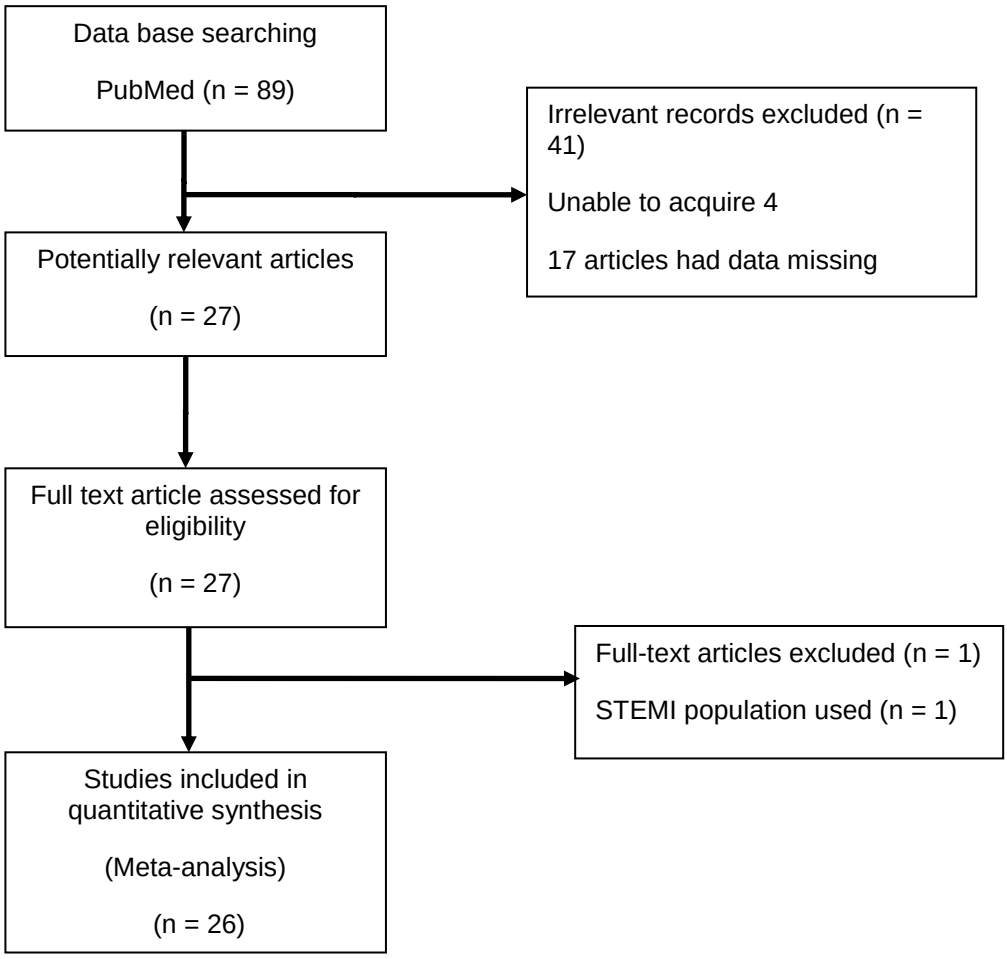

Figure 13: Selection of studies of Ischemia modified-albumin to rule out patients presenting to accident and emergency department with chest pain suggestive of acute coronary syndrome (Flow diagram adapted from PRISMA 2009).

2.3.4 Study population and definition of outcome

The final selected studies enrolled participants who presented to the ED with chest pain suggestive of AMI. All the patients who presented to ED with chest pain were subject to local triage protocol which includes clinical assessment, 12-lead electrocardiogram and physical examination. Cardiac troponin levels and IMA were measured at presentation. The final diagnosis was independently confirmed from medical record.

AMI was defined in accordance with 2007 ESC/ACCF/AHA guidelines(Hamm, 2011). In a clinical scenario acute AM6I describes the combination of necrosis of cardiomyocyte and the presence of acute myocardial ischaemia (Thygesen et al., 2012). To meet the current diagnostic criteria for AMI at least the clinician has to satisfy that a cardiovascular biomarker (preferably high sensitivity troponin I or T) is increased or decreased with at least one value above the 99th percentile of the upper reference limit and at least one of the followings:

1. Symptom of ischaemia.
2. New or presumed new significant ST-T wave changes or left bundle branch block on 12-lead ECG.
3. Development of pathological Q wave on ECG.
4. Imaging evidence of new or presumed new loss of viable myocardium or regional wall motion abnormality.
5. Intracoronary thrombus detected on angiography or autopsy.

Results

The program lists the results of the individual studies included in the meta-analysis: the estimate, its standard error and 95% confidence interval (CI). The pooled value for the estimate, with 95% CI, is given both for the fixed effects model and the Random effects model.

Random effects model

Under the random effects model (DerSimonian and Laird) the true effects in the studies are assumed to vary between studies and the summary effect is the weighted average of the effects reported in the different studies (Borenstein M, 2009).

The random effects model will tend to give a more conservative estimate (i.e. with wider confidence interval), but the results from the two models usually agree when there is no heterogeneity. When heterogeneity is present the random effects model should be the preferred model.

Forest plot

The Forest plot shows the estimate (with 95% CI) found in the different studies included in the meta-analysis, and the overall effect with 95% CI.

The random effects model will tend to give a more conservative estimate (i.e. with wider confidence interval), but the results from the two models usually agree where there is no heterogeneity. If the test of heterogeneity is statistically significant ($P < 0.05$), then more emphasis should be placed on the random effects model (Petrie,Bulman & Osborn, 2003).

When the option Marker size relative to study weight was selected, then the size of the markers that represent the effects of the studies vary in size according to the weights assigned to the different studies.

When the option Diamonds for pooled effects was selected then the pooled effects are represented using a diamond. The location of the diamond represents the estimated effect size and the width of the diamond reflects the precision of the estimate.

Heterogeneity

Cohran's Q

Q is the weighted sum of squares on a standardized scale. It is reported with a P value with low P-values indicating presence of heterogeneity. This test

however is known to have low power to detect heterogeneity and it is suggested to use a value of 0.10 as a cut-off for significance (Higgins,Thompson,Deeks *et al.*, 2003). Conversely, Q has too much power as a test of heterogeneity if the number of studies is large.

I^2 statistic

I^2 is the percentage of observed total variation across studies that are due to real heterogeneity rather than chance. It is calculated as I^2 = 100% x (Q - df)/Q, where Q is Cochran's heterogeneity statistic and df the degrees of freedom. Negative values of I^2 are put equal to zero so that I^2 lies between 0% and 100%. A value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity (Higgins,Thompson,Deeks *et al.*, 2003).

Funnel Plot

A funnel plot is a graphical tool for detecting bias in meta-analysis (Davey Smith,Egger & Phillips, 1997). In a funnel plot treatment effect is plotted on the horizontal axis and MedCalc plots the standard error on the vertical axis (Sterne & Egger, 2001). The vertical line represents the summary estimated derived using fixed-effect meta-analysis.

Two diagonal lines represent (pseudo) 95% confidence limits (effect ± 1.96 SE) around the summary effect for each standard error on the vertical axis. These show the expected distribution of studies in the absence of heterogeneity or of selection bias. In the absence of heterogeneity, 95% of the studies should lie within the funnel defined by these diagonal lines.

Publication bias results in asymmetry of the funnel plot. If publication bias is present, the smaller studies will show the larger effects. The funnel plot may not always be a reliable tool, in particular when the number of studies included in the analysis is small (Sterne,Sutton,Ioannidis *et al.*, 2011) .

Table 9: Meta-analysis results summary

Point variable		**Heterogenicity**			**Rank correlation**		**Regression test**	
	Overall effect (95% CI)	Q (p value)	df	I^2 (95%CI)	Kendal's Tau	P value	Eggers regression (95% CI)	P value
Sensitivity	77.73 (72.21 to 83.24)	14414.6 (< 0.0001)	25	99.83 (99.81 to 99.84)	-0.086	0.53	-8.73 (-23.0 to 5.5)	0.219
Specificity	72.71 (64.09 to 81.34)	39072.6 (< 0.0001)	25	99.94 (99.93 to 99.94)	-0.227	0.05	-2.66 (-24.3 to 19.08)	0.401
NPV	80.13 (73.18 to 87.08)	17928.8 (<0.0001)	23	99.87 (99.86 to 99.88)	-0.119	0.41	-1.32 (-19.61 to 16.9)	0.882
PPV	67.92 (58.47 to 77.39)	23232.00 (<0.0001)	20	99.91 (99.91 to 99.92)	-0.2	0.204	7.67 (-17.10 to 32.45)	0.524

2.3.5 Overall IMA assay sensitivity

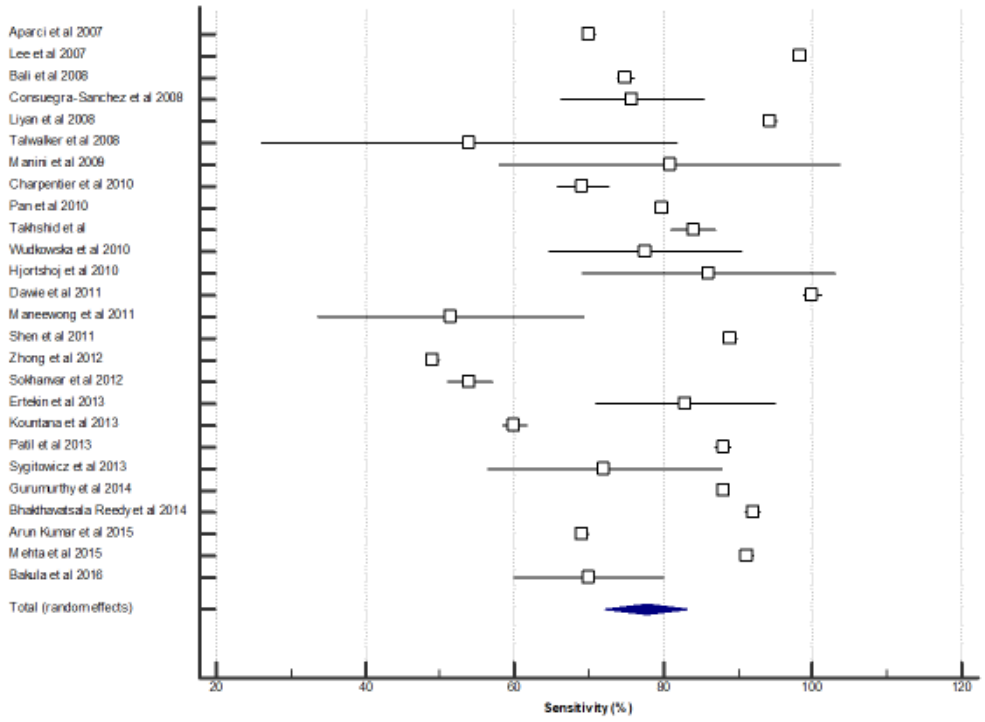

Figure 14: Over all IMA assay sensitivity 77.73% (95% CI, 72.21 to 83.24%).

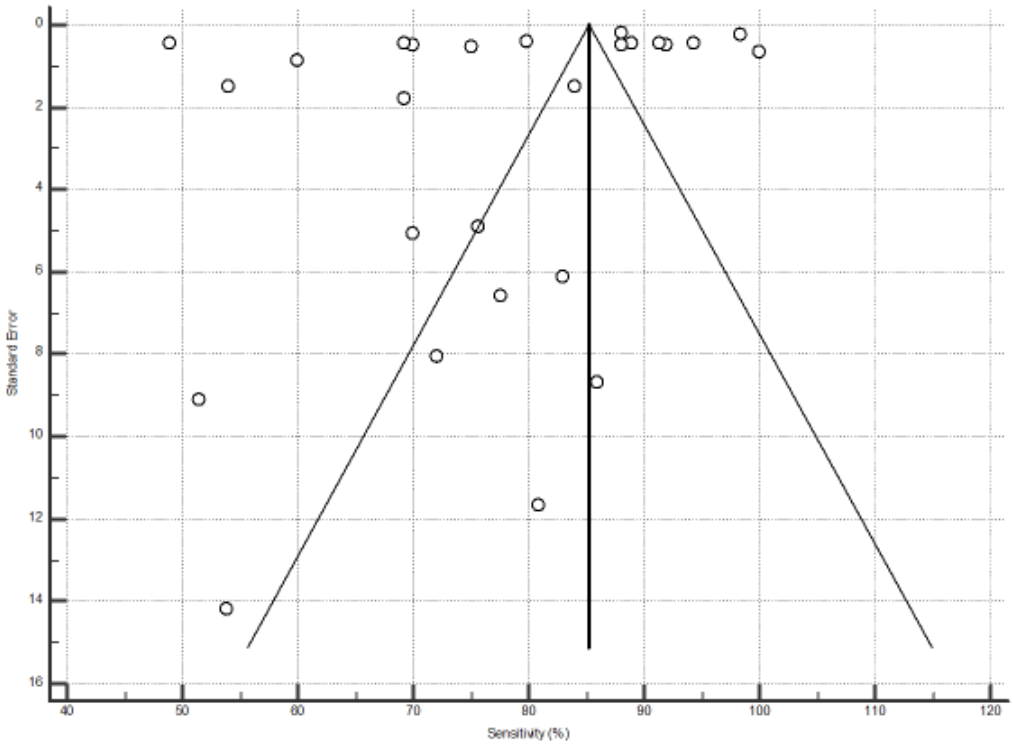

Figure 15: Funnel plots shows publication bias in the article included in the meta-analysis. The data is not well distributed in the funnel shaped area the asymmetry appear to suggest a publication bias. Increasing heterogeneity I^2 99.9; this bias may due to poor methodologies or analysis or study design. The sensitivity 77.73% (95% CI, 72.21 to 83.24%), specificity 72.71% (95% CI, 64.09 to 81.34%), NPV 80.13% (95% CI, 73.18-87.08%) and PPV 67.91% (95% CI, 58.47-77.39%) for IMA assay in diagnosing AMI at presentation.

2.3.6 Overall IMA assay specificity

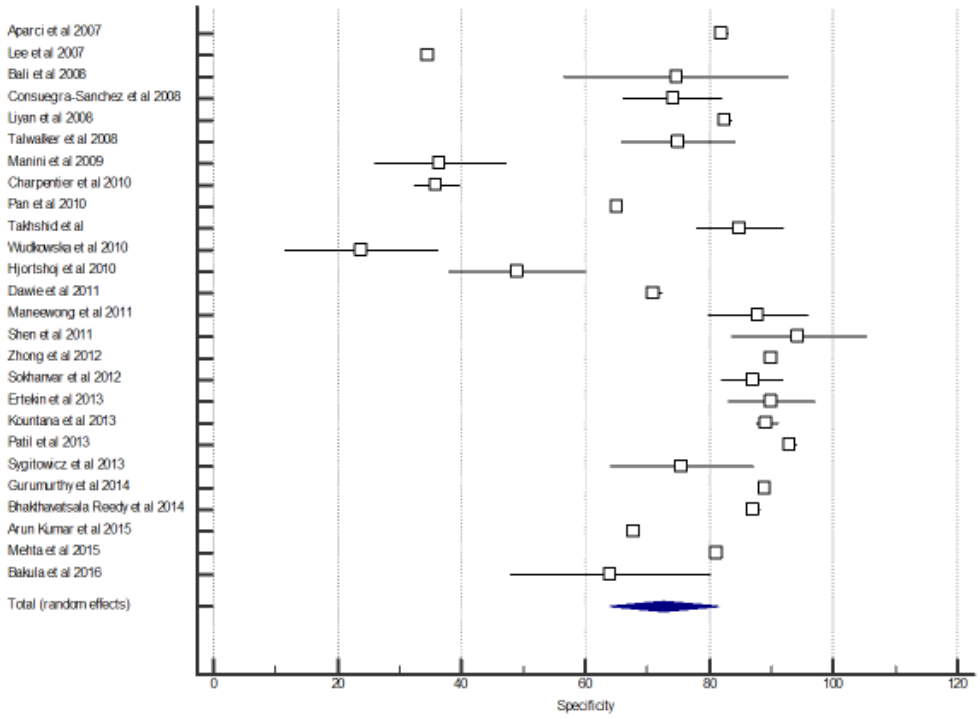

Figure 16: Over all IMA assay specificity 72.71% (95% CI, 64.09 to 81.34%).

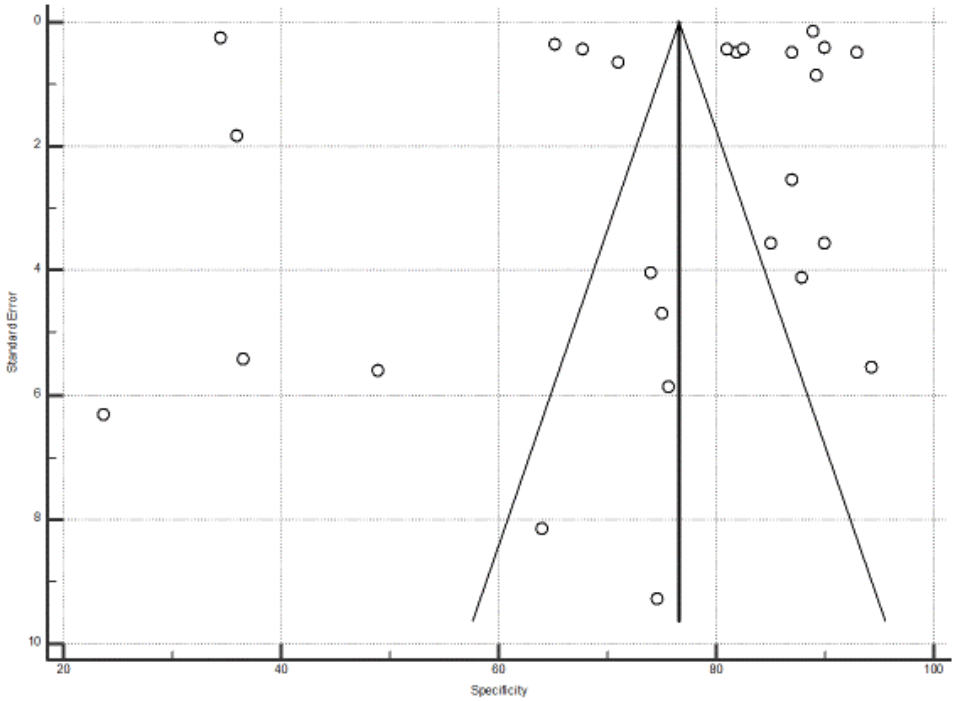

Figure 17: Funnel plots shows publication bias in the article included in the meta-analysis. The data is not well distributed in the funnel shaped area the asymmetry appear to suggest a publication bias. Increasing heterogeneity I^2 99.9; this bias may due to poor methodologies or analysis or study design. The sensitivity 77.73% (95% CI, 72.21 to 83.24%), specificity 72.71% (95% CI, 64.09 to 81.34%), NPV 80.13% (95% CI, 73.18-87.08%) and PPV 67.91% (95% CI, 58.47-77.39%) for IMA assay in diagnosing AMI at presentation

2.3.7 Overall IMA assay negative predictive value

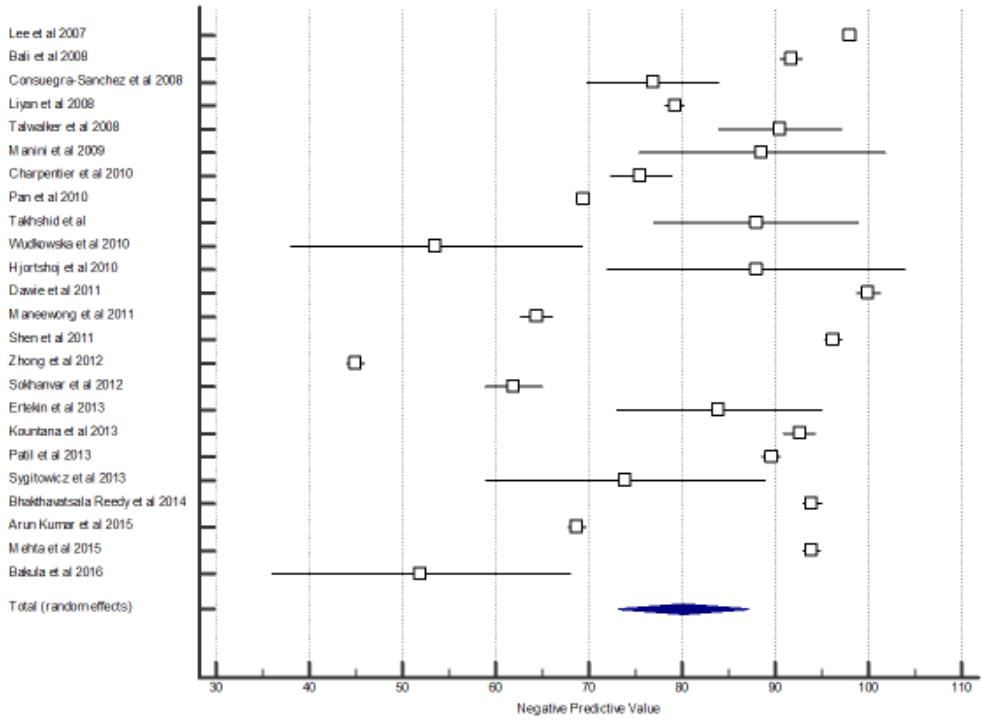

Figure 18: Over all IMA assay negative predictive value 80.13% (95% CI, 73.18 to 87.08%).

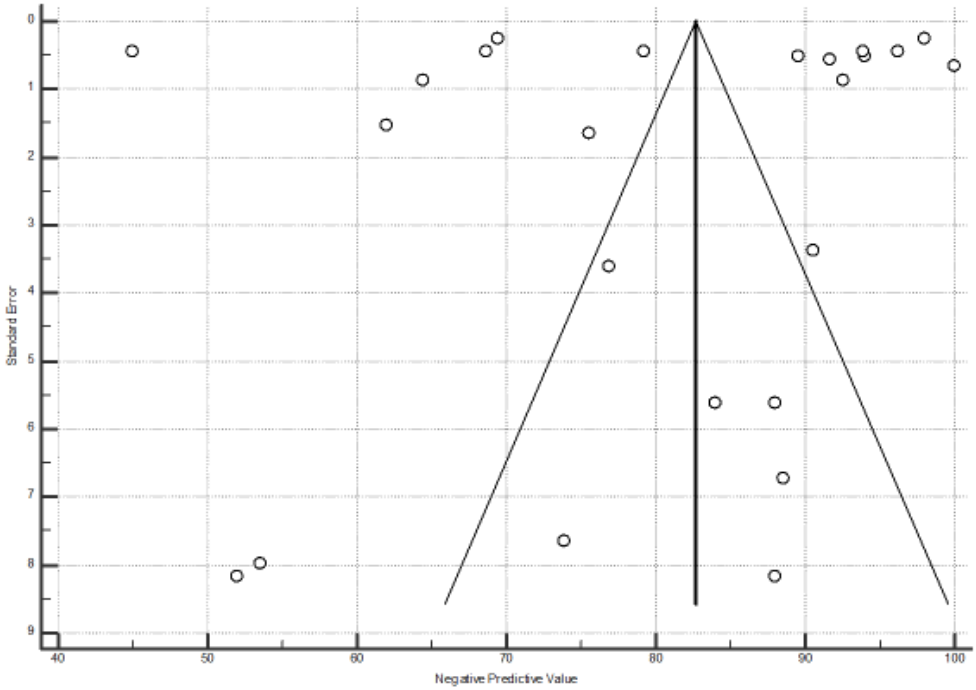

Figure 19: Funnel plots shows publication bias in the article included in the meta-analysis. The data is not well distributed in the funnel shaped area the asymmetry appear to suggest a publication bias. Increasing heterogeneity I^2 99.9; this bias may due to poor methodologies or analysis or study design. The sensitivity 77.73% (95% CI, 72.21 to 83.24%), specificity 72.71% (95% CI, 64.09 to 81.34%), NPV 80.13% (95% CI, 73.18-87.08%) and PPV 67.91% (95% CI, 58.47-77.39%) for IMA assay in diagnosing AMI at presentation.

2.3.8 Overall IMA assay positive predictive value

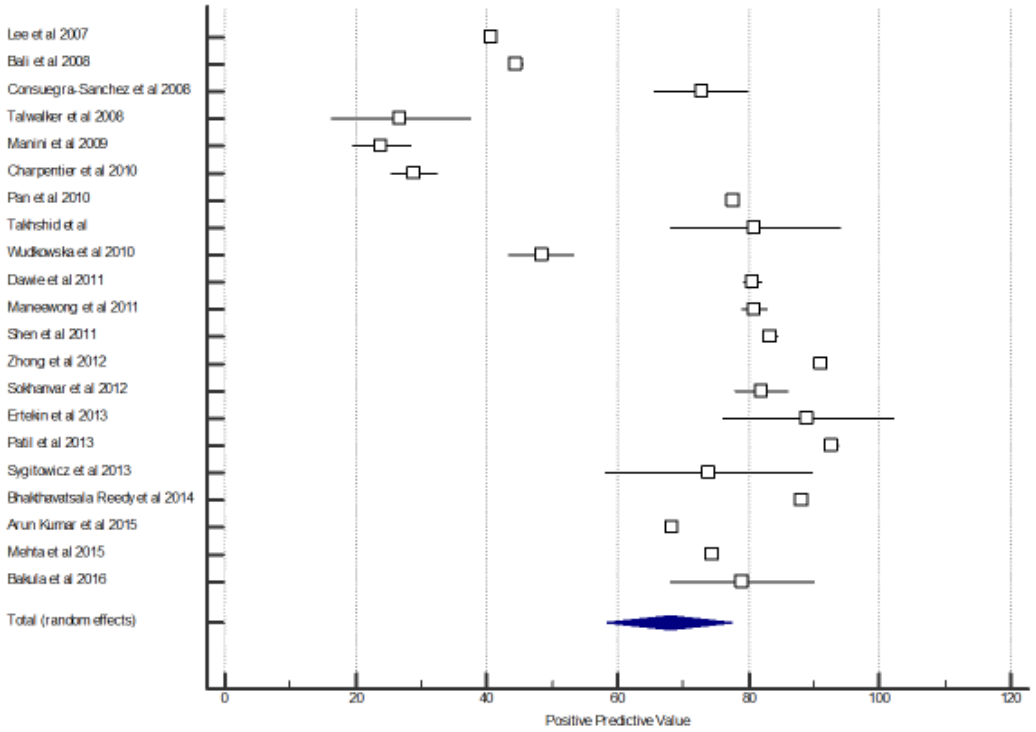

Figure 20: Overall IMA assay positive predictive value 67.92 (95% CI, 58.47 to 77.39).

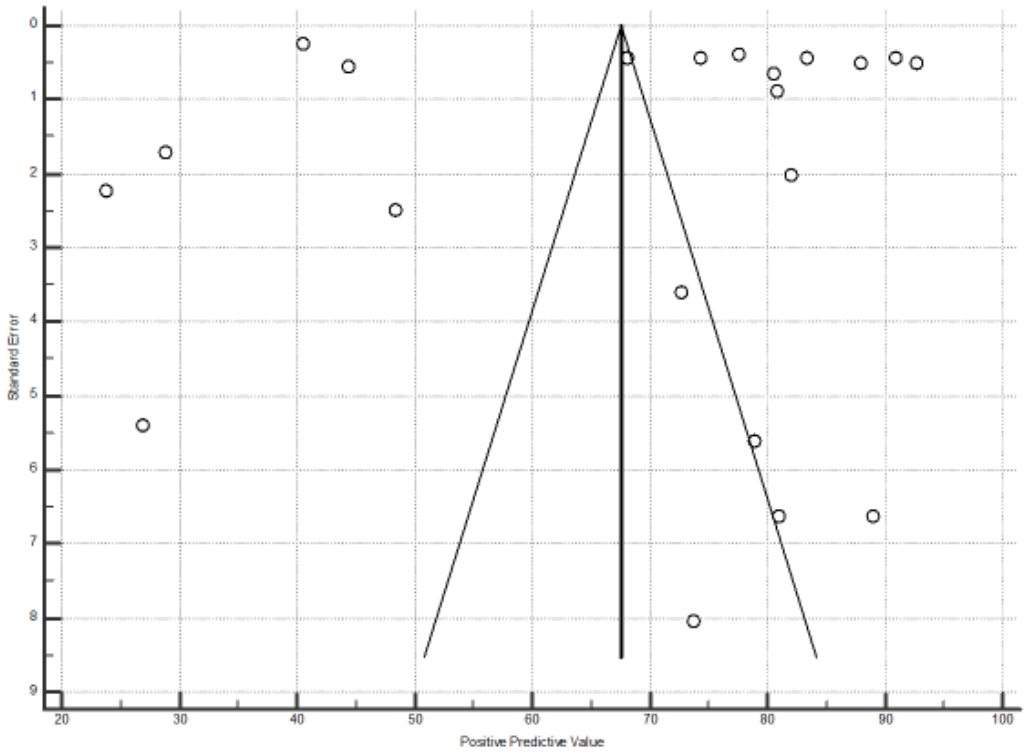

Figure 21: Funnel plots shows publication bias in the article included in the meta-analysis. The data is not well distributed in the funnel shaped area the asymmetry appear to suggest a publication bias. Increasing heterogeneity I^2 99.9; this bias may due to poor methodologies or analysis or study design. The sensitivity 77.73% (95% CI, 72.21 to 83.24%), specificity 72.71% (95% CI, 64.09 to 81.34%), NPV 80.13% (95% CI, 73.18-87.08%) and PPV 67.91% (95% CI, 58.47-77.39%) for IMA assay in diagnosing AMI at presentation

2.4 Discussion

There is no gold standard for detecting cardiac ischaemia, most studies that evaluate the utility of IMA assay has used cardiac troponin I or T, ECG, patients' history and patient's symptom at presentation as an outcome measure of ACS (Sinha, Roy, Gaze, Collinson, & Kaski, 2004b). Christenson et al. conducted a multicenter study involving 256 ACS patients, in which they examined cardiac troponin I from samples taken at admission and 6 to 24 hours after admission, and used it as an outcome measure; they found that IMA has a negative predictive value (NPV) of 96% and a positive predictive value (PPV) of 33% (Christenson et al., 2001).

Our meta-analysis (n = 4259) shows that the use of IMA assay to diagnose AMI at presentation to the ED resulted in good sensitivity and a good NPV. The summary sensitivity and specificity of IMA assay in diagnosing AMI at presentation was 77.73% (95% CI, 72.21-83.24%) and 72.71% (95% CI, 64.09-81.34%) respectively. The summary negative predictive value and positive predictive value IMA assay was 80.13% (95% CI, 73.18-87.08%) and 67.91% (95% CI, 58.47-77.39%) respectively. the previous meta-analysis found that IMA values when used alone has a 91% NPV and this increases to 97% when used with in combination with negative cTnT and normal or non-conclusive ECG (Peacock et al., 2006).

IMA levels change significantly (lower) after exercise-leg induced ischaemia in patients with peripheral vascular diseases (Roy et al., 2004). In addition, a study looking at healthy subjects also found that IMA levels decrease immediately after exercise and peak after 24 to 48 hours (Bhagavan et al., 2003). This observed interference with IMA concentration during exercise is believed to be caused by the presence of lactic acid (Zapico-Muniz et al., 2004).

The luck of IMA specificity and the potential increase of the false-positives test rate when used with other cardiovascular biomarkers and is particularly elevated in various diseases and conditions (table 10) might prove problematic. This low specificity is believed to be attributed to the fact that IMA assay may be capable of detecting subclinical ischaemia, which is not detected by the current conventional methods (Keating et al., 2006)

Table 10: Pathologies associated with increases in ischaemia modified-albumin concentration.

1. Cerebrovascular-ischaemic stroke, subarachnoid and intracranial haemorrhage
2. Peripheral vascular disease-arterial occlusion, deep vein thrombosis, mesenteric infarct
3. End-stage renal disease
4. Advanced liver cirrhosis
5. Acute infection
6. Malignancies
7. Systemic sclerosis
8. Intrauterine disorders-normal pregnancies, endometriosis, complicated deliveries.

Nevertheless, IMA's negative predictive value may be of value especially if we can exclude ACS patients (Roy & Kaski, 2000; Christenson,Duh,Sanhai *et al.*, 2001.

2.5 Limitations

The use of various methods, analyzers and different clinical cut-off points associated with IMA assay prevent an accurate meta-analysis. In addition, the use of previous troponin generations; this will eventually under estimate the sensitivity and specificity of IMA assay. Relevant studies in non-English speaking word were excluded. All studies used in this meta-analysis tested the diagnostic performance of IMA on the assumption of possible ACS. This is not appropriate comparison because currently no standarised test part from ECG measures cardiac ischaemia. The finding in this meta-analysis cannot be generalised.

2.6 Future considerations

Elevated IMA concentrations in non-cardiac and healthy subject warrant further studies into the levels of IMA in different clinical setting including the establishment of the optimal level of IMA in various organs during an ischaemic state (Kim et al., 2008b). Furthermore, IMA assay is needed to be compared to the current high sensitivity troponin I, because recent study suggested that highly sensitive troponin I may be used to exclude patients with AMI within 1 or 2 hours (Charpentier & Chenevier-Gobeaux, 2016). The reliability of IMA evaluation in real clinical setting including the cut-off point and its incremental value, additional cost to the healthcare system is still to be established. Nevertheless, IMA evaluation is still worth developing and improving, because currently IMA measurement is the only test available and licensed for the diagnosis of patients suspected of cardiac ischaemia. IMA assay in its current form is not reliable enough because of its low specificity.

Chapter 3

Material and methods

3.0 Overview and study design

The main aim of this thesis is to investigate the diagnostic potential of IMA assay in conjunction with other cardiac and novel biomarkers in assessing AMI. The IMA study is also an opportunity to assess the overall usefulness of IMA and a number of novel biomarkers in improving and contributing to the existing information when assessing AMI in ED environment.

3.1 Population

The present investigations in this thesis are a sub-study within a multicenter pragmatic randomised control trial called the Randomised Assessment of Treatment using Panel Assay of Cardiac Biomarkers (RATPAC). RATPAC trial is a multicenter pragmatic randomised control trial (RCT), primarily concerned with the economic evaluation of point-of-care testing (POCT) of cardiac biomarker panel in the management of patients with suspected, but not proven AMI (Goodacre,Bradburn,Cross *et al.*, 2011; Goodacre, 2011).

The hospitals participating (table 11) in the study were Barnesley District General Hospital, Derriford Hospital in Plymouth, Leeds General Infirmary, Leicester Royal Infirmary, Edinburgh Royal Infirmary and Frenchay Hospital in Bristol. These participating hospitals were selected to include a range of different setting, thus providing a current NHS practice and a realistic variation in facilities available to UK patients presenting with chest pain suggestive of ACS.

Table 11: Characteristics of the participating centers

Hospital	Annual ED attendances: 1 April 2008 to 31 March 2009	No. of acute medical beds*	ED facilities	On-site cardiology services
Barnesley	71,678	462	-	CCU, rapid access clinic
Derriford	85,341	240	CDU	CCU, angioplasty, cardiac
Leeds	109,362	491	CDU	surgery, rapid access clinic
Leicester	156,053	290	-	CCU, angioplasty, cardiac
Edinburgh	105,378	843	-	surgery, rapid access clinic
Frenchay	62,823	461	CDU	CCU, angioplasty, cardiac surgery, rapid access clinic
				CCU, angioplasty, cardiac surgery, rapid access clinic
				CCU, angioplasty

CCU = coronary care unit. CDU = clinical decision unit. * = excluding escalation beds

Potential participants were all recruited to the trial; and then excluded if they meet any of the exclusion criteria (table 12). Once the clinical staff identified and recruited the eligible patients these patients were then randomised into either those receiving a diagnostic assessment using POCT biochemical biomarkers panel (CK-MB (mass), myoglobin and troponin I on admission and 90 min after admission or those receiving standard laboratory based cardiac biomarker testing according to the protocol of the participating hospitals. Participating patients, career and clinicians were not blinded in the RATPAC study and day-to-day management of patients was left entirely to the discretion of clinicians.

3.2 Recruitment

The research nurses or participating hospital staff screened all the patients presenting with chest pain and excluded those that satisfied the exclusion criteria. The research nurses or ED staff explained the trial to the potential recruits and obtained their written consent. Once the participants consented to the trial they were randomised into a single randomisation sequence. The randomisation sequence was generated by the Clinical Trials Unit and accessed through a secure website to either receive the standard care or POCT.

3.3 The objectives of the Randomised Assessment of Treatment using Panel Assay of Cardiac Biomarkers

The RATPAC trial was designed to determine whether POCT in an ED setting could replace the current standard of care in terms of day-to-day management of patients presenting with chest pain suggestive of AMI. To achieve this objective the RATPAC trial evaluated the following: the proportion of patients successfully discharged home within 4 h after ED assessment, health utility and satisfaction with care, the use of coronary care beds and cardiac treatments. They also assessed the subsequent re-attendance at and/or re-admission to hospital, major adverse cardiac events include death, non-fatal AMI, life-threatening arrhythmia, emergency revascularisation or hospitalisation for AMI, and social care cost. Although, the decision to discharge or admit patients was based on the POCT protocol; the final decision in terms of care pathway was entirely left to the attending clinicians.

Furthermore, the RATPAC trial also allowed for secondary analysis of data with the aim to include TIMI and GRACE. In addition, the RATPAC trial had made a provision to create a bank of well characterised samples to be used

for research such as the assessment of novel cardiovascular biomarkers including IMA, copeptin and H-FABP (Collinson,Gaze,Thokala *et al.*, 2013).

3.4 Data management

Baseline demographic data and the final outcome were recorded including blood results, clinical details, and adverse events up to a month. Surviving participants were mailed a questionnaire at one month, measuring satisfaction with care, health utility, and social care resources used.

Trial data from follow-up (30 days) was systematically recorded by authorised personnel and entered into a fully audited online secure data base. Data management including a procedure for dealing with errors and quality control issues were dealt with in accordance with Sheffield Clinical Trials Research Unit (CTRU) standard operating procedures. The final classification of patients with AMI and major adverse cardiac events was blindly conducted by an independent expert cardiologist.

3.5 Ethical consideration

Ethical approval for the RATPAC was granted by Leeds East Research Ethics Committee (07/Q12506/22) and the subsequent reviews were conducted by the local participating hospitals ethics committee. The RATPAC trial complied with the Declaration of Helsinki and the Research Council Guidelines for Good Clinical Practice in Clinical Trial. The trial was registered with the International Clinical Trials Authority and sponsored by the University of Sheffield.

Potential participants were required to provide written consent, however in an emergency setting with limited time to consider trial information and its

potential benefit clinicians made no attempt to pressurise patients to participate in this study.

Samples required for the successful trial were made anonymous by assigning a unique prime number known as an identifier. Pre-prepared test packs were made available to the participating staff. These test packs contained the following: four primary sample tubes comprising two lithium-heparin for POCT and two serum separator gel tubes for the standard laboratory arm and for bank (Becton Dickinson, Oxford, UK). In addition, the serum samples were allowed to clot for 30 min at room temperature, centrifuged (5000 rpm) for 10 min and decanted into two labeled (with unique identifier) long storage tubes and then frozen at -20 ^{0}C before transfer to St George's Healthcare NHS Trust, Tooting, London, UK for further analysis. At 90 min after admission the process described for sample handling at admission was repeated.

3.6 Randomised Assessment of Treatment using Panel Assay of Cardiac Biomarkers trial

The RATPAC study was primarily concerned with the economic evaluation of POCT of a cardiac biomarker panel comprising of myoglobin, CK-MB and cardiac cTnI for the management of low risk patients with chest pain. Potential participants were excluded if they met any of the criteria given in table 12.

Table 12: Patient exclusion criteria

- ECG changes for myocardial ischaemia (> 1 mm ST deviation or > 3 mm inverted T wave)
- High risk ACS
- Known coronary heart disease.
- Recurrent episodes of cardiac type pain.
- Confirmed or suspected serious non- cardiac pathology (e.g. pulmonary, embolus)
- Co-morbidity or social problems that require hospital admission
- Obvious non-cardiac cause (e.g. pneumothorax or muscular pain more than 12 h since their most significant previous episode of pain.
- Participants who are unable to understand trial information or did not consent

Once the clinical staff had identified, obtained written consent and recruited the eligible patients; they were then randomised into receiving either:

1) Diagnostic assessment using POCT cardiac biomarker panel including CK-MB mass, myoglobin and cTnI, on admission (0 min) and at 90 min after admission.
2) Standard laboratory based diagnostic assessment according to the local protocol of the participating hospitals.

Participating clinicians were not blinded to the results and day-to-day management of patients was left entirely to the discretion of clinicians. Patient's recruitment was prospective; although, the present study and other biomarkers studies were retrospective.

The RATPAC cohort were assessed clinically an admission, fully characterised and further followed up at 30 days. Retrospective analysis of biomarkers in

this thesis is a component of the RATPAC study allowing the original trial criteria to be transferred to the present study (Bhandari, 2006).

3.7 Analytical methods

The biochemical analyses for the standard arm were conducted in accordance with the local participating hospital trusts protocols and the local equipment. However, the Stratus® CS Analyser for POCT was used by all the participating trusts.

The POCT for cardiac biomarker panel included CK-MB mass, myoglobin and cTnI; these multiple cardiac biomarkers were measured by trained ED staff using Stratus® CS analyser Siemens Healthcare Diagnostic (Camberley, UK) on admission and ninety minutes after admission.

3.8 Point-of-care testing assays across all sites.

The Stratus® CS was used to measure the following cardiac biomarkers, myoglobin, CK-MB mass and cTnI. The analytical characteristics of the assays for each analyte are summarised in table 13.

Table 13: Point-of-care testing analytical characteristics.

Analyte	Detection limit	Analytical range	Inter-assay CV%	Reference interval
Myoglobin	1 µg/L	1-900 µg/L	1.9-12.7 (56-308 µg/L)	21-98 µg/L (males)* 19-56 µg/L (females)* 20-82 µg/L (combined)*
CK-MB	0.3 µg/L	0.3-150 µg/L	0.15-1.27 (3.7-39.3 µg/L)	0.3-3.5 µg/L*
Troponin I	0.03 µg/L	0.03-50 µg/L	4.0-8.2 (0.067-0.344 µg/L)	0.07 µg/L (99th percentile) †

* = 95% reference interval. † = 99th percentile of the assay.

3.9 The present study secondary analysis

The high sensitivity cardiac troponin, myoglobin, copeptin, IMA and H-FABP were analysed at St George's Healthcare NHS Trust, manufacturer's recommendations including 99th percentiles and equipment types. The analytical characteristics of the assays for each analyte are summarised in Table 14 and 15. In 2014 IMA evaluation study (appendix 3 for supplementary data) was conducted by me at St Georges Healthcare NHS trust. London. Cobas Mira plus analyser was used the determine IMA concentrations for both the participant and reference range study participants. Sample selection and analysis for reference range suitability was entirely conducted by me at St Georges Healthcare NHS. London. Secondary outcome including the estimation of h-FABP, copeptin, hs-cTn and NT-pro-BNP were also analysed at St Georges Healthcare NHS, by separate team under the supervision of Dr David Gaze. Data analysis was conducted with the assistance of Dr David Gaze using Analyst-it software. My role also consists of categorising current project data in excel in accordance with project objectives i.e. matching complete paired samples, demographic and MACE and Dr Gaze role was to compute it on my behalf in analysed-it software and handover the graphs and data for analysis and interpretation by me. The statistical requirements such as ROC, sensitivity, specificity, NPV, PPV and the required combination with different biomarkers for the current project were also specified by me. Current project health economics was entirely conducted by me.

Table 14: Analytical characteristics of myoglobin, heart fatty-acid binding protein, IMA, NT-pro-BNP and copeptin.

Analyte	Detection limit	Upper limit	Analytical range	Coefficient of variance (CV %)	Percentile
Myoglobin	1.8 mg/L	700 mg/L	-	8.8% at 83 mg/L 9.4% at 119 mg/L 9.5% at 125.9 mg/L	66 mg/L (97.5th percentile)
H-FABP	1.5 mg/L	100 mg/L	-	9.1% at 3.1 mg/L 7.5% at 17.6 mg/L 9.8% at 44.1 mg/L	2.5 mg/L (95th percentile) 3.0 mg/L (99th percentile)
NT-pro-BNP	20 ng/L		35,000 ng/L	4.0-5.0 (32.1-40.9 ng/L)	Not applicable
Copeptin	4.8 pmol/L	-	4.8-500 pmol/L	12-17% at 20-50 pmol/L 6% above 50 pmol/L	17.4 pmol/L (97.5th percentile) 19.1 pmol/L (Males) (97.5th percentile) 12.9 pmol/L (Females) (97.5th percentile)
IMA	7 KU/L	211 KU/L	7-211 KU/L	5.09% at 56.67 to 66.57 kU/L 3.05% at 147.17 to 158.03 kU/L	

Table 15: Analytical characteristics of cTn according to manufacturers, in parentheses are the decision limit for diagnosis of AMI

Analyte	Detection limit	Upper limit	The claimed CV%	99th percentile	Participating hospitals & decision limit for diagnosis of AMI	Analytical platform
Roche® cTnT	3 ng/L	10,000 ng/L	13 ng/L	14 ng/L	Plymouth Derriford (20 ng/L)	Elecsys® 2010 system
Siemens® TnI	6 ng/L	50,000 ng/L	30 ng/L	40 ng/L	Barnesley (200 ng/L), Leeds (50 ng/L) and Leicester (60 ng/L)	ADVIA Centaur®XP system
Beckman® AccuTnI	1 ng/L	100,000 ng/L	60 ng/L	40 ng/L	Bristol Frenchay (60 ng/L)	Access 2 system®
Abbott® cTnI	10 ng/L	50,000 ng/L	32 ng/L	12 ng/L	Edinburgh (50 ng/L)	Architect i2000SR system®

CV% = Coefficient of variance

3.10 Core laboratory assays for the secondary objectives

As part of the RATPAC study secondary objectives, hs-cTnI, hs-cTnT, copeptin, NT-pro-BNP and H-FABP were evaluated and described by the RATPAC-Contemporary Biomarker evaluation (RATPAC-CBE) team (Collinson, Gaze, Thokala, & Goodacre, 2013). The analytical characteristics of IMA, NT-pro-BNP, H-FABP and copeptin are summarised in table 14.

3.11 Analytical characteristics

The measurements of myoglobin, CK-MB isoform mass, hs-cTn, copeptin, H-FABP, NT-pro-BNP and IMA were performed according to manufacturer's specification and standardised protocols. The evaluation of hs-cTnI was performed using Siemens ADVIA Centaur® XP system, Abbott ARCHITECT stat i2000SR System® and Beckman-Coulter Access® 2 system; whereas, hs-cTnT concentration was determined using Roche Diagnostics Elecsys® 2010 System. Although, most of these systems measure the same analyte; differences in detection mode, and assay protocol exist between manufacturers. Thus, each analyte detection method will be illustrated individually.

3.12 Brief description of the principle of sandwich immunoassay

Quantification of myoglobin, CK-MB mass, hs-cTnI, hs-cTnT, copeptin and NT-pro-BNP is based on a sandwich immunoassay (Figure 22). Sandwich type immunoassay uses a format of a pair of matched high-affinity monoclonal antibodies (Abs) with the appropriate label usually an enzyme such as streptavidin and biotin. This pair of matched high-affinity monoclonal Abs will usually consist of the primary Abs and its role is to bind to the antigen (Ags) for example CK-MB. The secondary Abs and its role is to lock the Ags to from a complex (sandwich) hence the use of term sandwich immunoassay.

Secondary antibodies are usually labeled with an excitable visible label that can be measured (Figure 22).

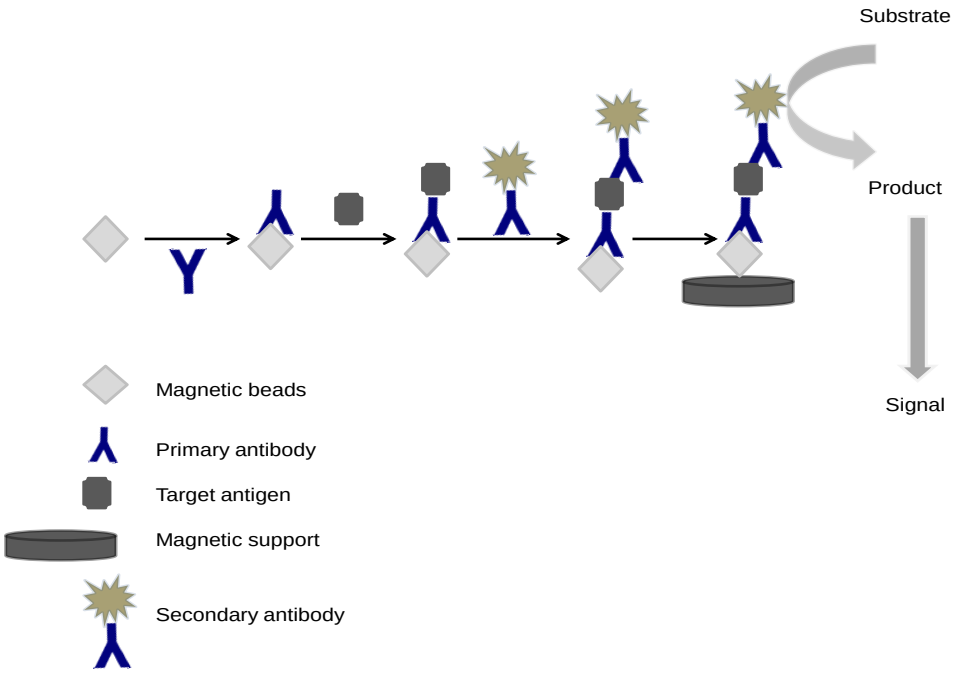

Figure 22: Principle of sandwich immunoassay. Primary anti-antibody labelled with a magnetic bead is left to bind to the target antigen. After incubation and wash the secondary enzyme labelled anti-antibody is added and allowed to form the immune-complex or sandwich. After incubation and washing to remove non-specific proteins the immune-complex is left to interact with a substrate and the signal emitted is proportional to the antigen concentration.

3.13 Evidence Cardiac Array® measured on the Evidence Investigator™

The analytical measurements of H-FABP, CK-MB mass and myoglobin were performed using the Evidence Cardiac Array® measured on the Evidence

Investigator™ (Randox Laboratories, Crumlin, UK). Evidence investigator™ allows the simultaneous quantification of multiple analyte from a single sample. The analytical characteristics are summarised in table 14. Evidence investigator™ quantification of H-FABP, CK-MB and myoglobin is based on sandwich chemiluminescent immunoassay. Evidence investigator™ and biochip technology is based on a solid phase ceramic chip containing 25 discrete test regions containing immobilised antibodies specific to H-FABP, CK-MB and myoglobin (Figure 23).

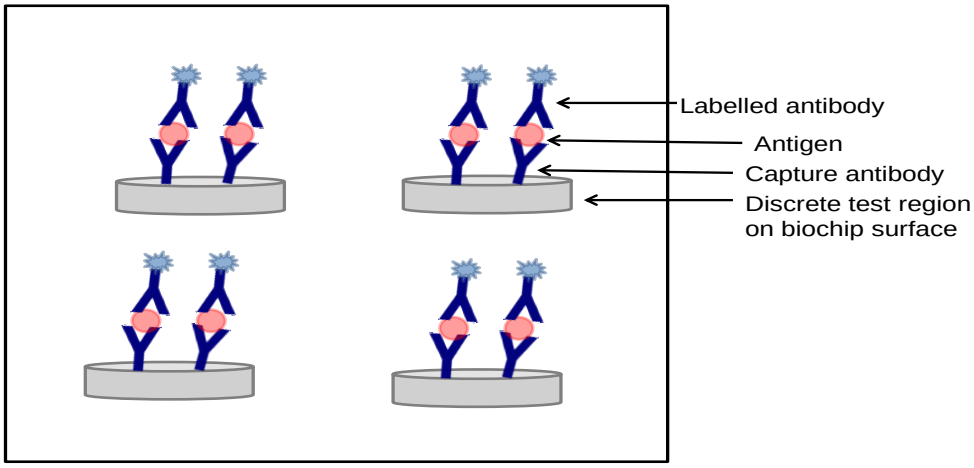

Figure 23: Biochip carrier allows the analysis of 9 patients simultaneously. Each biochip carrier is coated with capture agent which is usually an anti-antibody. The analyte repersents the antigen of interest, for example CK-MB. After incubation and washing stages the labelled secondary anti-antibody is added and allowed to incubate. The final step involves a wash and the addition of the substrates; the light emitted is proportional to the concentration of the antigen.

3.13.1 Assay protocol

Patient serum sample is diluted and 60 µL with unknown H-FABP, CK-MB and myoglobin is mixed, and transferred into the appropriate biochip wells (coated with the appropriate antibody) and allowed to incubate at 37 ^{0}C for 30 min. The biochip wells are washed 4 times with tris buffered saline to remove non-specific unbound proteins. Following the removal of the unbound proteins, 300 µL of enzyme horseradish peroxidase conjugate antibody is added to each well and allowed to incubate for 30 min at 37 ^{0}C. The biochip wells are washed (4 times) with tris buffer saline to remove the unbound horseradish peroxidase conjugate antibody. Once the unbound horseradish peroxidase conjugate antibody is removed a final wash is carried out to allow the biochip well to soak in the buffer for 15 s. Finally, 250 µL of working signal reagent-EV701 (1:1 of Luminol-EV701 and peroxide) is added to each biochip well while protect from light and the signal is measured. The light emitted is proportional to the concentration of the antigen.

3.14 Siemens ADVIA Centaur® XP system

3.14.1 Ultra-sensitive cardiac troponin I

The Advia Centaur® XP system TnI-Ultra assay is a three-site sandwich immunoassay using direct chemiluminometric technology for the quantification of human cardiac troponin I in serum or plasma (Figure 24). In order to reduce nonspecific binding an ancillary reagent is incorporated in the assay procedure. The Binary Lite reagent contains a polyclonal goat anti-troponin antibody labeled with acridinium ester and 2 biotinylated mouse monoclonal anti-troponin I antibodies. The solid phase reagents contain magnetic latex particles conjugated with streptavidin.

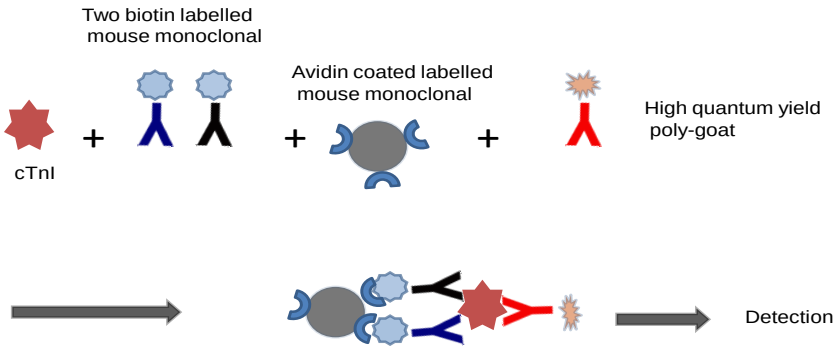

Figure 24: ADVIA Centaur TnI-Ultra assay three-site immunoassay using direct chemiluminometric technology (courtesy of Siemens Healthcare Diagnostics).

3.14.2 Assay principle

In the first step a 100 µL of patient sera with the unknown cTnI concentration is mixed with 50 µL of the ancillary reagent, 100 µL of Binary Lite reagent and further, two biotinylated mouse monoclonal anti-troponin I antibodies. The ancillary reagent helps to reduce nonspecific binding; whereas, the Binary Lite reagent which include polyclonal goat anti-troponin I antibody labeled with acridinium ester is designed to bind the unknown cTnI. The second step consist of the addition of two biotinylated mouse monoclonal anti-troponin I antibodies. The whole mixture is than allowed to incubate at 37 ^{0}C for 3.0 min. Finally, to immobilise the immune-complex a solid phase reagent of 150 µL which consists of magnetic latex beads conjugated with streptavidin is added and allowed to incubate at 37 ^{0}C for a further 6.3 min. The unbound proteins

are washed with tris buffer. Following the washing step 300 µL acids (reagent R1) and a 300 Base µL (reagent R2) is added to initiate and stop the chemiluminescent reaction. Direct relationship exists between the amount of cTnI in the sample and the amount of relative light unit (RLUs) detected.

3.15 Abbott ARCHITECT stat i2000SR System®

3.15.1 High sensitivity cardiac troponin I

The ARCHITECT stat hs-cTnI assay is a chemiluminescent microparticles immunoassay (CMIA) for the quantification of human cardiac troponin I in serum or plasma (Figure 25). Hs-cTnI quantification using ARCHITECT stat i2000SR System® is a two-step immunoassay using CMIA technology (Figure 25).

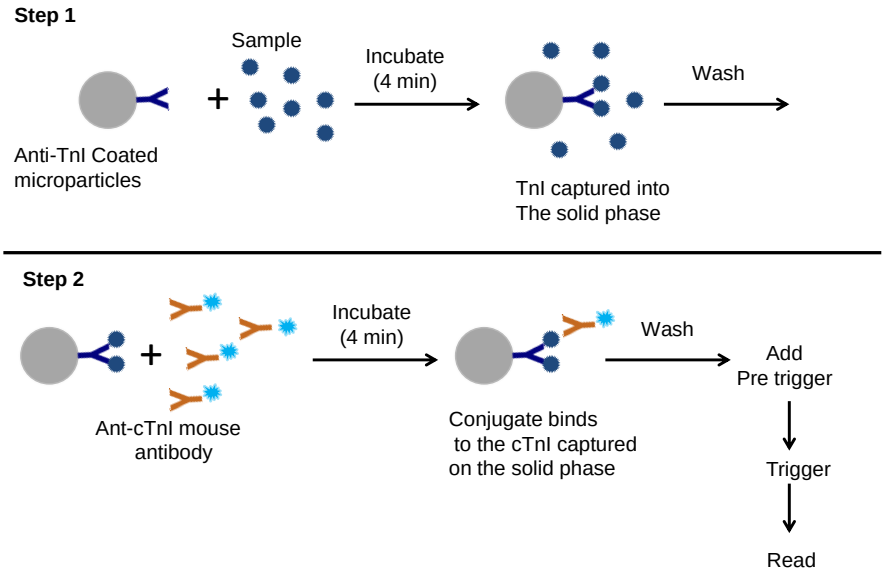

Figure 25: Abbott ARCHITECT stat highly sensitive troponin assay design (Produced from manufacturer insert Abbott Laboratories).

3.15.2 Assay principle

The first step consists of allowing patients sera and anti-troponin I antibody-coated paramagnetic microparticles (solid phase) to combine in a vessel. Troponin I present in the sample binds the anti-troponin I antibody-coated paramagnetic microparticles. After for 4 min incubation the unbound proteins are washed with a tris buffer. In the second step immediately after washing, anti-troponin I acridinium labeled conjugate is added and allowed to incubate for a further 4 min. After incubation the mix is washed with tris buffer. Pre-trigger and trigger solution is added. The resulting chemiluminescent reaction is measured as relative light units (RLUs). Direct relationship exists between

the amount of cTnI in the sample and the amount of RLUs detected. The concentration of the unknown cTnI is read relative to the standard curve.

3.16 Beckman Coulter Access® 2 system

3.16.1 High sensitivity cardiac troponin I

High sensitivity cardiac troponin I quantification using Beckman-Coulter Access® 2 system is a paramagnetic particle chemiluminescent immunoassay (Figure 26). The Access AccuTnI assay is a two-site immuno-enzymatic ("sandwich") assay.

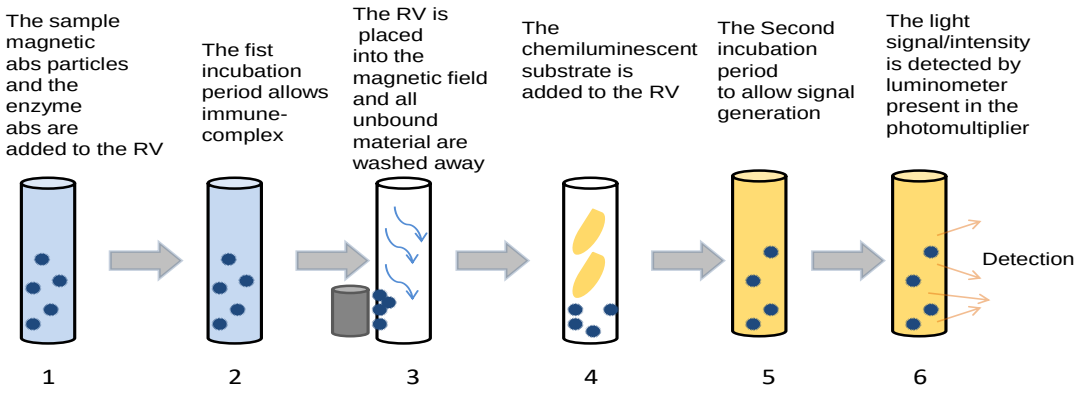

Figure 26: High sensitivity troponin I quantification by Beckman-Coulter Access 2 System using paramagnetic particle chemiluminescent immunoassay. RV, reaction vessel. (By Beckman-Coulter).

3.16.2 Assay principle

Patient's serum sample with unknown amount of cTnI is added to a reaction vessel, containing monoclonal anti-cTnI-antibody conjugated to alkaline phosphatase and paramagnetic particles coated with monoclonal anti-cTnI antibody. The unknown human cTnI binds to the anti-cTnI antibody on the solid phase, whereas the monoclonal anti-cTnI antibody conjugated to alkaline phosphatase reacts with different antigenic sites on cTnI molecule. After incubation, material bound to the solid phase are temporarily held by magnetic field to allow unbound material to be washed. The chemiluminescent substrate Lumi-Phos® 530 is added to the vessel. The light generated by the reaction is measured with luminometer. The light produced is directly proportional to the concentration of cTnI in the sample. The concentration of the unknown cTnI is determined from the stored multipoint calibration curve.

3.17 Roche Diagnostics Elecsys® 2010 System

3.17.1 High sensitivity cardiac troponin T

Hs-cTnT quantification using Roche Diagnostics Elecsys® is an electrochemiluminescent immunoassay "sandwich" (ECLIA) (Figure 27).

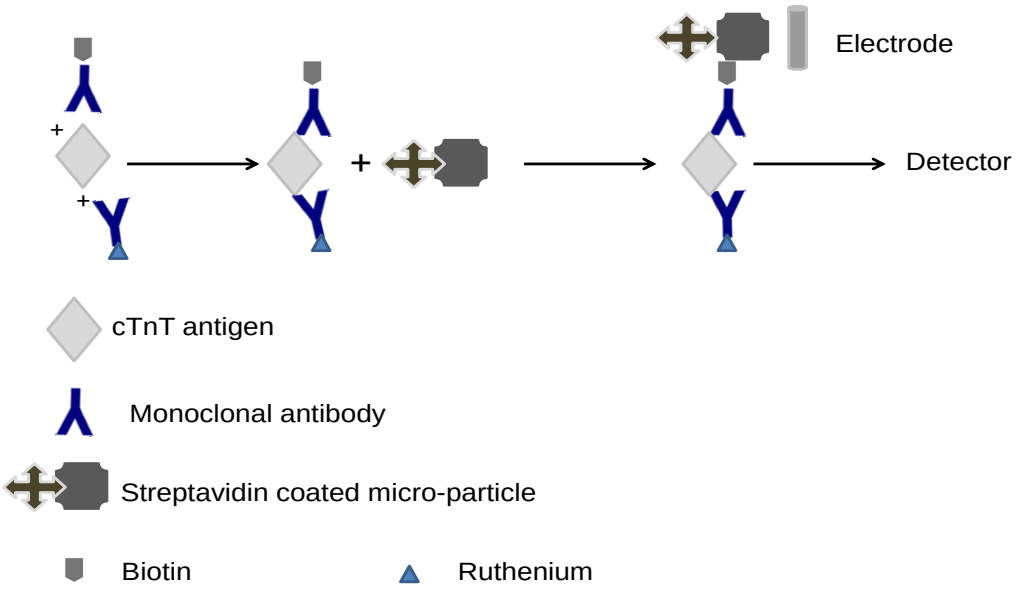

Figure 27: Hs-cTnT Roche is an electrochemiluminescent immunoassay "sandwich", utilising two different monoclonal antibodies with different label specifically directed against human cTnT. These two monoclonal antibodies recognise two different epitopes located in the center of human cTnT. The first monoclonal antibody recognises amino acid 125-131 and is labeled with ruthenium and the second recognises 136-147 and is labeled with biotin. Once the immune-complex is formed streptavidin coated micro-particle is added (solid phase). Finally, the application of a voltage causes the complex to emit light. The amount of light is proportional to the concentration of the unknown troponin in patient's sera. (By Roche Diagnostics).

3.17.2 Assay principle

The first incubation involves the introduction of 50 µL patient's sera with a biotinylated monoclonal anti-cTnT antibody and a monoclonal anti-cTnT antibody labeled with a ruthenium to form a complex. The second incubation involves the addition of streptavidin-coated microparticles; the complex becomes bound to the solid phase via the interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound particles are then washed with tris buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The results are determined by a 2-point calibration and a master multipoint (5-point) calibration curve.

3.18 Roche Diagnostics Elecsys® 2010 System

3.18.1 N-terminal pro B-type natriuretic peptide

N-terminal pro B-type natriuretic peptide quantification using Roche Diagnostics Elecsys® is an electrochemiluminescent immunoassay (ECLIA) (Figure 28).

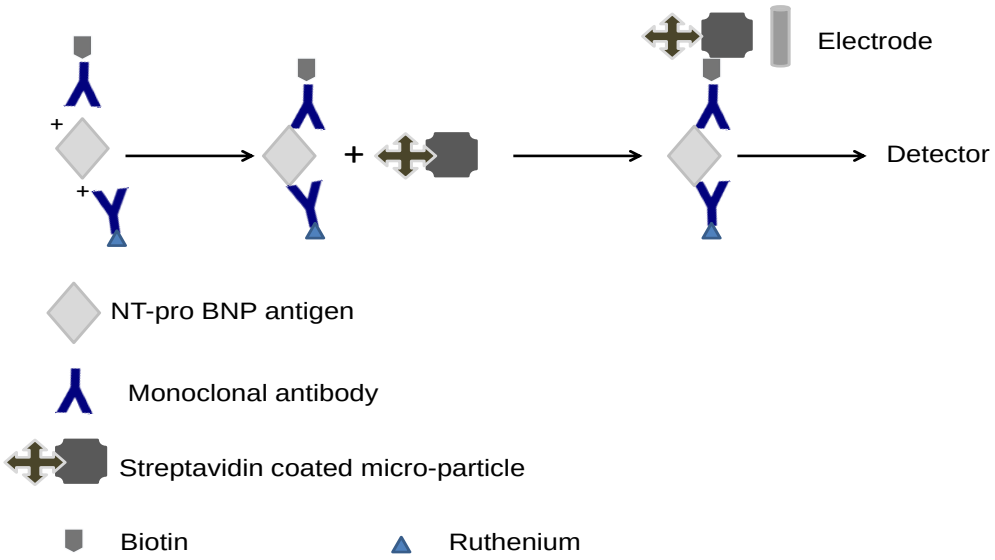

Figure 28: NT-pro-BNP Roche diagnostic is an electrochemiluminescent immunoassay “sandwich”**;** utilising polyclonal antibodies labeled with biotin and ruthenium and is specifically directed against human NT-pro-BNP. These polyclonal antibodies recognise the epitopes located in the N-terminal part (1-76) of NT-pro-BNP (1-108). The biotinylated and the ruthenium polyclonal anti-NT-pro-BNP form a complex with human NT-pro-BNP (By Roche diagnostic).

3.18.2 Assay principle

NT-pro-BNP quantification is a two steps sandwich immunoassay. The protocol is as follow:

The first incubation involves the introduction of 20 µL of patient’s sera with biotinylated polyclonal anti-NT-pro-BNP antibody and a polyclonal anti-NT-pro-BNP antibody labeled with a ruthenium. The second incubation involves the addition of streptavidin-coated microparticles; the complex becomes bound to the solid phase via the interaction of biotin and streptavidin. The reaction

mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound particles are then washed with tris buffer. Finally, the application of a voltage to the electrode induces chemiluminescent emission which is measured by a photomultiplier. The results are determined by a 2-point calibration and a master calibration curve.

3.19 The B-R-A-H-M-S Copeptin KRYPTOR®

3.19.1 Time-Resolved Amplified Cryptate Emission Technology

The B-R-A-H-M-S Copeptin KRYPTOR® (Brahms, Hennigsdorf, Germany) measured the C-terminal precursor portion of arginine vasopressin by a sandwich immunoassay. KRYPTOR® method uses Time-Resolved Amplified Cryptate Emission Technology (TRACE technology); which measure the signal from an immune-complex with time delay (Figure 29). TRACE technology is based on a non-radioactive energy transfer between a donor typically Europium (Eu) cryptate and an acceptor such as algal light collecting protein XL665. The assay uses two copeptin specific monoclonal antibodies labeled with either Eu or XL665. During the immuno-complex formation, the emission from the cryptate (Eu) and the absorption from the acceptor (XL665) cause an increased fluorescent signal of the cryptate and subsequently extend the life span of the acceptor signal. This immuno-complex interaction allows the measurement of temporarily delayed fluorescence. The signal is proportional to the concentration of the analyte to be measured. The analytical characteristic of the B-R-A-H-M-S Copeptin KRYPTOR® are summarised in table 14.

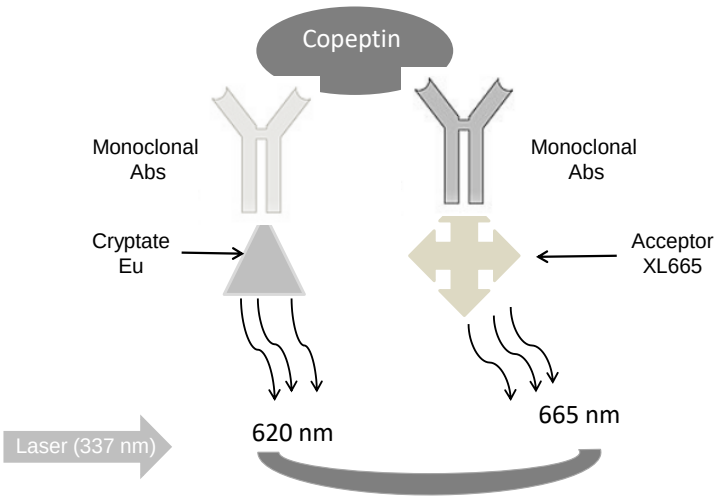

Figure 29: Copeptin detection using Eu cryptate labeled anti-human Abs and an anti-human Abs labeled with XL665. When the immuno-complex containing the protein of interest, in this case copeptin is excited with a nitrogen laser at 337 nm, the donor emits a long-life fluorescent signal (millisecond) in the range of 620 nm while the acceptor generates a short-life signal (nanosecond) at 665 nm. The combined signal generated from the immuno-complex is measured at 665 nm; the long-life signal is proportional to the concentration of the analyte to be measured. (Courtesy of Brahms, Hennigsdorf, Germany).

3.20 The albumin cobalt binding (ACB®) assay

3.20.1 Indirect colorimetric method

The original biochemical test for IMA was known as the albumin cobalt binding assay (ACB®). The assay is based on the principle that IMA cannot bind cobalt a consequence of damage to the N-terminus damage. The assay measures

the cobalt binding capacity of albumin in a sample of serum. A known amount of cobalt is added to the patient serum sample and allowed to interact with IMA if present. Dithiothreitol (DTT) is added which binds any remaining unbound cobalt and the colorimetric change is measured spectrophotometrically. In serum from non-ischaemic patients, cobalt binds to the N-terminus, leaving little free cobalt to react with DTT and form a colored product. Conversely, in serum obtained from ischemic patients, cobalt does not bind to the N-terminus of IMA, leaving more free cobalt to react with DTT and form a darker color. The absorbance produced by the darker color is proportional to the amount of IMA present in the serum (Figure 30).

3.20.2 Assay protocol

200 µL of patient sera is allowed to mix with 50 µL cobalt chloride (1 mg/L). Incubate at room temperature for 10 min. 50 µL Dithiothreitol (1.5 g/L) is added an allowed to incubate for a further 2 min. Finally, a 1.0 mL of NaCl (9.0 g/L) is added the solution to top the reaction. The absorbance is measured at 470 nm. The absorbency is proportional to the concentration the antigen in this case cobalt and DTT.

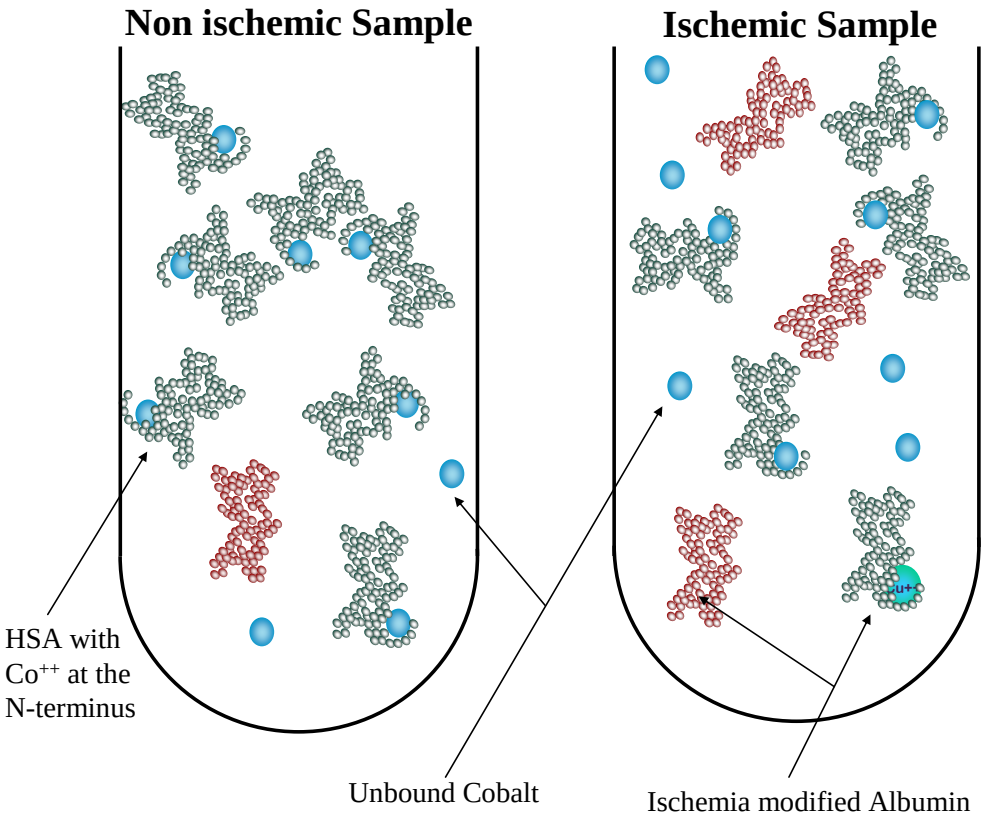

Figure 30: Measurement of ischemia modified-albumin by the Albumin Cobalt Binding Assay (ACB®). A known amount of cobalt chloride ($CoCl_2$) is added to a serum sample. Dithiothreitol is added which binds unbound Co^{++} causing a colorimetric change which can be read spectrophotometrically (Courtesy of Dr D Gaze St George's Healthcare. London).

3.21 Statistical methods

Non-parametric statistics were used to analyse demographics and patient characteristics. The receiver operating characteristics cure (ROC) and the calculation of the area under the curve (AUC) were used to examine individual biomarkers on admission and at 90 min after admission. ROC curve was also used to assess the diagnostic efficiencies of delta changes in absolute concentration and percentages change values (on admission minus 90 min values). Diagnostic test comparison was carried out using AMI as the dichotomous variable (negative or positive for AMI). Contingency tables (using

predefined cut-off values and delta values) for individual and multiple biomarkers were used to compare sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). All statistical analysis including, 95% confidence interval, Fisher exact tests were performed using Analyse-it® for Microsoft Excel (version 2.21; www.analyse-it.com).

3.22 Economic evaluation of ischaemia modified-albumin

3.22.1 Economic analysis

Economic analysis for the introduction of IMA was based on two scenarios. The first scenario cost minimization analysis (CMA) was used to establish whether a single cardiac biomarker i.e. IMA, H-FABP and copeptin would have the same diagnostic and prognostic efficiency compared to the existing cardiac biomarkers of necrosis such as hs-cTn or a panel of cardiac and novel biomarkers. The second scenario a decision-analysis and cost benefit analysis were used; the aim was to establish whether single biomarkers or a combination of different biomarkers using the delta changes could be used to achieve high diagnostic and prognostic efficiency.

This model applied (Figure 31) different scenarios in patients (n = 174) presenting to the ED with chest pain suggestive of AMI. The model also assumes that the sample was taken on admission and the results were available within 90 min. The model also assumes that medical staffs in the ED are available 24 h and a decision regarding patient's management is made within 1 h of the results being available. It was also assumed that hs-cTn is the "gold standard" for the diagnosis of AMI and the true positive would be confirmed at 10-12 h; whereas, the true negative and the false positive will be discharged at 10-12 h. Standard hs-cTn testing at 10-12 h as recommended by NICE (NICE, 2010). The cost accrued during a patient's management will depend on the number of tests carried out and the length of time spent in hospital. The use of hs-cTn is financially justified as an early accurate

diagnosis of AMI and its use can potentially reduce the duration of hospital admission. In addition, discharging patients on the same day attracts a tariff benefit in the National Health Service (NHS) system (Health, 2012-2013; Thokala,Goodacre,Collinson *et al.*, 2012).

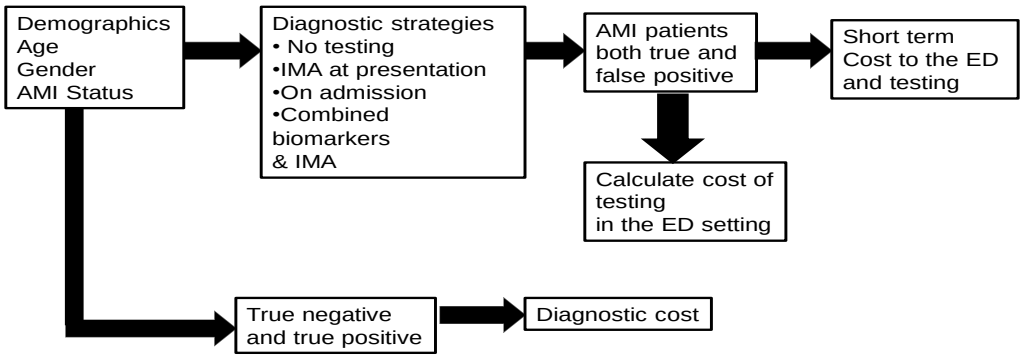

Figure 31: Basic cost-benefit analysis model structure. (St George's Healthcare NHS Trust. London)

The following diagnostic strategies were applied only to the cardiac biomarkers with the appropriate diagnostic efficiency i.e. the best sensitivity and specificity for the diagnosis of AMI:

1. Discharge without investigation.
2. IMA negative discharge.
3. Discharge if IMA and hs-cTn negative or admit if clinical suspicion exists.
4. Admit if both IMA and hs-cTn are positive.

3.22.2 Current study sample size calculation

Table 16: Current project sample size calculation using http://clincalc.com/stats/samplesize.aspx software.

Study parameter	
Population incidence	7%
Incidence study group	5%
Alpha	0.05
Beta	0.2
Power	0.8
Sample size	1168

In the current project n = 1168 evaluable subjects on admission and at 90 min after admission would be expected to have 80% power to detect a 5% absolute difference in primary outcome at the two significant levels of 5%. Unfortunately, the samples (n = 174) acquired in this project was under powered and not expected to provide 80% power to detect 2% absolute difference (2% vs. 4%) in major adverse cardiac events.

Chapter 4

Results

-

This chapter describes the results of the present study utilising conventional biomedical tests and a range of novel biomarkers that could potentially be used in the ED for the diagnosis and triage of low risk patients with chest pain suggestive of AMI.

4.1 Population

The RATPAC trial recruited 2263 patients between 30 January 2007 and June 2008; 1125 were randomised to POCT arm. Two sets of blood were drawn; the first on admission (sample 1) and the second at 90 min from admission (sample 2). Both sets of serum samples were measured by the participating center for conventional cardiac troponin I (AccuTnI) or T (Centaur cTnI), hs-cTnI (Beckman) or hs-cTnT (Roche). However, the following biomarkers including myoglobin, CK-MB mass, copeptin and H-FABP, and IMA were measured at St George's Healthcare NHS trust. The subjects were followed for major adverse cardiac events (MACE) and final outcomes were recorded as follows: 3% recorded AMI (36 patients), 1% death (6 patients), 2% ACS hospitalization without AMI (18 patients), <1% non-fatal AMI, 1% severe arrhythmia (6 patients), 1% emergency revascularization (10 patients). The final recorded event i.e. AMI between two arms was not statistically significant, despite the fact that the study found more patients with AMI from the POCT compared with standard arm (90/1125 vs. 72/1118).

Surplus samples were retained for the Randomised Assessment of Treatment using Panel Assay of Cardiac Biomarkers-Contemporary Biomarker Evaluation (RATPAC-CBE), in total 847 out of 850 participants had their cTn status established. The available data includes median age, final diagnosis, cardiac biomarkers results and MACE. They also had data regarding the median time (495 min range 95-46.6 min and the interquartile range (IQR) was 310-738 min) from the onset of chest pain until the last cTn measurement.

When considering all participants 33.5% had blood taken in less than 6 h from chest pain 65.4% were taken ≥ 6 h from the onset of chest pain. Thus, the majority of patients had their troponin measurement performed according to the ESC.

The Stratus® CS analyser was not considered to be a high sensitivity cardiac troponin assay; however, it satisfied the criteria as outlined by the guidelines of an acceptable assay and therefore was used in the RATPAC-CBE study (Apple, 2009).

The Roche Cobas MIRA® Plus analyser was used to measure IMA concentrations. Surplus blood samples of n = 645 (76%) were available for this study and n = 210 where insufficient for analysis. In total, 444 (69%) samples were included in the study and 348 (54%) samples had matched and full paired data i.e. two sets of blood were drawn on admission (sample 1) and the second samples at 90 min after admission (sample 2) and frozen at -20 ^{0}C until analysis (Figure 32). Samples for this study (n = 444) were organised into batches of 10 samples and analysed within 30 min of thawing at room temperature. Prior to sample thawing, external quality controls (low, medium and high) were included in every run. IMA assay was calibrated according to manufacturer recommendation.

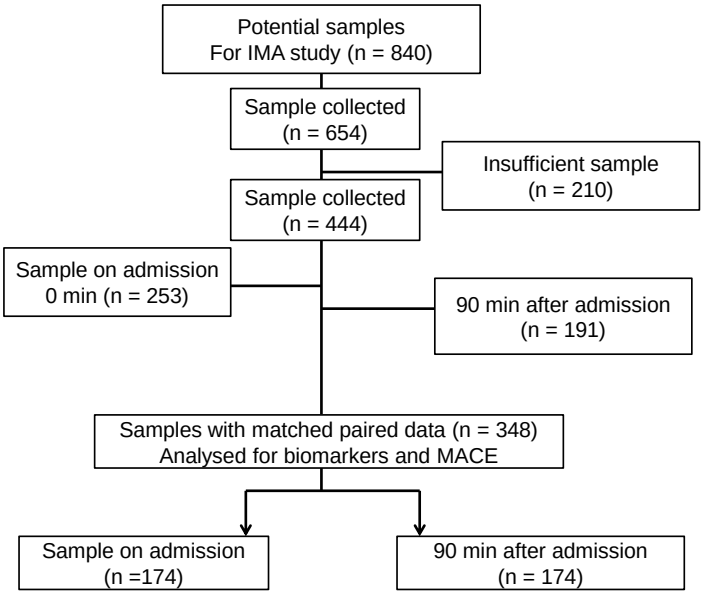

Figure 32: Sample available for IMA study. A total of 645 out of 840 unmatched samples were available for analysis, a further, n = 210 were insufficient and not analysed. The remaining n = 444 from which 253 were obtained on admission and another 191 at 90 min after admission. A total of n = 444 samples was analysed for hs-cTnT, hs-cTnI, cTnI (conventional), copeptin, H-FABP, myoglobin, CK-MB and IMA. A total of 348 out of the 444 available samples had a complete matched and paired data including concentration values for hs-cTnT, hs-cTnI, copeptin, H-FABP, myoglobin, CK-MB, IMA and final outcome of MACE on admission and 90 min after admission.

The simultaneous use of multiple biomarkers of cardiac injury (cTn), neurohormonal (NT-pro-BNP), cytoplasmic (myoglobin, CK-MB and H-FABP) and vascular stress (copeptin and IMA) was based on the argument that these biomarkers may rise before the release of hs-cTn and may also challenge the ECG when inconclusive (Giannitsis,Becker,Kurz *et al.*, 2010; Goodacre, 2011; Hjortshoj,Kristensen & Ravkilde, 2010).

The diagnostic strategies were compared with individual biomarkers values based on hs-cTnT > 99th percentile, CK-MB > 5 µg/L and myoglobin >95th percentile, delta CK-MB > 1.5 µg/L and myoglobin was defined as percentage of > 25% change from admission. These ranges of biomarkers were then compared with a combination of individual cytoplasmic, hs-cTn, conventional cTn and novel biomarkers on admission and at 90 min after admission. In addition, for each combination or delta value rule-in diagnostic of an AMI i.e. specificity and PPV and rule-out i.e. the sensitivity and NPV were calculated. The clinical cut-off for all biomarkers is summarised in table 17. Hs-cTnT was used as the predicate device as it is standardized. The lack of standardization of hs-cTnI is generally due to the heterogeneous nature of the circulating cTn, the monoclonal antibodies engineered specificity and commercial pressures (Clerico,Fortunato,Ripoli *et al.*, 2008). Currently, cTnI immunoassay does not directly correlate from technique to technique. Thus, consequently patient's results produced by a particular assay are not transposable (Chenevier-Gobeaux et al., 2015). Changing patient's management using different cTnI may become unreliable.

Table 17: Clinical cut-off values used to assess the diagnostic efficiency for all biomarkers.

Test	Manufacturer	Clinical cut-off
IMA	Inverness Medical	127 KU/L
AccuTnI (conventional)	Beckman	42 ng/L
hs-cTnI	Beckman	27 ng/L
TnI-Ultra(conventional)	Siemens	40 ng/L
Hs-cTnT	Roche	14 ng/L
H-FABP	Randox	2.5 mg/L
Myoglobin	Randox	66 mg/L
Copeptin	Randox	17.4 pmol/L
NT-pro-BNP	Siemens	125 ng/L
CK-MB	Randox	5 µg/L

The first phase of the evaluation aims at establishing reference population and the diagnostic efficiency of IMA measured on a Cobas Mira Plus analyser alone and utilising all the available data for hs-cTnI, hs-cTnT, copeptin, NT-pro-BNP, CK-MB (mass), myoglobin and H-FABP. The diagnostic efficiency of panel approach was compared with that of single biomarkers and single cardiac biomarker combinations utilising the final diagnosis. The second phase of the evaluation aims at assessing the cost-benefit of IMA assay alone and in combination with hs-cTnT.

4.1.1 Ischemia modified-albumin reference population

Sixty-six samples obtained from normal, healthy individuals were used to estimate the reference ranges for IMA. Normal, healthy individuals are defined as free of cardiac and renal disease (i.e. no history of myocardial infarction, heart failure, hypertension, dyslipidemia) and no history of cancer. The population will consist of 35% male and 65% female. Samples were analysed (Roche Cobas Mira Plus) for Ischaemia modified-albumin in replicate.

Redundant serum samples of only (n = 66) from patients attending general practice for routine checks were selected for inclusion in the reference range study. Samples were included and stored at -20 ^{0}C in the present study if they meet the exclusion criteria (table 18). The median age was 55 years (interquartile range 50 to 62 years), 23 (35%) subjects were male and 43 (65%) were female. There is no statistically significant difference between the two groups (p = 0.4) summary data in table 19.

Table 18: Exclusion criteria for reference population for the present study.

- Known coronary heart disease
- Known coronary heart disease.
- Confirmed serious non-cardiac pathology (e.g. pulmonary, embolus)
- Co-morbidity that require hospital admission
- Abnormal chemistry results (e.g. renal profile, liver profile and lipid profile)

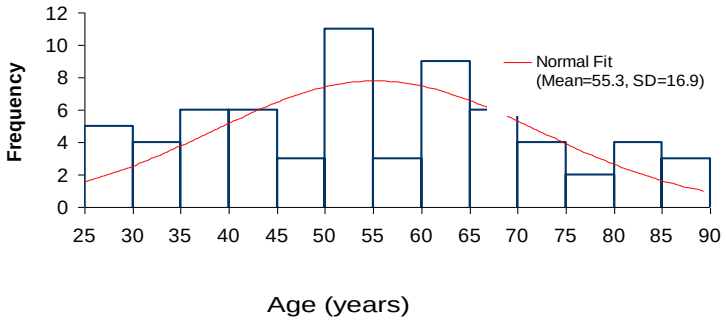

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Figure 33: Age distribution of reference range subjects (n = 66). The median IMA was 122 KU/L, (interquartile range, 121-124 KU/L). The data was non-parametrically distributed and there was no effect of gender on IMA concentration (p = 0.407) (table 19). The non-parametric 95% (CI) upper limit of normal reference interval was 127 KU/L (95% CI, 126-127 KU/L).

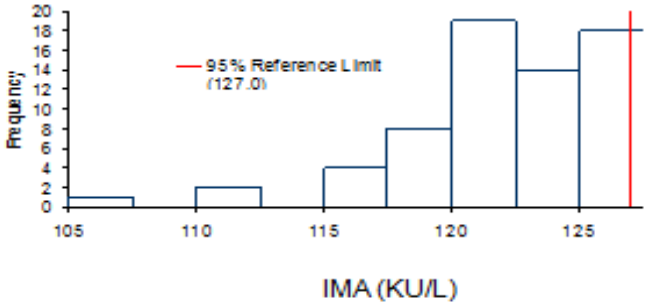


Figure 34: 95% (CI) Upper limit of Normal (URL) for IMA is 127 KU/L calculated from the reference population (n = 66).

Table 19: Control group summary data

	Proportion	Mean	SD	SE	P value
Female (n = 43)	65%	122.1	4.2	0.63	0.407
Male (n = 23)	35%	121.2	4.2	0.87	0.407

Table 19: Showed the proportion of female and male participation in the establishment of the reference interval. It also summarises the mean IMA concentration by gender. There is no statistically significant difference between the two groups (p = 0.4).

4.2 Analytical evaluation of IMA® assay, by Cobas Mira plus analyser

Aim

To evaluate the precision characteristic of the 3rd generation IMA® assay; according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2 (Second edition 2004). EP5-A2 document is designed to harmonise the laboratory methods for a comprehensive establishment of the precision capability of a method (the 3rd generation IMA® assay) it is also designed to help user to verify the validity of performance claims of manufacturers and can also be used by those laboratories how want to improve or modify an existing method i.e. changes in protocol steps or reagents.

4.2.1 Preliminary precision evaluation

4.2.1.1 Device familiarisation

This first step consists of five days and is designed to allow the operator to familiarise him/her self with the device characteristic including maintenance, reagent preparation, and program input. The device familiarization is also designed to highlight any major problem with the device (Roche Cobas Mira plus). Once the familiarisation period of the device (Cobas Mira plus analyser) is finished the evaluation of repeatability should commence. The NCCLS also recommend that the five operating days could be exceeded until data were obtained without operational difficulties.

Three IMA® samples (pooled from different patients) with three different concentrations (30/60 KU/mL, 66/105 KU/mL and > 211 KU/ml) will be evaluated for IMA. The data obtained will be calculated for standard deviation (SD) and the coefficient of variance (CV %); if significant discrepancy is found

between the manufacturers and the assessing laboratory, the manufacturers will be contacted and the experiment (analytical evaluation of IMA® assay) will be stopped until the source of the problem is found and resolved. In this stage even if problem is found the acceptability of the device will not be made based on these findings.

4.2.1.2 Protocol familiarisation period

The following steps should be performed each day for five days:

1. Analyse two run or batches per day
2. If run is rejected due to quality control non-conformance or operational errors/difficulties, an additional run after an investigation is carried out to identify and remedy the source of the problem.
3. Within each run or batches, analyse two aliquot of test material for each concentration used.
4. Include at least one control in each run.
5. Change the order of the analysis of quality control and test material each day or run.
6. To replicate the actual operation, include a least ten patients in each run if possible.
7. Segregate runs by at least two hours

4.2.1.3 Precision evaluation experiment

The precision evaluation protocol according to the NCCLS EP5-A2 documents requires twenty days to achieve. The NCCLS also recommend that the data collected during the five operating days without operational difficulties can be incorporated into the estimation of precision. Establishing the precision in twenty days, allows the incorporation of day-to-day impression introduced through analytical errors.

This involves estimating the repeatability and within-laboratory precision for a single device (Cobas Mira plus analyzer). The objective of the precision evaluation is to estimate the precision of Cobas Mira plus analyzer. The precision evaluation is designed to assess the precision of Cobas Mira plus analyzer when used over a long period of times, thus accounting for the influence of several source of variability including operator, temperature, and reagent stability.

The NCCLS recommend a minimum of twenty operating days (using single lot) to achieve an acceptable precision evaluation. ACB® evaluation using Cobas Mira analyzer is considered a short-run method since its duration is less than two hours. However, NCCLS also allows for 5 days precision evaluation if the device is used every day; which would be the case with Roche Cobas Mira plus if IMA assay is implemented.

Roche Cobas Mira plus analyzer requires calibration every 5 days or when internal control is outside the specified ranges or lot changes. Each day of the experiment requires two separate runs i.e. am and pm (or separated with at least two hours); each run must include two test samples with two different concentrations and at least two quality controls. The 3rd generation ACB® assay precision evaluation will include three quality controls i.e. Low, medium and high. Quality control procedure will be established using Westguard rules protocol. The data obtained will be calculated for standard deviation (SD) and the coefficient of variance (CV %) every five operating days of the experiment.

Data may not be rejected without good reason as this could underestimate the precision of the method/device.

4.2.2 Protocol of precision evaluation of IMA® assay (Cobas Mira analyzer).

The following steps shall be taken each day:

1. Analyse twenty aliquot of patient's sample with tree different concentration.

    a. Level Low (30 to 60 kU/mL) in duplicate twice day (at least two hours between run)

    b. Level medium (66 to 105 kU/mL) in duplicate twice day.

    c. Level high (110-136 kU/mL) in duplicate twice day

    d. Include quality control (R1) from ACB® in every run and a negative control (serum from healthy individual IMA < 30 kU/mL).

2. If run is rejected due to quality control non- conformance or operational errors/difficulties, an additional run after an investigation is carried out to identify and remedy the source of the problem.
3. Within each run or batches, analyse two aliquot of test material (kit control) for each concentration used.

4. Change the order of the analysis of quality control and test material each day or run.
5. Segregate runs by at least two hours
6. Plot the quality control values in the chart according to Westguard rules.

4.2.2.1 Linearity study

Linearity is the *"measure of the degree to which a curve approximates a straight line".* Linearity refers to overall system response (i.e., the final analytical answer rather than instrument output) (NCCLS, 1996). Linearity (Figure 35) is measured by testing levels of ACB® concentrations that are known and relative to each other.

Linear equation:

$$y = \alpha + \beta x$$

- The variables x and y are the generalised input and output variables, β is the slop and α is the intercept.

Slop (represented by β) the relationship between the change is y and the change is x between any two point (x_1, y_1 and x_2, y_2) along straight line.

$$\beta = \frac{\Delta y}{\Delta x} = \frac{y_2 - y_1}{x_2 - x_1}$$

There are many experimental approaches to evaluate linearity of a system (NCCLS, 1996). The NCCLS recommend up to five concentrations of the analyte (IMA) to undergo multiple measurement of four replicate and should

take one day to establish. The data obtained is plotted on the x and y axis and the outlier is visually inspected and if necessary removed. Once the satisfactory data is obtained the controlled independent variable model is applied for the detailed statistical evaluation of linearity. This includes the calculation of tolerance limits at various analyte (IMA) concentrations.

The sample matrix (the best hierarchy) that will be used to establish the ACB® linearity should be close to the sample used in real clinical setting. Thus, samples for ACB® linearity study will be drawn from patients. The patient specimen will contain ACB® level of 30% higher than the upper linearity limit that will be diluted with another patient's specimen with low IMA® concentration.

Analytical sequence

The analytical sequence should be random and separated by a blank if significant carryover is present.

Sample preparation and value assignment

The samples are coded as follows:

1. Two IMA pools of (10 mL) of sufficient volume for linearity evaluation are obtained.
2. The low concentration pool which is near the limit of linearity is code No. 1 on the other hand the highest IMA concentration pool is coded No. 5.
3. The intermediate concentration pools are made from the dilution of pool No. 1 & No. 5. These dilutions are related to each other and produce a constant interval. To produce an intermediate pools dilution the following procedure should be followed:

1. Pool (No. 2) is a mixture of 3 parts of the low pool (No. 1) and 1 part of the high pool (No. 5).
2. Pool No. 3 is a mixture of 2 parts of the low pool (No. 1) and 2 parts of the high pool (no. 5)
3. Pool No. 4 is a mixture of 1 parts of the low pool (No. 1) and 3 parts of the high pool (No. 5)

The concentration of pool No. 1 is C1 and the volume of No. 1 is V 1; moreover, the concentration of pool No. 5 is C 5 and the volume part of pool No. 5 is V 5. The concentration for each pool is defined by the following formula:

$$\text{Concentration} = (C1 * V1 + C5 * V5) \div (V1 + V5)$$

All the concentration and volume unit must be the same for each pool. Pre-analytical parameters such as mixing, protecting the pools from dehydration and general deterioration must be minimised or eliminated.

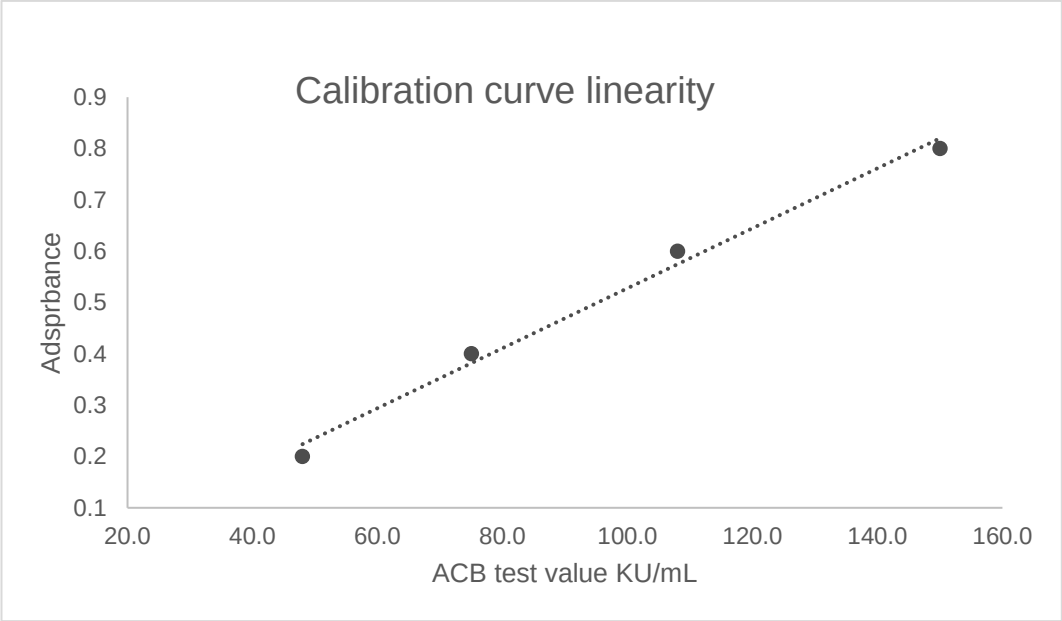


Figure 35: The ACB® test calibration curve is linear over the range of the calibration.

Table 20: ACB® assay imprecision study, reference ranges is between brackets.

Day	**Low control (30-60 kU/mL)**		**Mean**	**Medium control (66-105 kU/mL)**		**Mean**	**High control (110-136 kU/mL)**		**Mean**
	Dup 1	**Dup 2**		**Dup 1**	**Dup 2**		**Dup 1**	**Dup 2**	
1 (AM)	52	50	**51**	63	61	**62**	105	109	**107**
1 (PM)	46	48	**47**	67	66	**67**	108	110	**109**
2 (AM)	52	50	**51**	68	68	**68**	109	110	**110**
2 (PM)	48	49	**49**	64	66	**65**	109	104	**107**
3 (AM)	48	49	**49**	67	67	**67**	110	109	**110**
3 (PM)	49	51	**50**	66	64	**65**	106	111	**109**
4 (AM)	49	47	**48**	65	64	**65**	108	110	**109**
4 (AM)	50	47	**49**	65	66	**66**	110	110	**110**
5 (AM)	46	47	**47**	63	64	**64**	105	107	**106**
5(PM)	49	48	**49**	63	59	**61**	103	107	**105**
Mean		49			65			108	
STD		1.51			2.18			1.73	
CV%		3.11			3.36			1.60	
Manufacturers stated CV%									
CV%		5.4			5.7			4.4	

Table 20 showed that the imprecision study of the albumin cobalt binding assay (ACB®). CV% values for all three internal quality controls comparable to that specified by the manufacturers.

4.2 IMA concentration on admission and 90 min after admission

The difference in IMA concentration between admission and at 90 min after admission was statistically significant (p = 0.002) (Figure 36).

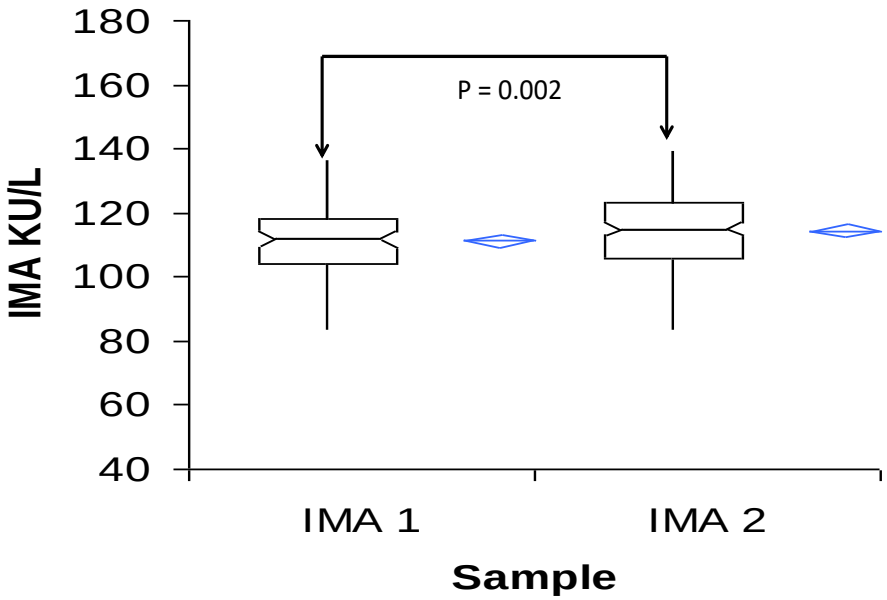


Figure 36: Distribution of IMA concentrations on admission (n = 174) and at 90 min after admission (n = 174). IMA 1 = on admission, IMA 2 = 90 min after

admission. There is a statistically significant difference between IMA concentration on admission and 90 min after admission (P = 0.002).

The box-and-whisker plot presented in Figure 36 above were used to present the preliminary data spread of IMA concentrations in both group of low risk patients presenting to ED with chest pain suggestive of AMI on admission and 90 min after admission. There is statistically significant difference between the two groups (p = 0.002).

Table 21: Final diagnostic categorisation of patients who participated in the present study.

Diagnostic categorisation	Final diagnostic IMA subset (n = 174), n (%)	Biomarkers Subset (n=840), n (%)	Final diagnostic All patients (n = 1125), n (%)
Median age (year)	52 (44-63)	54 (44-64)	53 (44-64)
Male	82 (47)	507 (60)	683 (61)
Female	92 (53)	343 (40)	442 (39)
Angina, no-ACS	16 (9)	72 (9)	83 (7)
Musculoskeletal pain	19 (11)	116 (14)	143 (13)
Gastro-oesophageal	21 (13)	100 (12)	124 (11)
ACS	7 (4)	68 (8)	90 (8)
Anxiety	7 (4)	31 (4)	36 (3)
Chest infection	5 (3)	-	-
Non-specific chest pain	69 (40)	279 (33)	361 (32)
Self-discharge	1 (1)	-	-
Others	20 (12)	154 (18)	228 (20)
Unknown	9 (5)	30 (4)	60 (5)
Normal ECG	131 (75)	-	-
Abnormal non-conclusive ECG	26 (15)	-	-
Pathological ECG	17 (10)	-	-

Values in parentheses represent percentage

4.3 Novel biomarker and conventional biomarker evaluation of the diagnostic accuracy for acute myocardial infarction

The diagnostic efficiency in detecting AMI on admission and 90 min after admission was calculated using the area under the curve (AUC) (Figure 37, 38, 39 and 40). On admission sample measured for novel biomarker of NT-pro-BNP and H-FABP were diagnostically efficient with AUC of 93% (95% CI, 88-97%) and 78% (95% CI, 68-89%) respectively (table 22). IMA on admission has a diagnostic efficiency with AUC of the ROC curve of 54% (95% CI, 43-66%) compared to 58% (95% CI, 35-81%).at 90 min after admission. In contrast, collectively the samples measured on admission and at 90 min after admission for cTn were the most diagnostically efficient biomarkers with AUC of the ROC curve ranging from 85%-90% and 84%-88% respectively.

Cardiac troponin measurement for diagnosis of AMI, were diagnostically superior to CK-MB, myoglobin and novel biomarkers on admission and 90 min after admission (table 22).

Table 22: Area under the ROC curve for all biomarkers.

Test (n = 253) admission	AUC %	95% Confidence interval (95% CI)
Myoglobin	76	(67-86)
CK-MB	71	(59-83)
AccuTnI	85	(76-94)
hs-cTnI	88	(81-95)
hs-cTnT	100	(-)
TnI-Ultra	90	(83-97)
H-FABP	78	(68-89)
Copeptin	58	(48-68)
NT-pro-BNP	93	(88-97)
IMA	54	(43-66)
Test (n = 191) 90 min after admission		95% Confidence interval (95% CI)
Myoglobin	72	(57-87)
CK-MB	62	(32-92)
AccuTnI	88	(71-100)
hs-cTnI	78	(54-100)
hs-cTnT	100	(-)
TnI-Ultra	84	(65-100)
H-FABP	65	(40-90)
Copeptin	63	(38-89)
NT-pro-BNP	52	(26-78)
IMA	58	(35-81)

(-) = predicate test

Table 22: The area under the curve (AUC) of each biomarker on admission and 90 min after admission. Hs-cTnT > 14 ng/L was chosen to classify patients having and AMI. The diagnostic performances of myoglobin, CK-MB, conventional cTn, hs-cTn, copeptin, NT-pro-BNP and IMA on admission and at 90 min after admission were compared. In this group IMA had a poor diagnostic efficiency on admission and at 90 min after admission with an AUC

of 54% (95% CI, 42-66%) and 58% (95% CI, 35-81%) respectively compared to other biomarkers. On admission and 90 min after admission conventional; cTn and hs-cTn were diagnostically superior compared to all biomarkers. However, NT-pro-BNP was also diagnostically superior on admission with AUC of ROC curve of 93% (95% CI, 88-97%) but a poor diagnostic efficiency at 90 min after admission with AUC of ROC curve of 52% (95% CI, 26-78%).

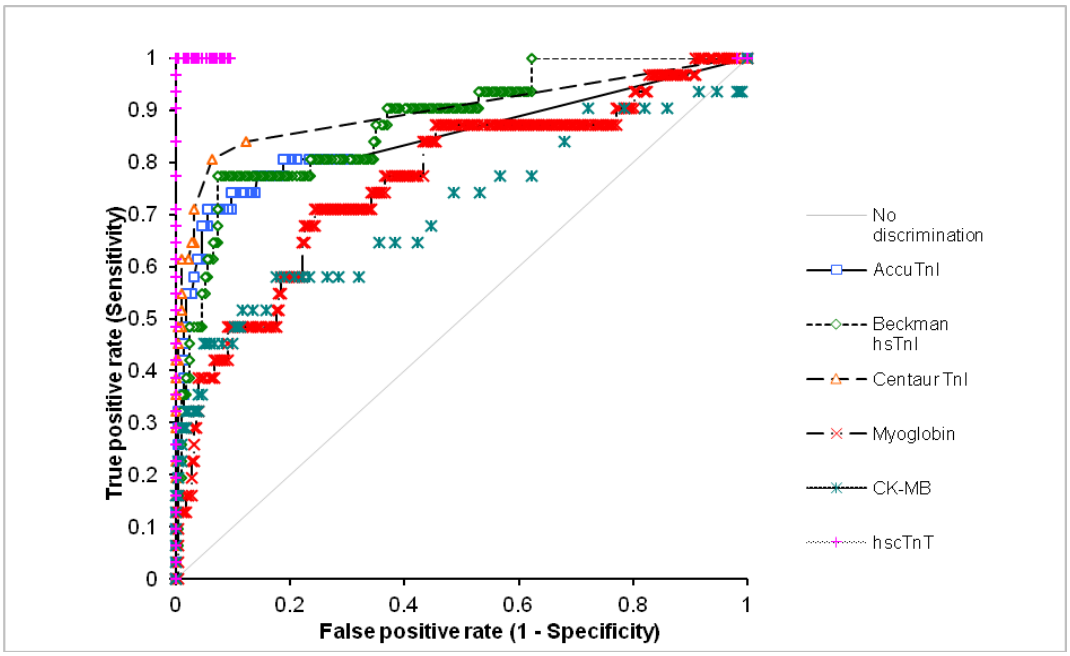


Figure 37: A ROC curve for Accu TnI 85% (95% CI, 76-94%), Beckman hs-TnI 88% (95% CI, 82-95%), Beckman TnI 90% (83-97%), myoglobin 76% (95% CI, 67-86%) and CK-MB 71 (95% CI, 59-83%) measurement on admission for the diagnosis of an AMI (n = 253). Hs-cTnT > 14 ng/L was chosen to classify AMI patients.

A ROC curve constructed as shown in figure 37 indicates the diagnostic performance of hs-cTn, Accu TnI, Beckman hs-TnI, CK-MB and myoglobin. The clinical cut-off point for all biomarkers are summarised in table 17. AUC results are summarised in table 22. Hs-cTnT > 14 ng/L was chosen to classify AMI patients.

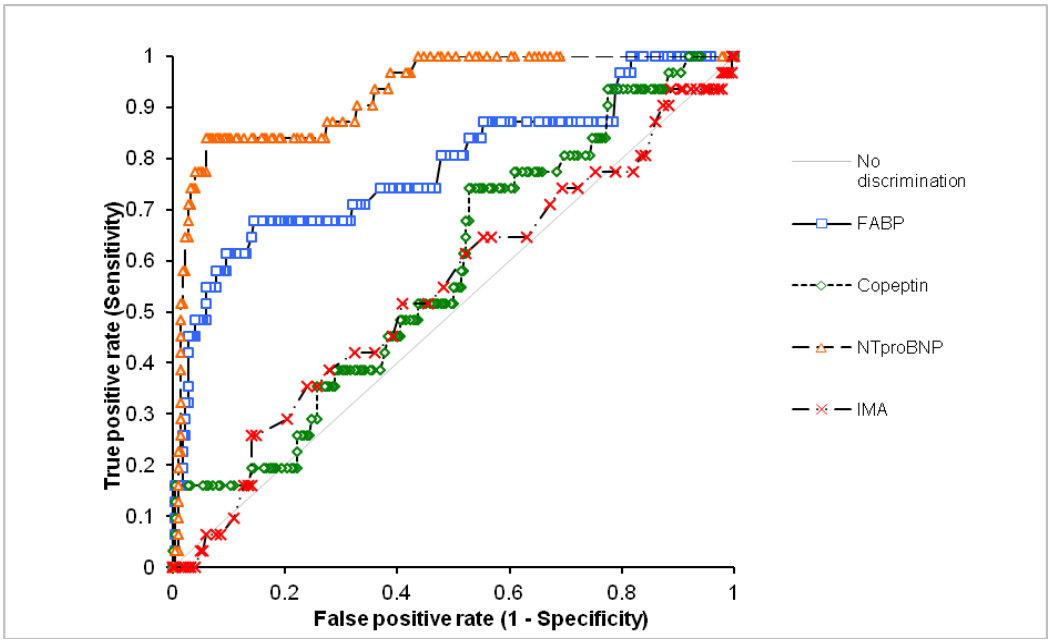


Figure 38 A ROC curve for novel biomarkers H-FABP 78% (95% CI, 68-89%), copeptin 58% (95% CI, 48-68%), NT-pro-BNP 93% (95% CI, 88-97%) and IMA 54% (95% CI, 43-66%) measurement on admission for the diagnosis of an AMI (n = 253). Hs-cTnT > 14 ng/L was chosen to classify AMI patients.

A ROC curve constructed as shown in figure 38 indicates the diagnostic performance of H-FABP, copeptin, NT-pro-BNP and IMA on admission. The clinical cut-off point for all biomarkers are summarised in table 17 Hs-cTnT > 14 ng/L was chosen to classify patients having and AMI.

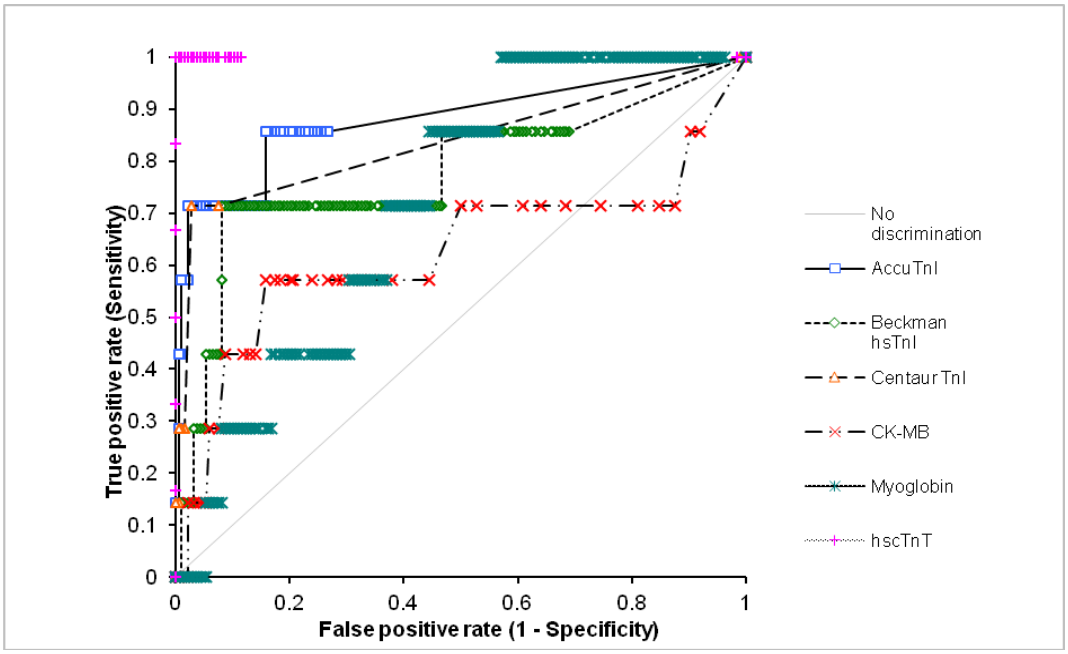


Figure 39: A ROC curve for biomarkers Accu TnI 88% (95% CI, 71-100%), Beckman TnI 77% (95% CI, 54-100%), Centaur TnI 84% (95% CI, 65-100%), CK-MB 62% (95% CI, 32-92%) and myoglobin 72% (95% CI, 57-87%) measurement for the diagnosis of AMI at 90 min after admission (n = 191). Hs-cTnT > 14 ng/L was chosen to classify AMI patients.

A ROC curve constructed as shown in figure 39 indicates the diagnostic performance of hs-cTn and convention cTn, CK-MB and myoglobin. The clinical cut-off point for all biomarkers are summarised in table 17. AUC results are summarised in table 22. Hs-cTnT > 14 ng/L was chosen to classify AMI patients.

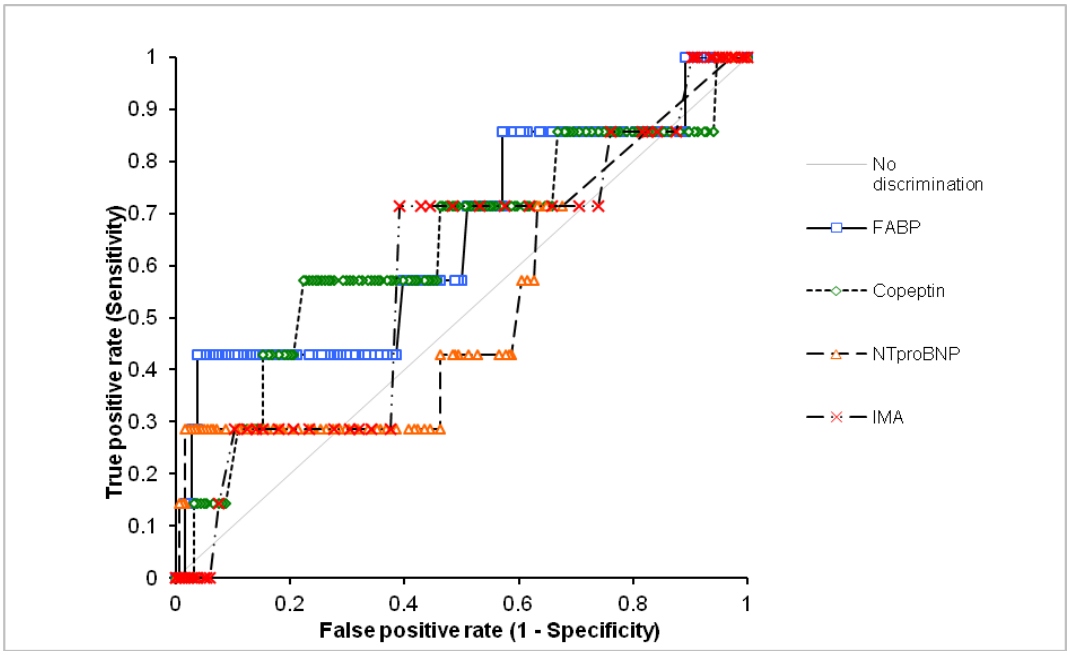


Figure 40: A ROC curve for novel biomarkers of h-FABP 51% (95% CI, 41-60%), copeptin 53% (95% CI, 38-68%), NT-pro-BNP 52% (95% CI, 38-66%) and IMA 57% (95% CI, 43-72%) measurement for the diagnosis of an AMI (n = 191). Biomarkers measured at 90 min after admission.

A ROC curve constructed as shown in figure 40 indicates the diagnostic performance of H-FABP, copeptin, NT-pro-BNP and IMA at 90 min after admission. The clinical cut-off point for all biomarkers are summarised in table 17. Hs-cTnT > 14 ng/L was chosen to classify patients having and AMI.

Table 23: Data used for the ROC curve construction when analysing various cardiac biomarkers at 90 min after admission. Hs-cTnT was used as a predicate test. SE is standard error. The ROC curve is presented as an area under the curve and 95% confidence interval (95% CI).

Test	Area	95% CI	SE
H-FABP	51	41 to 62	0.055
Copeptin	53	38 to 68	0.075
NT-pro-BNP	52	38 to 66	0.071
IMA	57	43 to 72	0.074

CI = Confidence interval. SE = Standard error

Summary data of biomedical biomarkers that are translated into ROC curve. (appendix 3 for additional data)

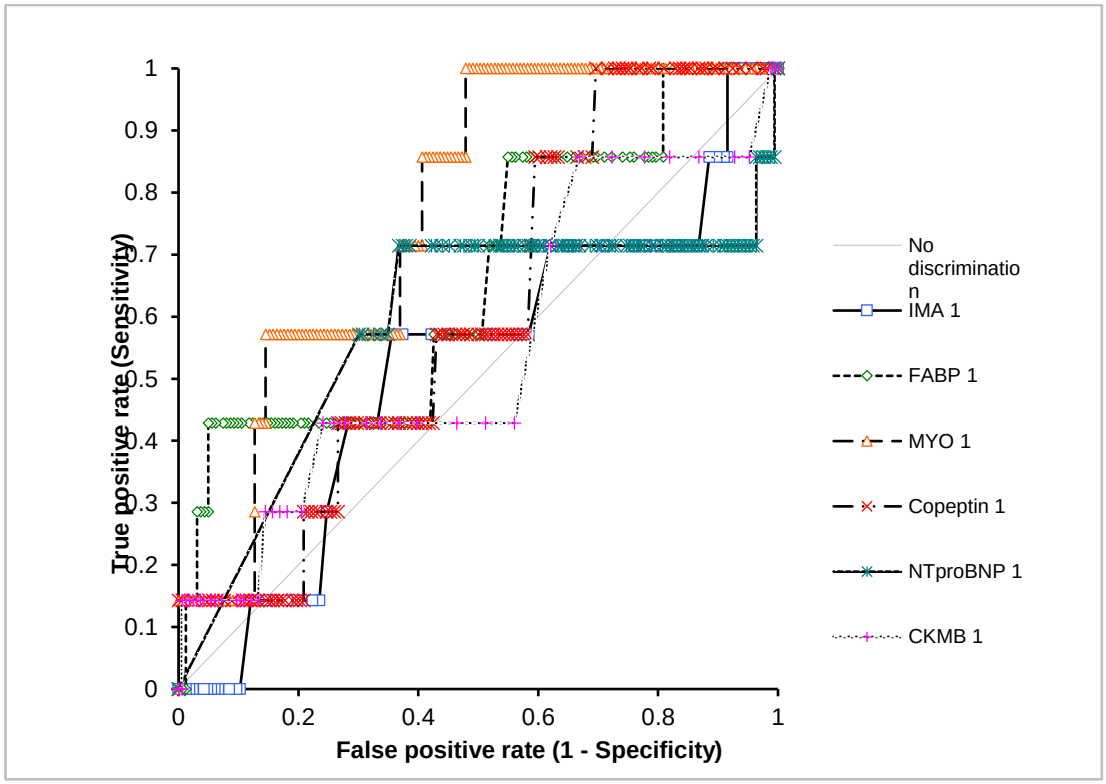


Figure 41: A ROC curve for novel biomarkers IMA 52% (95% CI, 18-76%), H-FABP 66% (43-89%), Myoglobin 76% (95% CI, 62-99%), copeptin 60% (95% CI, 41-89%), NT-pro-BNP 58% (95% CI, 29-87%) and CK-MB 55% (95% CI, 29-80%) measurement on admission for the diagnosis of an AMI (n = 174). Hs-cTnT > 14 ng/L was used to classify AMI patients.

Table 24: Data used for the ROC curve construction when analysing various cardiac biomarkers on admission. Hs-cTnT was used as a predicate test. SE is standard error. The ROC curve is presented as an area under the curve and 95% confidence interval (95%).

Test	Area	95% CI	SE
IMA	52	28 to 76	0.124
H-FABP	66	43 to 89	0.120
MYO	76	62 to 90	0.072
Copeptin	60	41 to 80	0.098
NT-pro-BNP	58	29 to 87	0.149
CK-MB	55	29 to 80	0.130

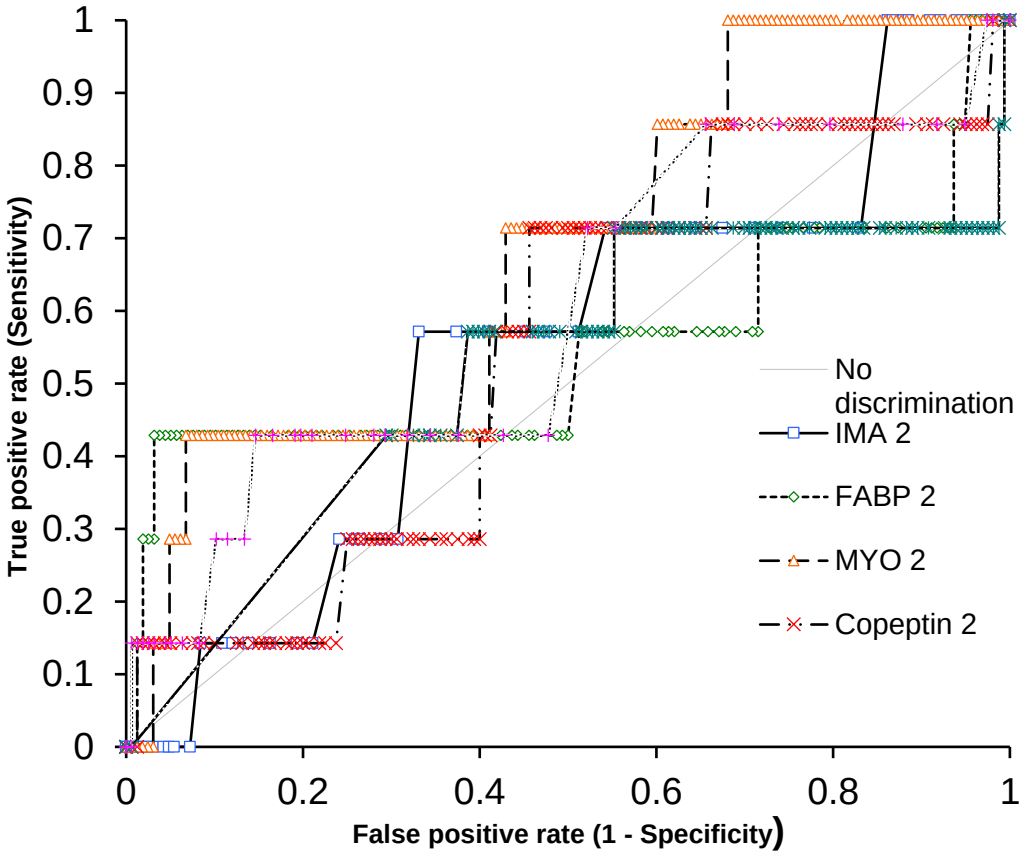


Figure 42: A ROC curve for novel biomarkers measurement at 90 min after admission for the diagnosis of an AMI (n = 174). Myoglobin 68% (95% CI, 47-88%), CK-MB 60% (95% CI, 34-85%), H-FABP 55% (95% CI, 23-87%) and Copeptin 55% (95% CI, 32-78%), NT-pro-BNP 52% (95% CI, 24-85%) and IMA 55% (95% CI, 32-87%). Hs-cTnT > 14 ng/L was used to classify AMI patients.

Table 25: Data used for the ROC curve construction when analysing various cardiac biomarkers at 90 min after admission. Hs-cTnT was used as a predicate test. SE is standard error. The ROC curve is presented as an area under the curve and 95% confidence interval (95%).

Test	Area	95% CI	SE
IMA	55	32 to 87	0.116
h-FABP	55	23 to 87	0.145
MYO	68	47 to 88	0.106
Copeptin	55	32 to 78	0.118
NT-pro-BNP	52	24 to 85	0.144
CK-MB	60	34 to 85	0.130

Table 26: Data used for the ROC curve construction when analysing various cardiac biomarkers on admission. Hs-cTnT was used as a predicate test. SE is standard error. The ROC curve is presented as an area under the curve with 95% confidence interval (95%).

Test	Area	95% CI	SE
Accu TnI 1	65	44-86	0.107
Beckman hs-TnI 1	57	36-78	0.107
Centaur TnI 1	52	52-65	0.069

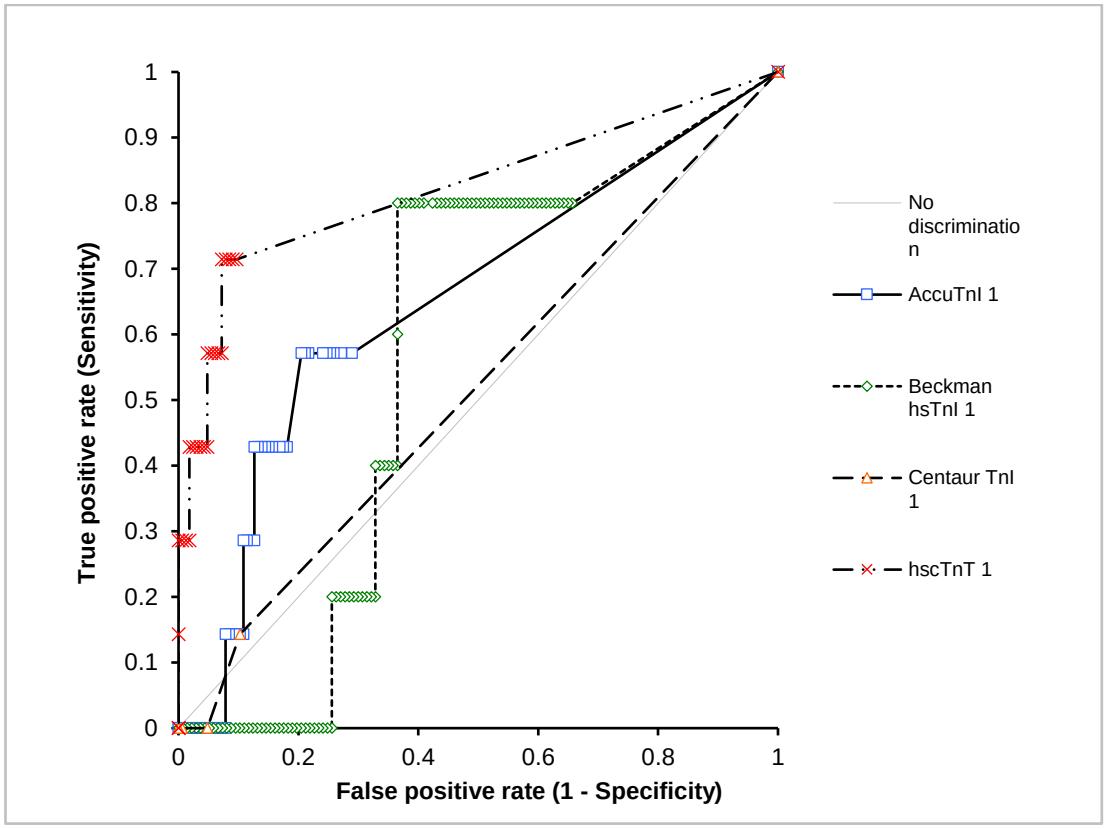


Figure 43: A ROC curve for novel biomarkers measurement on admission for the diagnosis of an AMI (n = 174). Accu TnI 65% (95% CI, 44-86%), Beckman hs-TnI 57% (95% CI, 36-78%) and Centaur TnI 52% (95% CI, 52-67%). Hs-cTnT > 14 ng/L was used to classify AMI patients.

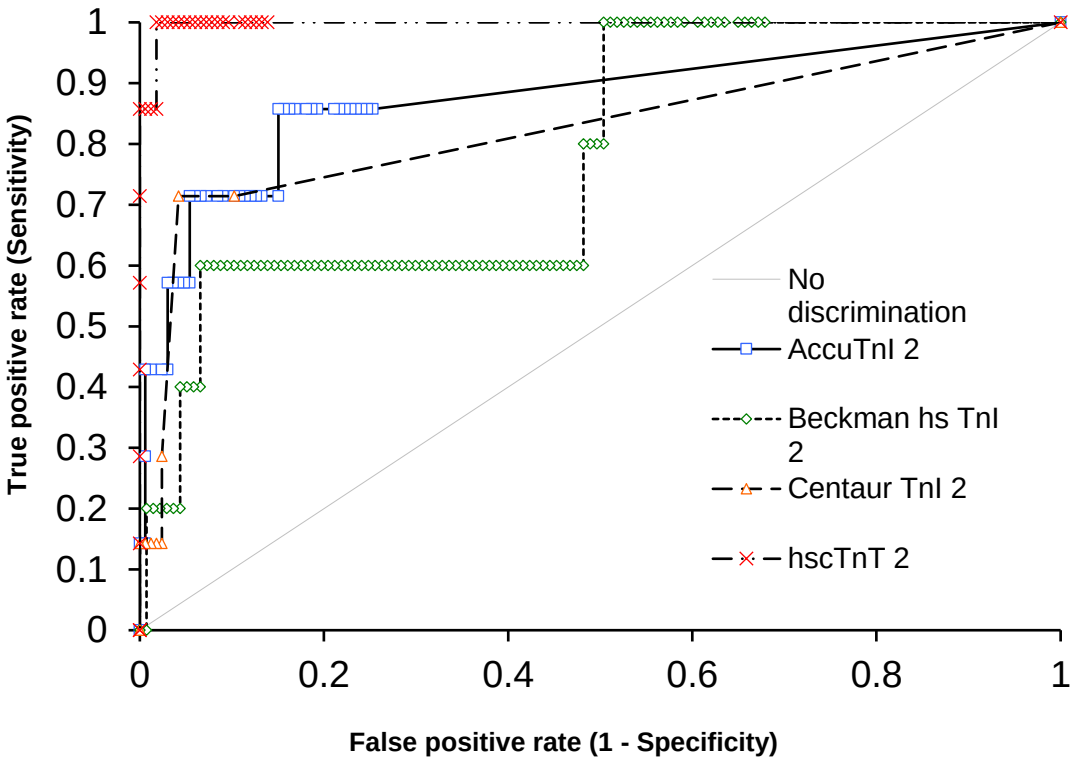


Figure 44: A ROC curve for novel biomarkers measurement at 90 min after admission for the diagnosis of an AMI (n = 174). Accu TnI 65% (95% CI, 44-86), Beckman TnI 57% (95% CI, 36-78) and Centaur TnI (95% CI, 52-65%). Hs-cTnT > 14 ng/L was used to classify AMI patients.

Table 27: Data used for the ROC curve construction when analysing various cardiac biomarkers on admission. Hs-cTnT was used as a predicate test. SE is standard error. The ROC curve is presented as an area under the curve with 95% confidence interval (95%). Hs-cTnT was used as a predicate, hence area under the cure of 1.00 (95% 0.99 to 1.00).

Test	Area	95% CI	SE
Accu TnI 2	88	71 to 1.00	0.086
Beckman hs-TnI 2	78	56 to 1.00	0.113
Centaur TnI 2	82	63 to 1.00	0.098
Hs-cTnT 2	1.00	99 to 1.00	0.003

Hs-cTnT is the predicate test

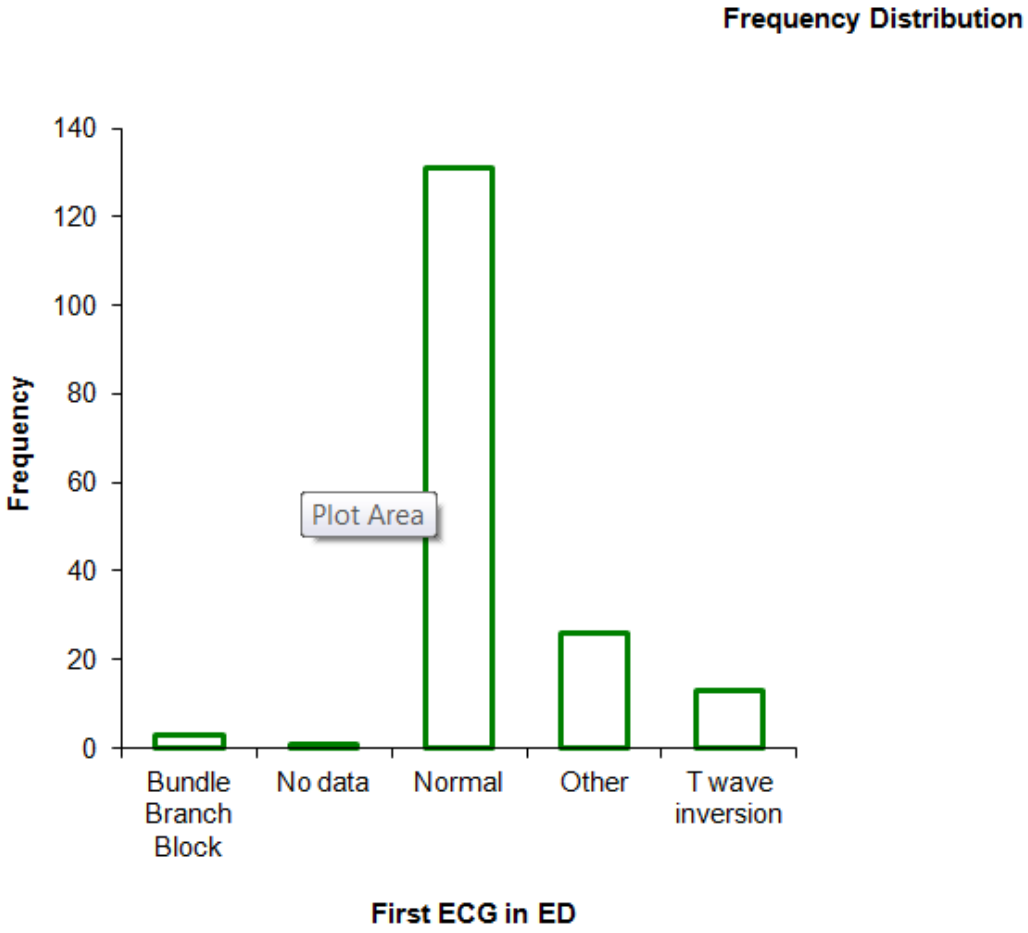


Figure 45: Current project participants (n = 174) ECG finding on admission. Bundle brunch block 1.7%, no data 0.6%, normal 73.5%, other 14.9% and T-wave inversion 7.5%.

Table 28: Current project summary data of ECG finding on admission

First ECG in ED	n	Proportion
Bundle branch block	3	0.017 (1.7%)
No data	1	0.006 (0.6%)
Normal	131	0.753 (75.3%)
Other	26	0.149 (14.9 %)
T wave inversion	13	0.075 (7.5%)

Table 28: Approximately 75% (n=131) of the current project participants had a normal ECG on admission to ED with chest pain suggestive of ACS. Only 1.7% (n = 3) bundle blanch block (BBB). 7.5% (n = 13) had a t-wave inversion.

4.4 Combined novel biomarker and conventional biomarker evaluation of the diagnostic accuracy for acute myocardial infarction

The combination of the diagnostic efficiency of IMA, hs-cTn, cTn, H-FABP, NT-pro-BNP and copeptin to rule-in or rule-out diagnosis of AMI was assessed by the construction of contingency tables utilising the 99th percentile cut-off for hs-cTnT and the 95th percentile cut-off for H-FABP, IMA and copeptin (table 29). The combined sensitivity and specificity of all biomarkers are summarised in table 29.

Table 29: Combined diagnostic efficiency of IMA and other biomarkers

Test (n = 253)	**Sensitivity % (95% CI)**	**Specificity% (95% CI)**	**PPV**	**NPV**
IMA + Myoglobin	32 (21-45)	93 (88-96)	55	84
IMA + CK-MB	38 (23-55)	91 (87-94)	39	91
IMA + AccuTnI†	49 (33-65)	93 (89-96)	52	92
IMA + hs-cTnI	47 (32-63)	94 (89-96)	55	91
IMA + hs-cTnT	71(56-82)	100 (98-100)	100	94
IMA + TnI-Ultra†	54(39-67)	96 (93-98)	74	91
IMA + H-FABP	41 (27-56)	93 (89-96)	55	89
IMA + Copeptin	13 (4-38)	88 (83-91)	6	94
IMA + NT-pro-BNP	52 (39-65)	98 (94-99)	84	89
Test (n = 191)	**Sensitivity % (95% CI)**	**Specificity% (95% CI)**	**PPV**	**NPV**
IMA + Myoglobin	6 (2-18)	97 (93-99)	29	82
IMA + CK-MB	-	96 (92-99)	-	90
IMA + AccuTnI	14 (5-35)	98 (94-99)	43	90
IMA + hs-cTnI	8 (2-25)	97 (93-99)	29	88
IMA + hs-cTnT	16 (13-46)	99 (97-100)	86	91
IMA + TnI-Ultra	15 (7-31)	98 (96-100)	71	85
IMA + H-FABP	12 (4-30)	98 (94-99)	43	88
IMA + Copeptin	-	96 (92-98)	-	91
IMA + NT-pro-BNP	8 (2-24)	97 (93-99)	29	87

† = non-sensitive troponin assay. Values in parentheses represent 95% confidence interval. PPV = positive predictive value. NPV = negative predictive value.

Table 29: presents the combined sensitivity and specificity of each parameter. Hs-cTnT > 14 ng/L was chosen to classify patients having and AMI. The diagnostic performance of IMA plus Centaur cTnI on admission has a sensitivity of 54% (95% CI, 39-67%) and a specificity of 96% (95% CI, 93-98%); the NPV and PPV were 91% and 74% respectively. The combined diagnostic efficiency of IMA and other biomarkers at 90 min after admission were very poor with sensitivity of < 50%; in contrast the specificity was very good ranging from 91%-98%. On admission the combined diagnostic efficiency for IMA plus hs-cTnT had sensitivity and a specificity of 71% (95 CI, 56-82%) and 100% (95% CI, 98-100%) respectively.

4.5 Prognostic role of ischemia modified-albumin when compared with other cardiac biomarkers

Receiver operating characteristic curve (Figure 46, 47, 48 and 49) at admission and 90 min after admission were constructed using the combined MACE of death, readmission with AMI, urgent revascularisation and readmission with unstable angina as the dichotomous variable.

Table 30: Receiver operating characteristic curve, for conventional cardiac biomarkers for the prediction of MACE. Biomarkers measured on admission and 90 min after admission.

Biomarkers	Area under the curve (AUC)	95% confidence interval (95% CI)
Sample 1 (n=253)		
TnI-Ultra	53	(43-64)
AccuTnI	53	(41-64)
hs-cTnI	52	(40-64)
hs-cTnT	52	(43-61)
CK-MB	59	(47-71)
Myoglobin	57	(46-69)
Sample 2 (n = 191)		
TnI-Ultra	56	(47-65)
AccuTnI	64	(52-75)
hs-cTnI	55	(40-71)
hs-cTnT	54	(45-64)
CK-MB	55	(41-69)
Myoglobin	52	(40-64)

Table 30: represents the AUC of each conventional biomarker for the prediction of MACE of death, readmission with AMI, urgent revascularisation and readmission with unstable angina as the dichotomous variable.

Table 31: Receiver operating characteristic curve, for novel biomarkers for the prediction of MACE. Biomarkers measured on admission and 90 min after admission.

Biomarker	Area under the curve (AUC)	95% confidence interval (95% CI)
Sample 1 (n = 253)		
IMA	52	(39-66)
H-FABP	50	(40-61)
Copeptin	59	(46-73)
NT-pro-BNP	51	(38-65)
Number of no MACE = 234 Number of MACE = 19		
Sample 2 (n = 191)		
IMA	57	(43-72)
H-FABP	51	(41-62)
Copeptin	53	(38-68)
NT-pro-BNP	52	(38-66)
Number of no MACE = 172 Number of MACE = 19		

Table 31: Represent the AUC of each novel biomarker for the prediction of MACE of death, readmission with AMI, urgent revascularisation and readmission with unstable angina as the dichotomous variable.

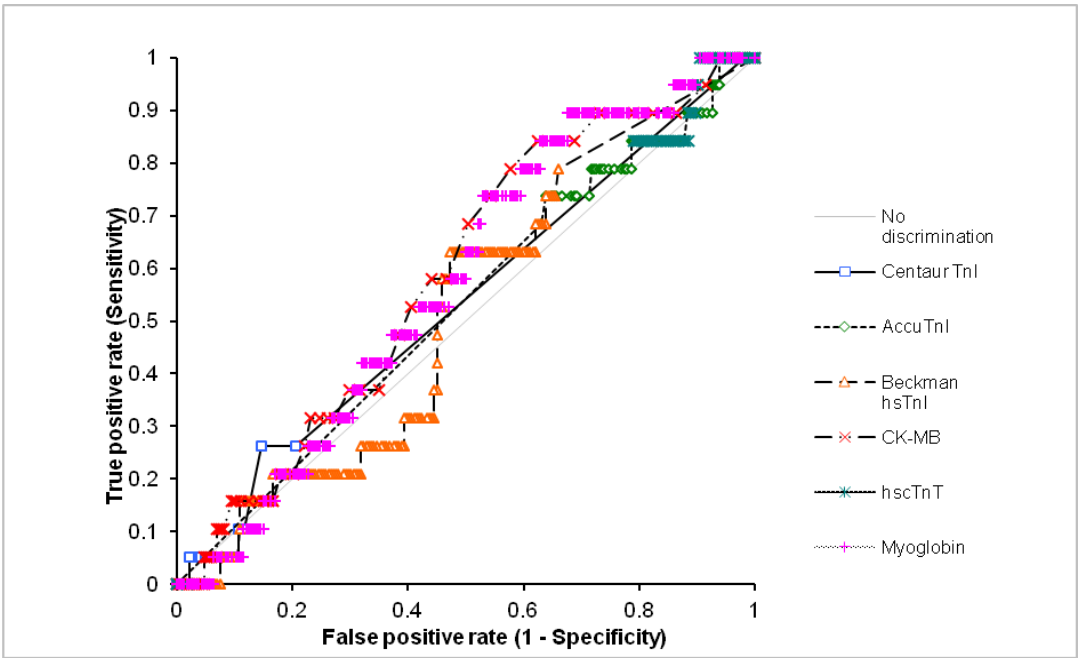


Figure 46: A ROC curve for conventional biomarkers measurement for the prediction of MACE (n = 253). Biomarkers measured on admission. The following biomarkers of necrosis Centaur TnI 53% (95% CI, 48-64%), Accu TnI 53% (95% CI, 41-64%), Beckman hs-TnI 52% (95% CI, 40-64%), hs-TnT 52% (95% CI, 43-63%), CK-MB 59% (95% CI, 47-71%), and myoglobin 57% (95% CI, 46-69%), were assessed for the predication of MACE. Revascularisation, UA, readmission with AMI and/or cardiac death was used as an outcome measure

A ROC curve constructed as shown in Figure 46 indicates the predictive value for hs-cTn, conventional troponin, CK-MB and myoglobin; the AUC of each novel biomarker for the prediction of MACE of death, readmission with AMI, urgent revascularisation and readmission with unstable angina as the dichotomous variable. The AUC values are summarised in table 31.

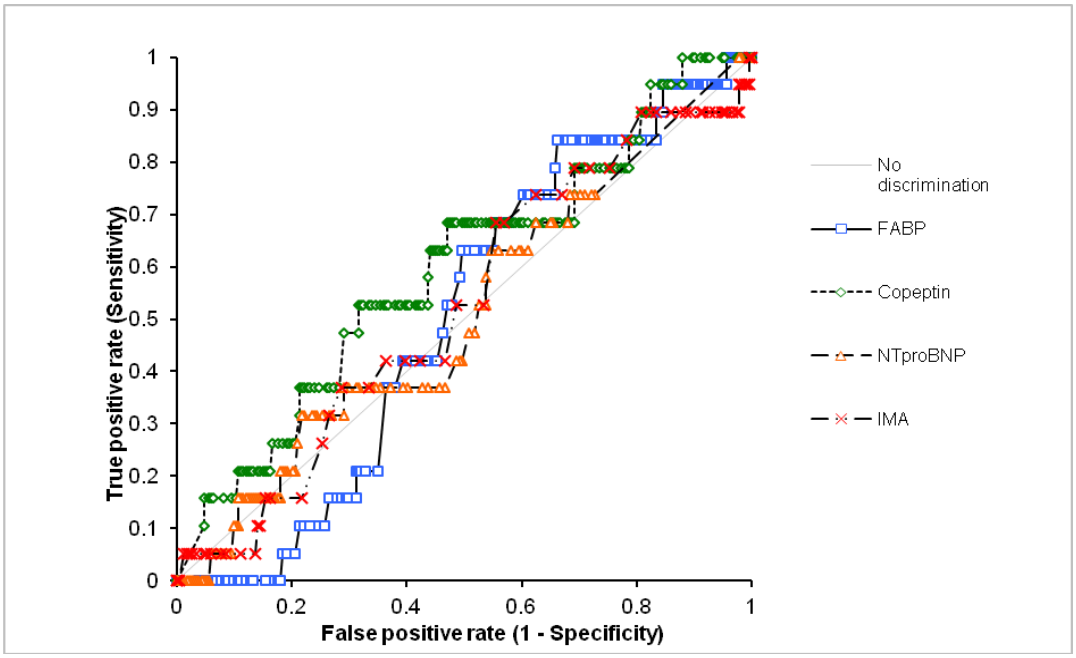


Figure 47: Receiver operating characteristic curve for novel biomarkers IMA 52% (95% CI, 39-66%), H-FABP 50% (95% CI, 40-62%), copeptin 59% (95% CI, 46-73%) and NT-pro-BNP 51% (95% CI, 38-65%) measurement for the prediction of MACE (n = 253). Biomarkers measured on admission. The following novel biomarkers of necrosis and cardiac ischaemia h-FABP, copeptin, NT-pro-BNP and IMA were assessed for the predication of MACE. Revascularisation, UA, readmission with AMI and/or cardiac death was used as an outcome measure.

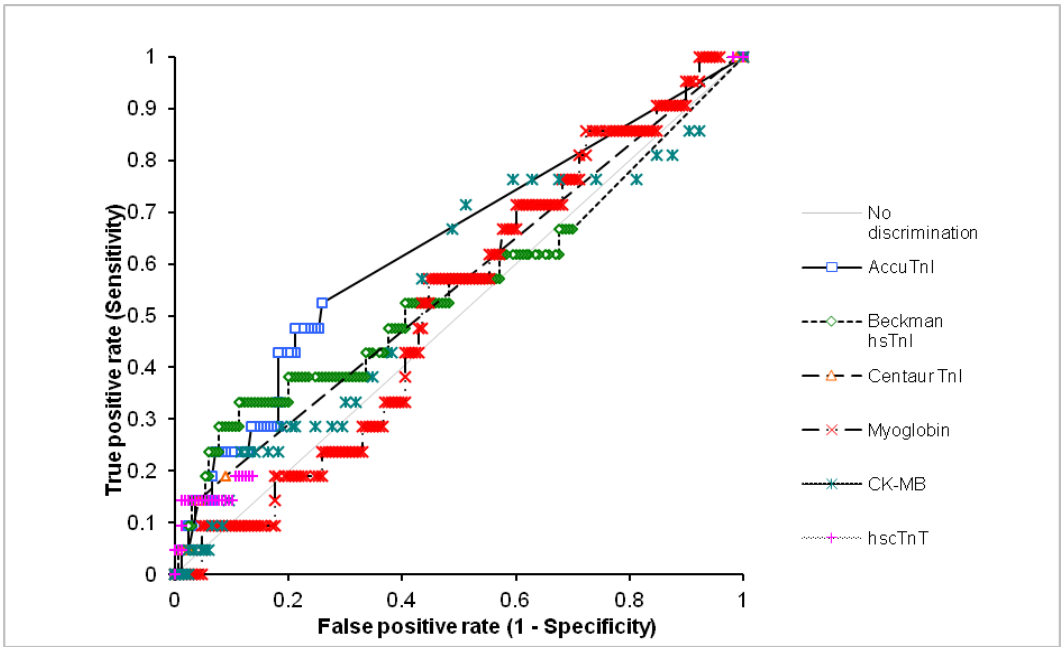


Figure 48: Receiver operating characteristic curve for conventional biomarkers measurement for the prediction of MACE (n = 191). Biomarkers measured on admission. The following biomarkers of necrosis Beckman TnI 56% (95% CI, 47-65%), Centaur TnI 53% (95% CI, 47-65%), Accu TnI 52% (95% CI, 40-64%), hs-TnT 52% (95% CI, 43-61%), CK-MB 59% (95% CI, 47-71%) and myoglobin 57% (95% CI, 46-69%) were assessed for the predication of MACE. Revascularisation, UA, readmission with AMI and/or cardiac death was used as an outcome measure.

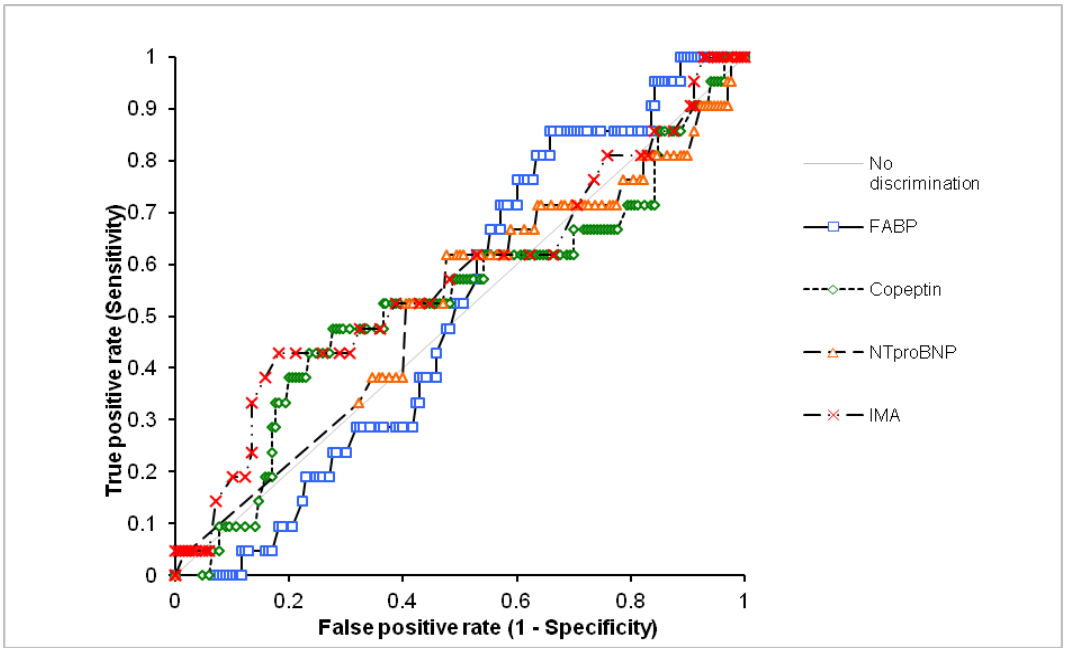


Figure 49: Receiver operating characteristic curve for novel biomarkers measurement for the prediction of MACE (n = 191). Biomarkers measured on admission. The following novel biomarkers of necrosis and cardiac ischaemia h-FABP 50% (95% CI, 40-61%), copeptin 59% (95% CI, 46-73%), NT-pro-BNP 51% (95% CI, 38-65%) and IMA 52% (95% CI, 39-66%) were assessed for the predication of MACE. Revascularisation, UA, readmission with AMI and/or cardiac death was used as an outcome measure.

4.5.1 Diagnostic efficiency of delta IMA concentration and other biomarkers, in paired samples

Delta values were calculated using a total of 174 patients with the full data set.

Receiver operating characteristic (ROC) curve on admission and at 90 min after admission were constructed using delta concentration values for all biomarkers (table 32 and 33). The delta values were calculated using the following equation:

ΔIMA = 100 x IMA (on admission) – IMA (90 min after admission) ÷ IMA concentration on admission

Table 32: Area under the curve of the absolute delta values and the percentage delta values of individual conventional and high sensitivity cardiac troponin biomarkers.

Assays (n = 174)	Area under the curve (AUC)	95% confidence interval (95% CI)
∆hs-cTnI	61	(33-89)
∆hs-cTnI%	56	(24-88)
∆TnI-Ultra	84	(67-100)
∆TnI-Ultra%	84	(67-100)
∆hs-cTnT	72	(35-100)
∆hs-cTnT%	72	(36-100)
∆AccuTnI	80	(56-100)
∆AccuTnI %	73	(47-98)

Table 32: As shown above represent the AUC of the absolute delta values and percentage delta values of troponins. Centaur TnI and Accu cTnI both has the best AUC for absolute delta value of 84% (95% CI, 67-100%) and 80% (95% CI, 56-100%) respectively. The AUC for the percentage delta values of Centaur TnI and Accu cTnI were 84% (95% CI, 67-100%) and 73% (95% CI, 47-98%) respectively. The global harmonised standardisation for cTnI is yet to be achieved.

Table 33: Area under the curve of the absolute delta value and the percentage delta values of individual novel biomarkers including myoglobin and CK-MB.

Analyte (n = 174)	Area under the curve (AUC)	95% confidence interval (95% CI)
ΔIMA	57	(39-76)
ΔIMA%	57	(38-76)
ΔH-FABP	55	(25-86)
ΔH-FABP%	58	(30-87)
ΔMyoglobin	60	(31-89)
ΔMyoglobin%	62	(35-88)
ΔCopeptin	53	(38-68)
ΔCopeptin%	54	(38-69)
ΔNT-pro-BNP	69	(49-89)
ΔNT-pro-BNP%	66	(47-84)
ΔCK-MB	61	(36-86)
ΔCK-MB%	66	(47-86)

Table 33: As shown above represent the AUC of the absolute delta values and percentage delta values of novel biomarker, myoglobin and CK-MB. NT-pro-BNP and CK-MB both has the best AUC for absolute delta value of 69% (95% CI, 49-89%) and 61% (95% CI, 36-86%) respectively. The AUC for the percentage delta values of NT-pro-BNP and CK-MB were 66% (95% CI, 47-84%) and 66% (95% CI, 47-86%) respectively. The AUC for IMA for both

absolute delta value and percentage delta values were 57% (95% CI, 39-76%) and 57% (95% CI, 38-76%) respectively.

4.6 IMA comparative predictive value on admission in the diagnosis of acute myocardial infarction using hs-cTnT as a predicate

Table 34: IMA diagnostic efficiency characteristics on admission; combined AMI and hs-cTnT were used as a predicate test.

IMA 1 by Biochem Dx AMI hs-cTnT	Mean	95% CI	SE	SD
AMI (n=7)	111.9	101.1 to 122.6	4.4	11.65
Non-AMI (n=166)	111.4	109.3 to 113.4	1.05	13.59

Table 35: IMA diagnostic efficiency characteristics on admission; combined AMI and hs-cTnT were used as a predicate test.

IMA by Biochem Dx AMI hs-cTnT	1st Quartile	Median	95% CI	3rd Quartile	Max	IQR
AMI (n=7)	100.5	116	95 to 127	119.7	127	19.2
Non-AMI (n=166)	104.5	112	109 to 114	118.2	150	14.2

Table 34 & 35: As shown above represent IMA diagnostic efficiency in detecting AMI on admission; when AMI and hs-cTnT were used as a predicate. The IMA values are presented as mean and median. 95% CI and quartile is also calculated.

4.7 IMA comparative predictive values on admission and at 90 min after admission in the diagnosis of acute myocardial infarction using IMA as a predicate

Table 36: IMA diagnostic efficiency characteristics at 90 min after admission; combined AMI and hs-cTnT used as a predicate.

IMA by Biochem Dx AMI hs-cTnT	Mean	95% CI	SE	SD
AMI (n=7)	112.7	103.4 to 122.0	3.79	10.03
Non-AMI (n=166)	111.6	112.5 to 116.7	1.05	13.55

Table 36: As shown above represent IMA diagnostic parameters at 90 min after admission; when AMI is used as a predicate. The IMA values are presented as mean, 95% CI, standard error (SE) and standard deviation (SD).

Table 37: IMA diagnostic efficiency characteristics at 90 min after admission; combined AMI and hs-cTnT used as a predicate.

IMA 2 by Biochem Dx AMI hs-cTnT	min	1st Quartile	Median	95% CI	3rd Quartile	Max	IQR
AMI (n=7)	98	105.8	110	98 to 125	123	125	17.7
Non-AMI (n=166)	59	106.0	115	113 to 117	123.5	160	17.0

Table 37: As shown above represent IMA diagnostic parameters at 90 min after admission; when AMI and Hs-cTnT were used as a predicate. The IMA values are presented as median, 95% CI, and quartile.

Table 38: Comparison of the magnitude of putative biomarkers on admission.

Contrast	Difference	95% CI			P value
IMA v H-FABP	-0.14	-0.53	to	0.25	0.4882
IMA v MYO	-0.24	-0.51	to	0.03	0.0771
IMA v Copeptin	-0.08	-0.35	to	0.19	0.5430
IMA v NT-pro-BNP	-0.06	-0.42	to	0.29	0.7303
IMA v CK-MB	-0.03	-0.37	to	0.31	0.8747
H-FABP v MYO	-0.10	-0.25	to	0.04	0.1654
H-FABP v Copeptin	0.06	-0.22	to	0.33	0.6930
H-FABP v NT-pro-BNP	0.08	-0.40	to	0.55	0.7511
H-FABP v CK-MB	0.11	-0.06	to	0.29	0.2062
MYO v Copeptin	0.16	-0.05	to	0.37	0.1290
MYO v NT-pro-BNP	0.18	-0.22	to	0.58	0.3762
MYO v CK-MB	0.22	0.05	to	0.39	0.0128
Copeptin v NT-pro-BNP	0.02	-0.42	to	0.46	0.9255
Copeptin v CK-MB	0.06	-0.15	to	0.26	0.5895
NT-pro-BNP v CK-MB	0.03	-0.47	to	0.54	0.8933

Table 38: showed a statistically significant difference between Myoglobin and CK-MB on admission (P = 0.01). As indicated above the remaining biomarkers did not demonstrate a statistically significant difference P > 0.5.

Table 39: Comparison of the magnitude of putative biomarkers at 90 min after admission.

Contrast	Difference	95% CI	P value
IMA v H-FABP	0.00	-0.25 to 0.25	0.9899
IMA v MYO	-0.13	-0.41 to 0.16	0.3781
IMA v Copeptin	0.00	-0.38 to 0.38	0.9983
IMA v NT-pro-BNP	0.03	-0.33 to 0.39	0.8759
IMA v CK-MB	-0.05	-0.36 to 0.25	0.7434
H-FAB v MYO	-0.13	-0.33 to 0.07	0.2128
H-FABP v Copeptin	0.00	-0.40 to 0.40	0.9953
H-FABP v NT-pro-BNP	0.03	-0.48 to 0.53	0.9158
H-FABP v CK-MB	-0.05	-0.28 to 0.18	0.6552
MYO v Copeptin	0.13	-0.12 to 0.38	0.3205
MYO v NT-pro-BNP	0.16	-0.29 to 0.60	0.4898
MYO v CK-MB	0.08	-0.05 to 0.21	0.2505
Copeptin v NT-pro-BNP	0.03	-0.43 to 0.49	0.9034
Copeptin v CK-MB	-0.05	-0.28 to 0.17	0.6551
NT-pro-BNP v CK-MB	-0.08	-0.56 to 0.40	0.7423

Table 39: As indicated above all biomarkers did not demonstrate a statistically significant difference $P > 0.5$.

4.8 Summary

The introduction of any novel biomarker into prime time use within the ED, must demonstrate the clinical usefulness with the aim of influencing patients management in a cost and effective manner. Presently hs-cTn are the most successfully biomarkers in an ED setting. The present study suggests that the combined diagnostic efficiency of IMA plus hs-cTnT or I may be clinically useful. The current study found no correlation exists between participant ages

and the concentration of different biomarkers on admission and at 90 min after admission. When using the combine diagnostic efficiency of hs-cTnT, and IMA as a predicate for the diagnosis of AMI, this was not statistically significant p > 0.5. On the other hand, hs-cTnT was statistically significant (p = < 0.0001) when predicting the final diagnosis of AMI. Myoglobin and CK-MB on admission were statistically significant (p = 0.013). A single reliable biomarker of cardiac ischaemia remains to be established.

4.9 RATPAC proposed sample size

The RATPAC trial estimated 3130 participants will be needed for the trial. Similar randomised trial estimated that 50% of the control group would be discharged (Goodacre,Nicholl,Dixon *et al.*, 2004). A total of 1565 participants in each arm and an estimated 80% power to detect a 5% an absolute difference in primary outcome (50% vs. 55%) at the two-sided significance level of 5% were expected. The sample for the two arms is expected to provide 80% power to detect 2% absolute difference (2% vs. 4%) in major adverse events e.g. non-fatal AMI or death. Based on previous studies the RATPAC team estimated that at least six hospitals will be required to recruit 550 suitable participants per year (Goodacre,Mason,Arnold *et al.*, 2001; Goodacre,Nicholl,Dixon *et al.*, 2004). Previous study has demonstrated an expected response rate of 70-80% for postal questionnaire (Goodacre,Nicholl,Dixon *et al.*, 2004).

In the current project we calculated (Appendix 5) n = 1168 evaluable subjects on admission and 90 min after admission would be expected to have 80% power to detect a 5% absolute difference in primary outcome at the two significant levels of 5%. Unfortunately, the sample (n = 174) acquired in this project were under powered. Thus, the samples used in this project were not expected to provide 80% power to detect 2% absolute difference (2% vs. 4%) in major adverse.

4.10 Summary of correlations between participant ages and various cardiac biomarkers

Table 40: Summary of correlations between participant ages and various cardiac biomarkers

Participants age vs., biomarkers concentration	R^2 value
Age vs. IMA on admission	0.0039
Age vs. IMA min after admission	0.0052
Age vs. Accu TnI on admission	0.0105
Age vs. Accu 90 min after admission	0.0031
Age vs. Beckman hs-c TnT on admission	0.0054
Age vs. Beckman hs-c TnT 90 min after admission	0.0054
Age vs. Centaur TnI on admission	0.0004
Age vs. Centaur TnI 90 min after admission	0.0011
Age vs. h-FABP on admission	0.0206
Age vs. h-FABP 90 min after admission	0.0031
Age vs. Myoglobin on admission	NC
Age vs. Myoglobin 90 min after admission	0.0031
Age vs. Copeptin on admission	0.0014
Age vs. Copeptin 90 min after admission	0.0001
Age vs. Roche hs-cTnT on admission	NC
Age vs. Roche hs-cTnT 90 min after admission	0.0031
Age vs. NT-pro-BNP on admission	0.0008
Age vs. NT-pro-BNP 90 min after admission	0.0031
Age vs. CK-MB on admission	0.0036
Age vs. CK-MB 90 min after admission	0.0040

NC = Not calculated

Table 40: As shown above represent correlation of participant age and different biomarkers on admission and 90 min after admission. No correlation exists between age and putative biomarkers.

4.11 Summary of correlation studies of participants biomarkers concentrations on admission and 90 min after admission

Table 41: Summary of correlations between biomarkers on admission and 90 min after admission

Biomarker concentration on admission vs., 90 min after admission	R^2 value
IMA on admission vs. IMA 90 min after admission	0.43
Accu TnI on admission vs. Accu 90 min after admission	0.003
Beckman hs-c TnT on admission vs. Beckman hs-c TnT 90 min after admission	0.012
Centaur TnI on admission vs. Centaur TnI 90 min after admission	0.0003
h-FABP on admission vs. h-FABP 90 min after admission	0.71
Myoglobin on admission vs. Myoglobin 90 min after admission	0.86
Copeptin on admission vs. Copeptin 90 min after admission	0.002
Roche hs-cTnT on admission vs. Roche hs-cTnT 90 min after admission	0.21
NT-pro-BNP on admission vs. NT-pro-BNP 90 min after admission	
CK-MB on admission vs. CK-MB 90 min after admission	0.94

Table 41: As shown above represent correlation of different biomarkers on admission and 90 min after admission. A statistically significant ($p = 0.002$) correlation between Copeptin on admission vs. Copeptin 90 min after admission. Also, a statistically significant ($p = 0.05$) correlation exist between Accu TnI on admission vs. Accu 90 min after admission, Centaur TnI on admission vs. Centaur TnI 90 min after admission and Beckman hs-c TnT on admission vs. Beckman hs-c TnT 90 min after admission (table 41).

Table 42: Current project participant ECG finding by gender.

Participants (n = 174)	Normal ECG	BBB	T-wave inversion	AMI	ER	Hospitalisation from ACS	ECG ischaemia
Male	92	1	6	-	2	1	6
Female	82	2	7	3	-	1	7
Total days in hospital;	One male for 37 days One female for 7 days						

ER = Emergency revascularisation. BBB = Bundle branch block

Table 42: As shown above represent the current study participants clinical finding including ECG finding and days in hospital.

Table 43: Current summary data of positive patients, per biomarker on admission and at 90 min after admission.

Assay	Number of positive patients (n = 174)
IMA on admission	21 (13%)
IMA min after admission	14 (9%)
Accu TnI on admission	0
Accu 90 min after admission	0
Beckman hs-c TnI on admission	4 (4%)
Beckman hs-c TnI 90 min after admission	4 (4%)
Centaur TnI on admission	0
Centaur TnI 90 min after admission	0
h-FABP on admission	9 (5%)
h-FABP 90 min after admission	9 (5%)
Myoglobin on admission	14 (8%)
Myoglobin 90 min after admission	15 (9%)
Copeptin on admission	0
Copeptin 90 min after admission	0
Roche hs-cTnT on admission	6 (3%)
Roche hs-cTnT 90 min after admission	6 (3%)
NT-pro-BNP on admission	8 (8%)
NT-pro-BNP 90 min after admission	9 (5%)
CK-MB on admission	4 (4%)
CK-MB 90 min after admission	2 (2%)

Table 43: As shown above represent the number of participants with results above the clinical cut-off for each biomarker on admission and at 90 min after admission. IMA test scored 13% or 21 positive results in the current study. Copeptin, Centaur TnI and Accu TnI failed to identify positive participant. Hs-TnT was used a predicate.

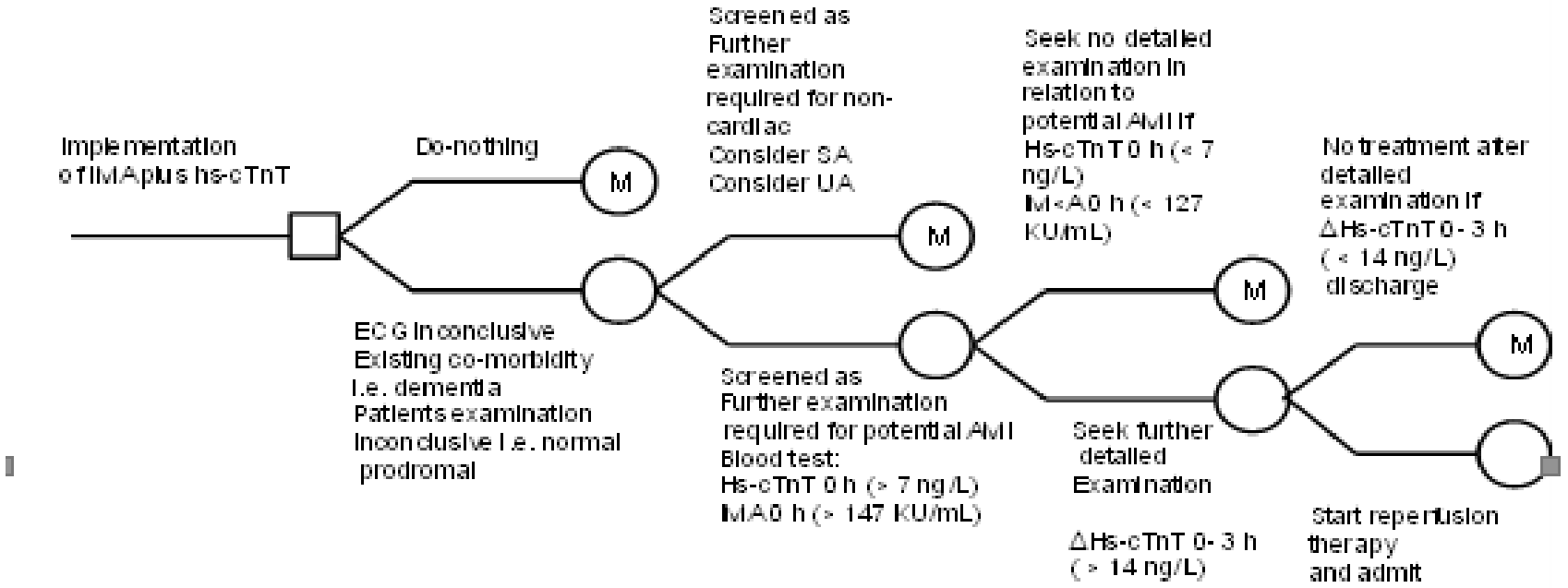


Figure 50: Decision tree (M = Markov model) Adapted from Kondo et al. Clin Exp Nephrol 2012.

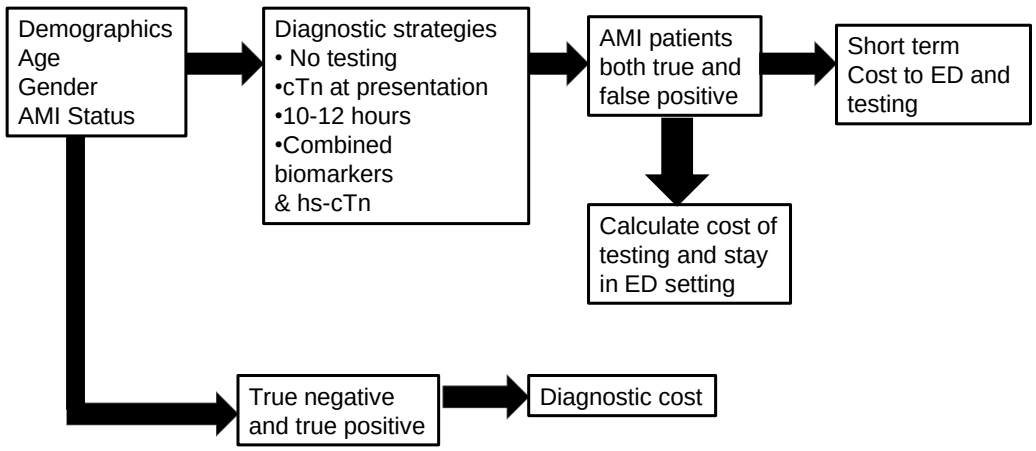


Figure 51: Basic cost-effective analysis model structures

4.12 The cost-effectiveness planes

The incremental cost and the incremental effect can be visualised using the incremental cost-effectiveness plan (figure 52)

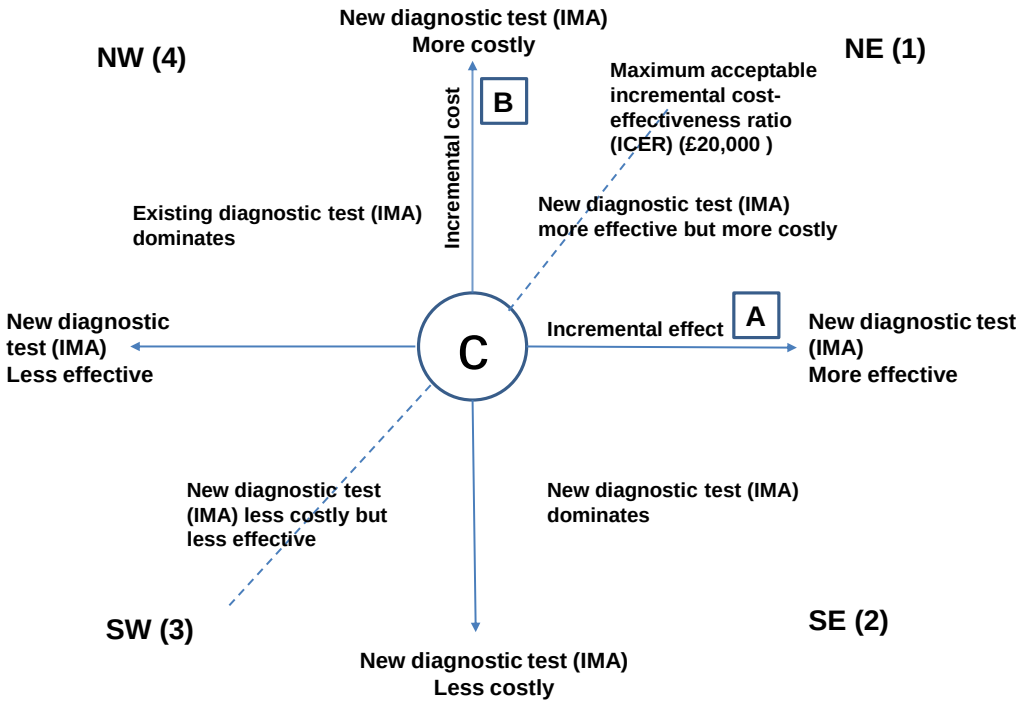


Figure 52: The cost-effectiveness plane for ischaemia-modified albumin (IMA). (adapted from Applied Methods of CEA in Health Care by Alistair Gray page 12).

The horizontal axis divides the plan according to incremental cost with a positive above and a negative below; on the other hand, the vertical axis divides the plane according to incremental effect the positive the right and the negative to the left. The overall design divides the incremental cost effectiveness plane into four quadrants. The role of each quadrant helps

improve and have an implication on the overall decision. Interventions falling in the southeast quadrant with negative cost and positive effect are considered cost-effective. In contrast, interventions falling in the northwest quadrant with positive cost and negative effect are not considered cost-effective and would be costlier.

If we consider interventions that their ICER fell in the northwest quadrants, with positive cost and positive effects, or the southwest quadrants, with negative costs and negative effects are considered equivalents and usually both interventions are considered equal.

4.12.1 Incremental effect of Ischaemia modified-albumin assay

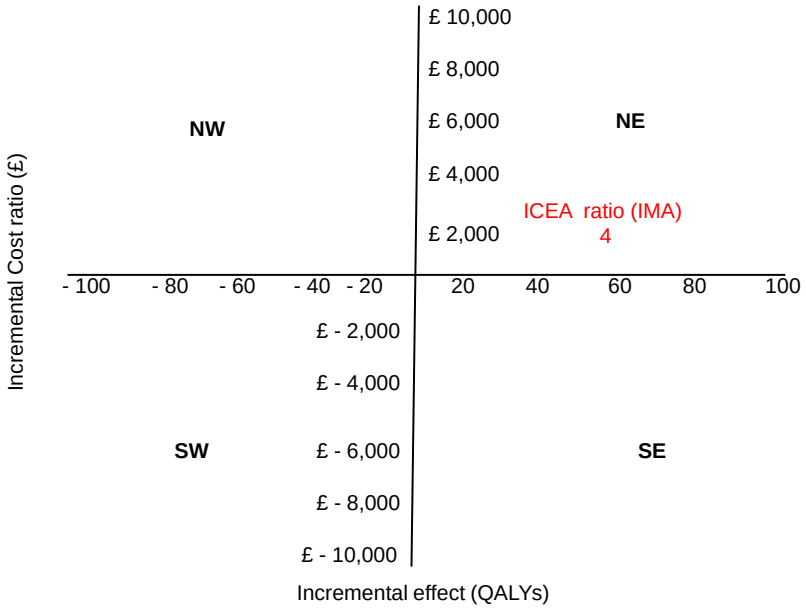


Figure 53: The incremental cost-effectiveness plane. NE = northeast quadrant; NW = northwest quadrant; SE = Southeast quadrant; SW = southwest quadrant.

Table 44: Summary cost estimation of IMA and hs-cTnT on admission

Test	Test Cost (£) per 174 patients	Effectiveness cost (£) Per high risk patients	Incremental cost effectiveness ratio
Hs-cTnT	696.00	464.00	-4.2
IMA	4,698.00	1,424.00	4.2

Table 44: IMA ICER fall in the north eats quadrant with a positive value of 4.2.

4.13 The cost-effectiveness-analysis of using ischaemia modified-albumin assay

The objective

- To evaluate the cost-benefit analysis of using IMA test alone or in combination with other biomarkers for the diagnosis of AMI on admission.
- To evaluate the best strategies of using IMA in combination with other biomarkers.

The cost for myoglobin, CK-MB to the trust is lower than hs-cTn; however, only hs-cTn is required by the universal definition of AMI. At St George's Healthcare NHS Trust, myoglobin, CK-MB and hs-cTnT tests can be measured using the existing analyser. In contrast, IMA requires a standalone analyser or optimisation and integration into the current instrument; thus, additional cost. It is well documented that measuring myoglobin, CK-MB and

hs-cTn as a cardiac panel has no diagnostic efficiency superior to that of single hs-cTn test (Goodacre,Bradburn,Cross *et al.*, 2011). The cost effectiveness analysis shows that measurement of hs-cTn alone was the most-effective diagnostic strategy (Goodacre,Bradburn,Cross *et al.*, 2011). Previous studies and the present study showed that measuring IMA as part of a cardiac panel may be useful (Mehta,Marwah,Ghosh *et al.*, 2015; Pan,Tong,Lin *et al.*, 2010; Patil,Banker,Padalkar *et al.*, 2013). Combined IMA plus hs-cTnT or Centaur cTnI measurement at admission has a superior sensitivity and specificity compared to other biomarkers on this study (table 49). At St George's Healthcare NHS Trust, hs-cTnT; is offered as routine using Roche platform. Moreover, IMA measurement can also be carried out in house using a Roche Cobas Mira plus or Hitachi 911 (Roche) analyzers. Currently, Hitachi 911 is capable of interfacing with the current hospital information system ensuring continuity of clinical governance and does not require special training.

Based on the present study, combined IMA plus hs-cTnT measurement on admission was the most diagnostically efficient panel therefore it may be useful in assessing low risk patients. Thus, the cost-benefit analysis was based on the following strategies:

1. Discharge patient without treatment or testing
2. Discharge patient with IMA negative
3. Discharge patients if IMA plus hs-cTnT are negative.

The sensitivity and specificity of strategies tested are listed in table 45.

Table 45: Combined sensitivity and specificity used in cost-benefit analysis.

Strategy	Sensitivity (95% CI)	Specificity (95% CI)
No testing	0	1
IMA plus hs-cTnT	70% (55-82%)	100% (98-100%)

4.12.2 Cost benefit analysis

The ED staffs are required to diagnose and refer AMI patients to the cardiac unit within 90 min or less after admission (door-to-balloon time) (Amsterdam,Wenger,Brindis *et al.*, 2014). It is therefore reasonable to suggest that the cost-benefit analysis could be based on the assumption of doctor on demand scenario. Moreover, the cost benefit analysis is also based on the assumption that 174 present study patients will be treated. Using this cohort allows a complete data measurement on admission and 90 min after admission.

The current strategy of using hs-cTn as the first diagnostic test for AMI in low risk patients presenting to the ED with chest pain suggestive of AMI; will require at least two or more hs-cTn measurement and it could take up to 12 h to rule-in or rule-out AMI. IMA testing (early biomarker) diagnostic window of opportunity passes after 90 min. The cost of measuring IMA on admission is higher than hs-cTn (table 47). However, IMA assay can only be cost effective if measured only on admission and in combination with hs-cTnT. The reason for this is that hs-cTnT alone is capable of detecting patients AMI over time. As demonstrated by the AUC, IMA as a standalone test cannot compete with the diagnostic efficiency of hs-cTn on admission or 90 min after admission. However, combined IMA assay with Centaur cTnI or hs-cTnT may be useful and could be used to rule-out AMI. The total cost of each patient's management increases as sensitivity and specificity decreases and more patients require conventional admission, 3 h, 6 h, 10 and 12 h, hs-cTn testing. Cost of tests per manufacturer and cost benefit analysis are summarised in table 47.

4.12.3 Cost benefit analysis calculation

The cost used to calculate the health economics in this study is provided by the manufacturers (table 46). The current total cost of using hs-cTnT, if measured on admission, 3 h, 6 h and 12 h is approximately £16.00 per patients; whereas IMA test is £27.00 per test per patient when measured only once on admission. The calculation is based on the assumption that IMA can only be accepted if low risk patients presenting with chest pain suggestive of AMI could be discharged in < 90 min and with testing IMA just on admission. Thus, potential saving due to reduced cost of observation, beds occupation (£300 per day), crowding and expensive investigations such as imaging.

Table 46: Cost per test as provided by manufacturer.

Assay	Manufacturer	Cost per sample
AccuTnI	Beckman	£7.40
Hs-cTnI	Beckman	£7.40
TnI Ultra	Siemens	£6.20
hs-cTnT	Roche	£4.00
NT-pro-BNP	Siemens	£19.65
H-FABP	Randox	£4.95
Myoglobin	Randox	£2.78
CK-MB	Randox	£2.56
ACB® (IMA®)	Inverness Medical	£27.00
Copeptin	Randox	£15.00

4.12.3.1 Current cost for screening n = 174 low risk patients on admission and 3 h after admission, using hs-cTnT alone

- Cost exclude overhead
- Hs-cTnT cost = £4.00 per test

- Total Cost (hs-cTnT) = (174 x £4.00 = £696.00) = £696.00 x 2 = £1,392.00

4.12.3.2 Cost per high risk patients when using hs-cTnT

- £1,392.00 ÷ 3 (high risk patients) = £464.00

4.12.3.3 Total cost for screening n = 174 low risk patients using IMA and hs-cTnT on admission

- Hs-cTnT cost = £4.00 per test
- Total Cost (hs-cTnT) = (174 x £4.00 = £696.00)
- IMA cost = £27.00 per test
- Total cost (IMA) = (£27.00 x 174 = £4,698.00)
- The total cost of screening this cohort of patients (n = 174) is
  £ 4,698.00 + £696.00 = £5,394.00
  OR £31.00 per patient

4.12.3.4 Single IMA testing on admission scenario

- Cost of assays excluding overhead
- IMA strategy for treating 174 low risk patients
- 3 patients had the final diagnosis of AMI, thus three high risk patients

4.12.3.5 Calculation of cost benefit of treating one high risk patient using IMA testing:

Cost benefit = Cost of recommended strategy – cost of current strategy ÷ 3 high risk patients

= £4,968.00 – 696.00 ÷ 3 = £ 1,424.00 per high risk patient

The introduction of IMA test as a standalone will cost the healthcare system an estimated £ 1,424.00 per high risk patient or £31 per screened patients compared to £8 when hs-cTnT is measured on admission and 3 h after admission as per current protocol.

4.12.3.6 Cost benefit per panel based on screening 174 patients on admission

- Cost per combined IMA plus hs-cTnT per panel per patient: £27.00 + £4.00 = £31.00
- Total cost for screening n = 174 using the combined diagnostic efficiency of IMA plus hs-cTnT is: £31.00 x 174 = £ 5,394.00

4.12.3.7 Calculation of cost benefit of treating one high risk patient using IMA plus hs-cTnT panel

Cost benefit = Cost of recommended strategy – cost of current strategy ÷ 3 high risk patients

= £5,394 – 696 ÷ 3 = £ 1,566 per high risk patient

Combined IMA plus hs-cTnT if introduced will cost £1,566 per high risk patients. The total cost for screening 174 low risk patients when using combined IMA plus hs-cTnT is as follow: £31 x £174 = £ 5,394.00.

Table 47: Cost benefit analysis on admission when using combined diagnostic efficiency of IMA and other biomarkers.

Test combination	**Cost per test**	**Current cost on admission (£) per panel**	**Cost benefit on admission (£)**	**Saving per high risk patients (£)**
No Testing	0	0	0	889.00*
IMA admission	27.00	27.00	1,334.00	-1,102.00
IMA + Myoglobin	2.78	29.78	1,495.24	-1,263.24
IMA + CK-MB	2.56	29.56	1,482.48	-1,250.48
IMA + Accu cTnI	7.40	34.40	1,763.20	-1,531.20
IMA + Beckman cTnI	7.40	34.40	1,763.20	-1,531.20
IMA + cTnT (Roche)	4.00	31.00	1,566.00	-1334.00
IMA + Centaur cTnI	6.20	33.20	1,693.60	-1,461.60
IMA + H-FABP	4.95	31.95	1,621.10	-1,389.20
IMA + Copeptin	15.00	42.00	2,204.00	-1,972.00
IMA + NT-pro-BNP	19.65	46.65	2,473.70	-2,241.70

(-) indicate extra cost to the healthcare system per high risk patients. (*) £889.00 saving per patient prevented from admission (Byrne, 2014).

Table 47 above compared the cost benefit analysis of IMA testing alone and in combination with other biomarkers. Single IMA testing on admission will cost the healthcare system an additional £ 1102.00 per high risk patients in every 174 patients admitted. The total cost to the healthcare system in term of screening 174 patients is £4,698.00.

4.12.3.8 Number needed to treat

From 444 available samples, a total of 348 (78%) had complete matched and paired (i.e. sample available on admission and 90 min after admission) data including concentration values for hs-cTnT, hs-cTnI, copeptin, H-FABP, myoglobin, NT-pro-BNP, CK-MB, IMA and outcome of MACE. The number needed to treat (NNT) was based on n = 174 patients who, on admission, had a complete data set. The current project found 7 cases of MACE when IMA plus hs-cTnT was used to predict the outcome of MACE. In contrast the RATPC reported 4 cases. Moreover, NNT was calculated using the combined diagnostic efficiency of IMA plus hs-cTnT, as IMA alone was not diagnostically efficient. Thus, NNT calculation of IMA assay alone was not warranted.

NNT was calculated using the outcome e.g. MACE. The outcome (MACE) as established by the RATPAC study was considered the experimental events rate (EER) whereas IMA plus hs-cTnT diagnostic outcome was considered the controlled events rate (CER).

Number needed to treat calculation:

Table 48: Summary for NNT

Variable	IMA + hs-cTnT (n = 174)	RATPAC (n = 174)
Total MACE	7	4
	CER	EER

Number needed to treat (NNT) = 1 ÷ absolute risk reduction (ARR)

= 1/ARR

ARR = Controlled events rate (CER) – Experimental events rate (EER)

ARR = CER – EER

= 4% – 2% = 2%

NNT = 1/0.02 = 50

In order to diagnose and treat one patients at leats 50 patients (£1,550.00) needed to be screened for ACS using the combined diacnostic efficiency of IMA plus hs-cTnT.

4.14 Summary finding

The introduction of IMA assay as a standalone test provides no additional clinical information superior to that of hs-cTn and cost more than hs-cTnT or I strategy. In addition, as it stands IMA assay alone is not suitable for the diagnosis of ACS or ischaemic cardiac patients in an ED setting. However, the combined diagnostic efficiency of IMA plus hs-cTnT may be useful.

Table 49: Diagnostic efficiencies of individual biomarkers on admission (sample 1) and 90 minutes (sample 2).

	Sensitivity		Specificity		PPV		NPV	
Sample (1)	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
IMA	100	(59-100)	10.2	(6.1-15.9)	4.5	(1.8-9.0)	100	(80.5-100)
AccuTnI	0	(0-41)	98.2	(94.0-98.6)	0	(0-70)	95.9	(91.7-98.3)
Beckman hs-TnI	0	(0-52)	98.5	(94.8-99.8)	0	(0-84.2)	94.4	(91.9-98.8)
Centaur TnI	14.3	(9.4-57.9)	89.8	(84.1-93.9)	5.6	(0.1-27.3)	96.1	(91.8-98.6)
H-FABP	28.6	(3.7-71)	65.7	(91.3-98.2)	22.2	(2.8-60)	96.9	(92.9-99.0)
Myoglobin	14.3	(0.4-57.9)	96.4	(92.3-98.7)	14.3	(0.4-57.9)	96.4	(92.3-98.7)
Copeptin	57.1	(18.4-90.1)	46.2	(38.2-54.3)	4.5	(1.2-11.1)	96.1	(88.9-99.2)
hs-cTnT	28.6	(3.7-71)	100	(97.8-100)	100	(15.8-100)	97.1	(93.3-99.0)
NT-pro-BNP	28.6	(3.7-71)	96.4	(92.3-98.7)	25	(3.2-65.1)	97	(93.1-99.0)
CK-MB	14.3	(0.4-57.9)	98.2	(94.8-99.6)	25	(0.6-80.6)	96.4	(92.4-98.7)
Sample (2)	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
IMA	0	(0-41.1)	86.7	(80.6-91.5)	0	(0-15.4)	95.4	(90.7-98.1)
AccuTnI	42.9	(9.9-81.6)	97.6	(93.9-99.3)	42.9	(9.9-80.6)	97.6	(93.9-99.3)
Beckman hs-TnI	20	(0.5-51.6)	97.8	(93.7-99.5)	25	(0.6-80.6)	97.1	(92.7-99.2)
Centaur TnI	71.4	(29-96.3)	89.8	(84.1-93.9)	22.7	(7.8-45.4)	98.7	(95.3-99.8)
H-FABP	42.9	(9.9-81.6)	96.2	(91.9-98.6)	33.3	(7.5-70.1)	97.4	(93.6-99.3)
Myoglobin	14.3	(0.4-57.9)	95.7	(91.4-98.3)	12.5	(9.3-52.7)	96.3	(92.1-98.6)
Copeptin	71.4	(29-96.3)	52.5	(44.5-60.4)	6.2	(2.0-13.8)	97.7	(91.9-99.7)
Hs-cTnT	85.7	(42.1-99.6)	100	(97.8-100)	100	(54.1-100)	99.4	(96.7-100)
NT-pro-BNP	28.6	(3.7-71.0)	95.7	(91.4-98.3)	22.2	(2.8-60)	96.9	(92.9-99)
CK-MB	14.3	(9.4-57.9)	9.94	(96.5-100)	50	(1.3-98.7)	96.3	(92.1-98.6)

IMA, ischaemia modified albumin; PPV, positive predictive value; NPV, negative predictive value; 95%CI, 95% confidence interval; CK-MB, creatine kinase isoenzyme MB; h-FABP, heart fatty-acid binding protein.

Table 50: Combined performance of cardiac biomarkers

Sample 1	**Sensitivity %**		**Specificity %**		**Positive likelihood**		**Negative likelihood**	
Tests	**95%CI**		**95%CI**		**95%CI**		**95%CI**	
IMA + Copeptin	71.4	(0.36-0.92)	46.4	(0.39-0.54)	1.332	(0.82-2.17)	0.616	(0.19-2.0)
IMA + hs-TnT	42.9	(0.16-0.75)	88	(0.82-0.92)	3.557	(1.38-9.19)	0.650	(0.34-1.24)
IMA + h-FABP	29	(0.08-0.64)	83.3	(0.77-0.88)	1.714	(0.51-5.81)	0.857	(0.53-1.38)
Sample 2								
IMA + Copeptin	71.4	(0.36-0.92)	55.4	(0.48-0.63)	1.60	(0.97-2.63)	0.52	(0.15-1.67)
IMA + hs-TnT	1.0	(0.06-1.0)	76	(0.69-0.82)	4.15	(3.1-5.4)	-	-
IMA + h-FABP	57.1	(0.25-0.84)	87.3	(0.82-0.92)	4.51	(2.11-9.66)	0.49	(0.21-1.16)

Table 50: Shows that IMA plus copeptin has the highest sensitivity on admission and at 90 min after admission 71.4% (95% CI, 0.36 to 0.92%) and 71.4 (0.36-0.92) respectively. The positive likelihood and the negative likelihood were 1.33% (95% CI, 0.82 -2.17) and 0.62 (95% CI, 0.19 – 2.0) respectively.

Chapter 5

Discussion

5.0 Introduction

This thesis describes the potential diagnostic value of the current conventional cardiac biomarkers and the novel biomarkers in detecting low risk patients with AMI. This chapter includes the main discussion on the findings of IMA assay, conventional biomarkers and novel biomarkers.

To reduce misdiagnosis of patients with AMI and the unnecessary investigations; healthcare professionals have considered the possibility of combining established cardiac biomarkers such as hs-cTn and novel biomarkers including biomarkers of ischaemia (whole blood choline), coronary plaque destabilisation (myeloperoxidase), coronary plaque formation (homocysteine), myocardial necrosis (H-FABP)), inflammatory biomarkers (cytokines), oxidative stress (IMA), and vascular stress (copeptin) for the diagnosis of AMI especially NSTEMI (Apple,Smith,Pearce *et al.*, 2009; McLean & Huang, 2012). Among these novel biomarkers outlined above, only the albumin cobalt binding (ACB®) assay, to determine IMA, has been CE marked in Europe and approved by the FDA in the USA for the detection of myocardial ischaemia. The FDA authorised the use of IMA assay in an ED setting and in the clinical scenario where cTn and ECG are both negative. Since IMA assay has been authorised by the FDA, there remains a number of unresolved issues regarding its specificity and its molecular structure (Collinson & Gaze, 2008). The ACB® assay is an indirect measurement of IMA; thus, in real terms IMA as a protein has not being directly measured (Bhagavan,Lai,Rios *et al.*, 2003).

Currently copeptin is authorised by NICE to aid the diagnosis of AMI when cTn and ECG are both negative. Although, copeptin was authorised by NICE for the use in an ED setting, a number of issues including, its kinetic release, and its low specificity are still unresolved (Nemec,Koller,Nickel *et al.*, 2010; Nickel,Bingisser & Morgenthaler, 2012). Thus, currently copeptin and IMA are not offered as a routine test in ED across the UK.

There is some evidence to suggest that a multi-biomarker approach may be useful in ACS patient's management especially in the ED (Apple,Pearce,Chung *et al.*, 2007; Morrow & Braunwald, 2003). The following biomarker, NT-pro-BNP (Brown,Sease,Robey *et al.*, 2007), copeptin (Reichlin,Hochholzer,Stelzig *et al.*, 2009), H-FABP (Haltern,Peiniger,Bufe *et al.*, 2010) and IMA have been investigated (Collinson,Gaze,Bainbridge *et al.*, 2006; Peacock,Morris,Anwaruddin *et al.*, 2006). Collectively these biomarkers are known to increase during the inflammatory process and during cardiac ischaemia. Previous studies have suggested that increases in these biomarkers upstream from biomarkers of necrosis may help in the diagnosis of AMI (Figure 54) (Morrow & Braunwald, 2003; Pai,Pischon,Ma *et al.*, 2004; Vasan, 2006).

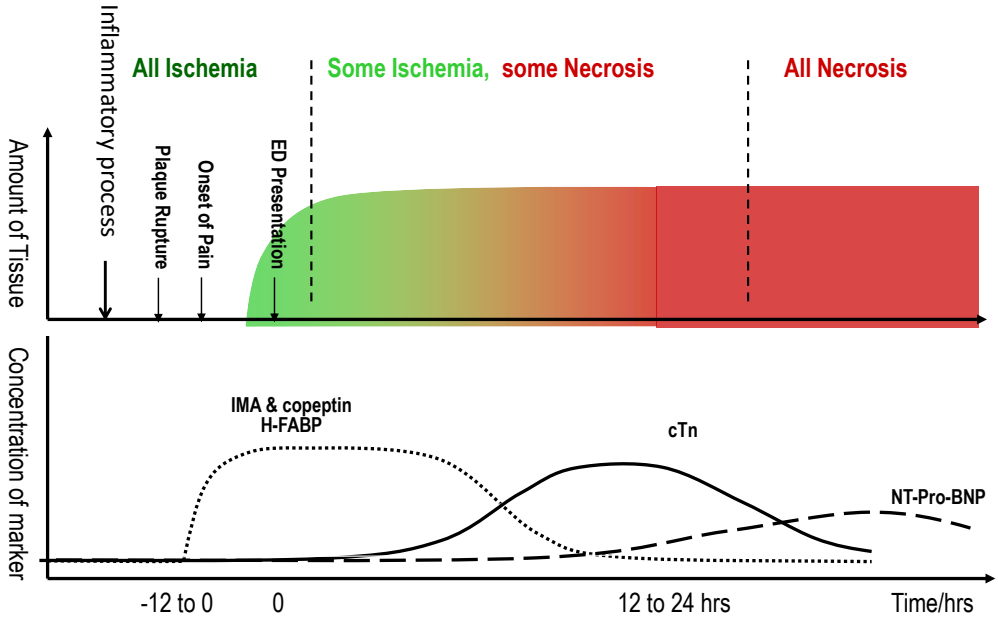


Figure 54: The kinetic release of IMA (dotted line) and cTn (solid line) and natriuretic peptide (dashed line) [bottom panel], in relation to time and tissue damage [top panel]. (By Dr D Gaze. St George's Healthcare NHS. London).

The rational for using these biomarkers is the possibility to improve the diagnosis efficiency of ACS, potentially improving the rule-out and rule-in protocol and help shorten the patient's hospital stay thus saving resources (Roberts, 1998). Prior to this study and to the best of my knowledge no research group has compared IMA plus hs-cTn in the diagnosis of low risk patients with AMI. Moreover, studies regarding the evaluation of the diagnostic efficiency IMA plus H-FABP and IMA plus NT-pro-BNP are scares. Therefore, a better understanding of this biomarker in combination with latest generation of hs-cTn assays is required before they could be considered for prime-time use.

Before commencing the present study, it was necessary to establish the reference interval for IMA assay. The current proposed IMA reference interval as determined by the manufacturer was carried out using the Cobas Mira plus analyser (Roche) and determined in 283 apparently healthy individuals. The IMA concentrations ranged from 52-116 KU/L. The 95th percentile was at 85 KU/L. Although, the preliminary clinical cut-off for IMA to rule-out ACS is 85 KU/L, manufacturers (ISCHEMIA Technologies, Denver Colorado) suggested a higher value of 100 KU/L should be used. They argued that reference population and the 85 KU/L value was not corrected for age, smoking and gender. Using different instrument (Beckman Coulter LX-20) IMA concentrations for a reference population of 110 KU/L was also used for the diagnosis of AM (Maguire,O'Sullivan,Ryan *et al.*, 2006). Although, the present study did not look at IMA concentrations in different ethnic groups; our findings are in agreement with one study that found that gender had no significant influence on IMA concentrations; whereas race (black vs. Caucasian) has. It must be noted that both studies used a small cohort of volunteers (Govender,De Greef,Delport *et al.*, 2008).

The optimum upper ACB® decision limit for evaluating diagnostic sensitivity, specificity and predictive value has not yet been established. The ACB® test manufacturers recommend that laboratories should establish their own IMA clinical cut-off (Inverness, 2005). In the present study the reference population

(n = 66) the median IMA was 122 KU/L, (interquartile range 121-124 KU/L). The median IMA value was higher than previously published data (Beetham,Monk,Keating *et al.*, 2006). However, this observed difference in concentration compared to recommended clinical cut-off (85 KU/L) may be due to the pre-analytical variable including temperature and long-term sample storage. Samples stored at - 20 ^{0}C yielded IMA values on average 3 units higher than those analysed within 2.5 h (mean 90.5 vs. 87.5; $P < 0.001$) (Beetham,Monk,Keating *et al.*, 2006). Another, study also found that storage at -20 ^{0}C may increase IMA concentration by as much as 10% (Maguire,O'Sullivan,Ryan *et al.*, 2006). In the present study, samples were stored for at least four years, thus the difference in clinical cut-off may be accounted for by storage conditions. However, throughout the present study we used the IMA clinical cut-off of 127 KU/L for diagnosis of AMI.

In practical terms samples sent from ED to the laboratory at St George's Healthcare NHS Trust are considered urgent and the local guidelines require trust to analyse ED samples within 45 min. However, the current turnaround time for ED samples and in particular cTn is approximately 120 min. In a real scenario, these samples may be subject to delays due to work load, staffing issues and pre-analytical errors. Therefore, any delay in sample receipt may affect IMA results.

5.1 Clinical utility of ischemia modified-albumin and high sensitivity cardiac troponin

In our study the combined sensitivity and specificity of IMA plus hs-cTnT on admission was 71% (95% CI, 56-82%) and 100% (95% CI, 98-100%) respectively; the NPV was 94%. The good diagnostic efficiency of hs-cTn when combined with IMA may be due to the lower detection limit of hs-cTnT. Since the introduction of hs-cTn immunoassay the sensitivity has increased from 83% (95% CI, 76-90%) to 95% (95% CI, 90-98%), (Reichlin et al., 2009).

There is a number of studies that have found that the combined diagnostic efficiency of IMA and conventional cTn at presentation has an excellent sensitivity close to 100% for predicting the final diagnosis of AMI (Collinson, 2006; Keating,Benger,Beetham *et al.*, 2006). Contrary to these previous studies; our study found that the combined diagnostic efficiency of IMA plus conventional cTn on admission and at 90 min after admission ranged from 49%-54% and 14%-15% respectively. The reason for this difference is twofold: firstly, the definition of time zero is taken at admission which can greatly vary according to geographic location and local triage protocols, thus the diagnostic efficiency of cTn may vary between studies. Also, the population used in this study is a very low risk cohort with low probability of AMI. Secondly, in this study the median time from onset of chest pain to the last troponin measurement was 495 min or 8:25 h (interquartile range (IQR) 310-738 min). Samples taking at 3 h or more after the last onset of chest pain may be unsuitable for IMA analysis. At this stage the IMA diagnostic window may be missed, and therefore IMA concentration may be normal (Cho,Choi,Kim *et al.*, 2007; Dusek,St'asek,Tichy *et al.*, 2006; Quiles,Roy,Gaze *et al.*, 2003; Sinha,Vazquez,Calvino *et al.*, 2006; Zapico-Muniz,Santalo-Bel,Merce-Muntanola *et al.*, 2004). On the other hand, even samples that were collected in < 6 h would have an effect on the circulating concentration of various biomarkers including copeptin, NT-pro-BNP, hs-cTn, H-FABP, myoglobin and CK-MB. The kinetic release of all the mentioned biomarkers is optimal in the first 2 to 4 h of the onset of chest pain (Eggers,Oldgren,Nordenskjold *et al.*, 2004; Kavsak,MacRae,Lustig *et al.*, 2006; Tucker,Collins,Anderson *et al.*, 1997).

Various studies confirm that the diagnostic sensitivity of hs-cTn ranges between 82%-100% (Keller,Zeller,Ojeda *et al.*, 2011; Keller,Zeller,Peetz *et al.*, 2009; Reichlin,Hochholzer,Bassetti *et al.*, 2009). In agreement with this observation, the present study found that the collective diagnostic efficiency of the conventional cTn ranged from 84%-88% on admission and from 78%-84% at 90 min after admission. In contrast, the diagnostic efficiency of hs-cTn ranged from 85%-100% on admission and 78%-100% 90 min after admission.

Moreover, in agreement with previous findings hs-cTn is still the most reliable method for assessing low risk patients presenting to ED with chest pain (Apple, 2011; Mills,Churchhouse,Lee *et al.*, 2011).

Generally, the findings in this study are in agreement with other studies. As demonstrated in a cumulative meta-analysis of these studies, the NPV of IMA can be used to rule-out ACS patients (Peacock,Morris,Anwaruddin *et al.*, 2006). In our study, the IMA assay has an excellent NPV, which could be integrated into a risk score in conjunction with NT-pro-BNP and cTn (Bali,Cuisset,Giorgi *et al.*, 2008). Although, the IMA assay have a good NPV this does not necessary translate into a practical triage tool in an ED setting, its NPV merely indicates the disease prevalence in the studied population (Sbarouni,Georgiadou,Panagiotakos *et al.*, 2008). CTnT coupled with NPV of IMA was found to be an independent predictor of the risk of developing ACS (Talwalkar,Bon Homme,Miller *et al.*, 2008). In the present study, IMA, hs-cTn and the conventional cTn were not strong predictors of MACE at 30 days with AUC of the ROC curve varying from 52-53% on admission and 54-64% at 90 min after admission. The reason for this is not clear. One probable explanation could be that the present study consists entirely of very low risk patients with low probability of MACE at 30 days.

Our study found, that the diagnostic efficiency of IMA concentration on admission had an AUC of ROC curve of 57% (95% CI, 43-66%) and 58% (95% CI, 35-81%) at 90 min after admission. The low diagnostic performance of IMA assay may be explained by the fact that IMA is not cardiac-specific and has a limited diagnostic window (Abboud,Labreuche,Meseguer *et al.*, 2007; Talwalkar,Bon Homme,Miller *et al.*, 2008). Based on this finding IMA testing alone is not suitable for assessing chest pain patients.

The utilisation of delta values in the present study did not improve the diagnostic efficiency of all biomarkers (table 32 & 33). The rate of change (delta value) of myoglobin has been claimed to improve the diagnostic sensitivity (Straface,Myers,Kirchick *et al.*, 2008). Conversely, in the present

study a delta changes in both absolute and percentage change in myoglobin and CK-MB did not significantly helped to improve the diagnostic efficiency of AMI (table 327 33). In agreement with our study a systematic-review of the diagnostic efficiency of myoglobin and CK-MB concluded that these biomarkers do not have an incremental value for early diagnosis of AMI (Keller,Zeller,Peetz *et al.*, 2009).

The evaluation of novel biomarkers including IMA was challenging given that in some cases most studies were heterogeneous in regards to methodology one study that used hs-cTn and IMA also used mixed ACS patients (Mehta,Marwah,Ghosh *et al.*, 2015), serum sampling, the impact of the new definition of AMI, the introduction of hs-cTn, definition of clinical end point i.e. ACS or AMI. In addition most studies looking at novel biomarkers were observational (Lin,Yokoyama,Rac *et al.*, 2012).

5.2 Novel biomarkers in assessing acute myocardial infarction

5.2.1 Copeptin

In the present study, copeptin was diagnostically comparable on admission to IMA with an AUC of ROC curve of 58% (95% CI, 48-68%) compared to 54% (95% CI, 43-66%) respectively. At 90 min after admission copeptin was also diagnostically comparable to IMA with AUC of ROC curve of 63% (95% CI, 38-89%) and 58% (95% CI, 35-81%) respectively. Various studies found that copeptin is elevated in STEMI patients vs. NSTEMI; this difference is thought to be due to an acute endogenous stress and may suggest that copeptin elevation is of cardiac origin (Charpentier,Maupas-Schwalm,Cournot *et al.*, 2012; Gu,Voors,Zijlstra *et al.*, 2011; Reichlin,Hochholzer,Stelzig *et al.*, 2009). The utilisation of low risk patients in the present study may contribute to this low sensitivity. Copeptin is a stress biomarker; thus, various haemodynamic conditions can influence its distribution in the circulation (Katan & Christ-Crain,

2010). In addition, the lower diagnostic efficiency of copeptin reflects the fact that this biomarker cannot detect ischaemic myocardial infarction (Staub,Morgenthaler,Buser *et al.*, 2009). Moreover, the combined diagnostic efficiency of IMA plus copeptin did not improve the diagnosis of AMI with the AUC of ROC curve of 55% (95% CI, 38-71%). Other studies suggested that copeptin may be used as a diagnostic and prognostic biomarker in patients admitted to the ED with a range of diseases such as diabetes mellitus, polycystic kidney disease, septic shock and HF (Dobsa & Edozien, 2013; Nickel,Bingisser & Morgenthaler, 2012). Importantly, the interpretation of copeptin concentrations must be considered in clinical setting and patient history.

The role of copeptin in the prediction of MACE is not well documented; however, in patients with CAD, copeptin was not a suitable predictor of MACE over three years (Schnabel,Schulz,Messow *et al.*, 2010). The present study also suggests that copeptin is not a suitable predictor of MACE with the AUC of ROC curve on admission of 59% (95% CI, 46-73%) and 53% (95% CI, 38-68%) at 90 min after admission.

In conclusion, the present study is in agreement with various studies that suggest that copeptin is a biomarker of non-specific stress response and cannot safely be used in an ED setting to rule-in or rule-out the diagnosis of AMI (Nemec,Koller,Nickel *et al.*, 2010; Nickel,Bingisser & Morgenthaler, 2012).

5.2.2 Heart fatty-acid binding protein

H-FABP is detected early in the circulatory system as soon as the myocardial membrane is compromised; thus H-FABP may be used as an early biomarker of cardiac ischaemia and necrosis (Chan,Sanderson,Glatz *et al.*, 2004; McLean & Huang, 2012). In the present study moderate diagnostic efficiency of H-FABP with AUC of ROC curve of 78% (95% CI, 68-89%) may be due to

the utilisation of very low risk patients. It is probable that H-FABP is released to the circulation before any necrotic damage.

H-FABP was diagnostically superior on admission to IMA with an AUC of ROC curve of 78% (95% CI, 68-89%) compared to 54% (95% CI, 43-66). H-FABP was also diagnostically efficient than IMA at 90 min after admission with AUC of 65% (95% CI, 40-90%) and 58% (95% CI, 35-81%) respectively. In agreement with our study McMahon and colleagues, studied 1128 unselected patients presenting to the ED with chest pain found that H-FABP had sensitivity at 0-3 h of 64.3% and 3-6 h of 85.3% (McMahon,Lamont,Curtin *et al.*, 2012). A similar study found that H-FABP did predict ACS diagnosis (OR 4.65; 95% CI 2.39-9.04) with specificity at 96.8% (95% CI, 95.4-98.1%) and sensitivity of 13.5% (95% CI, 10.9-16.1%) (Charpentier,Ducasse,Cournot *et al.*, 2010). In agreement with our study, Charpentier and colleagues, concluded that H-FABP did not add significant additional information superior to the currently available diagnostic tools for NSTEMI management ($p = 0.40$).

The combined diagnostic efficiency of IMA plus H-FABP on admission had a sensitivity and specificity of 41% (95% CI, 27-56%) and 93% (95% CI, 89-96%) respectively, the NPV was 89%. Conversely, the combined diagnostic efficiency of IMA and H-FABP at 90 min after admission had a very poor sensitivity and very good specificity of 12% (95% CI, 0.4-30%) and 93% (95% CI, 94-99%) respectively the NPV was 88%. A similar study found that the combined sensitivity of IMA and H-FABP did not provide significant information for ACS diagnosis (Charpentier et al., 2010). This low sensitivity at 90 min after admission could be explained by the ability of the heart tissue to switch energy utilisation from H-FABP to glucose during ischaemia may also lower the diagnostic efficiency of H-FABP (Arumugam,Sreedhar,Thandavarayan *et al.*, 2016).

The prognostic value for the outcome of MACE at 30 days on admission for H-FABP was comparable to IMA with AUC of ROC curve of 50% (95% CI, 40-61%) and 52% (95% CI, 39-66%) respectively. The prognostic value of H-

FABP at 90 min was also comparable to IMA with an AUC of ROC curve of 51% (95% CI, 41-62%) and 57% (95% CI, 43-72%) respectively. Various studies appear to suggest that H-FABP evaluation in ACS patients could predict long term mortality after one year (Kilcullen,Viswanathan,Das *et al.*, 2007; Viswanathan,Kilcullen,Morrell *et al.*, 2010). The present data demonstrates that H-FABP was a poor prognostic biomarker of MACE. The reasons for this could be two-fold. Firstly, the population studied was low risk with a low prevalence of MACE and secondly follow up was relatively short at 30 days, compared to one year in previous studies (Kilcullen,Viswanathan,Das *et al.*, 2007; Viswanathan,Kilcullen,Morrell *et al.*, 2010).

Published studies show that the diagnostic efficiency of H-FABP lacks the require methodological design including the inappropriate use of mixed population with higher percentage of patients diagnosed with ACS (O'Donoghue et al., 2006) or undefined population; one study used a STEMI cohort and other did not use hs-cTn (Body,McDowell,Carley *et al.*, 2011; Chan,Sanderson,Glatz *et al.*, 2004; Gururajan,Gurumurthy,Nayar *et al.*, 2010; McCann,Glover,Menown *et al.*, 2008). In support of our study a meta-analysis also suggests that H-FABP testing as a standalone test does not fulfill the diagnostic criteria in term of sensitivity, specificity and the predictive values for a safe rule-out protocol in patients suspected of AMI (Bruins Slot,Reitsma,Rutten *et al.*, 2010). As described above the H-FABP testing either alone or in combination with IMA does not add an incremental value for the diagnosis of AMI comparable to that already achieved by hs-cTn measurement. Presently H-FABP is not routinely available within the mainstream healthcare system in the UK.

5.2.3 N-terminal pro B-type natriuretic peptide

Measurement of NT-pro-BNP has been linked and was proposed as an early diagnostic test for patients presenting with chest pain (Bassan,Potsch,Maisel *et al.*, 2005). The Acute Decompensated Heart Failure National Registry

(ADHERE) registry, which includes information about 84,872 patients with acute heart failure (AHF) and cTn concentration at admission, found that 6% of this cohort had elevated cTn (Fonarow,Peacock,Horwich *et al.*, 2008). Another study found no correlation exists between elevated BNP concentration and cTnT (Yamamoto,Sato,Yasutake *et al.*, 2006). The mechanism of the elevation of BNP concentration in ACS patients is not fully understood (Salama,El-Moniem,El-Hefney *et al.*, 2011b). One explanation is that ischaemic attack during or pre-AMI may contribute to heart wall stretch inducing the neuro-hormonal activation (Marumoto,Hamada & Hiwada, 1995). This hypothesis is supported by the detection of BNP gene expression in ischaemic and infarct region of the heart (Ndrepepa,Braun,Schomig *et al.*, 2007).

In the present study, the NT-pro-BNP on admission was the most diagnostically efficient biomarker with an AUC of ROC curve of 93% (95% CI, 88-97%). A similar study also found that NT-pro-BNP on admission was diagnostically better then cTnT and CK-MB (Salama,El-Moniem,El-Hefney *et al.*, 2011a). The present study found that at 90 min after admission NT-pro-BNP has a poor diagnostic efficiency, but comparable to IMA with an AUC of ROC curve of 52% (95% CI, 26-78%) and 58% (95% CI, 35-81%) respectively. In addition, the present study demonstrated that the combined diagnostic efficiency of IMA plus NT-pro-BNP had a sensitivity and specificity in the diagnosis of AMI on admission of 52% (95% CI, 39-65%) and 98% (95% CI, 94-99%) respectively. Whereas, the combined diagnostic efficiency of IMA and NT-pro-BNP at 90 min after admission had a sensitivity and specificity for the diagnosis of AMI of 8% (95% CI, 2-24%) and 98% (97% CI, 93-99%) respectively. The low diagnostic efficiency of NT-pro-BNP at 90 min after admission can reflect cardiac ischaemic episode resolution i.e. restored circulation to the myocardium. In addition, this low performance of NT-pro-BNP can be explained by the fact that BNP is not stored in the cardiomyocyte rather it is expressed, thus it cannot be detected immediately after an

ischaemic episode because BNP is subject to DNA expression which may take hours to achieve (Hama,Itoh,Shirakami *et al.*, 1995).

Contrary, to previous studies, the present study demonstrated that NT-pro-BNP is not suitable for the prediction of MACE with an AUC of ROC curve on admission of 51% (95% CI, 38-65%) and 52% (95% CI, 38-66%) at 90 min after admission (Bassan,Tura & Maisel, 2009; Haaf,Reichlin,Corson *et al.*, 2011). The reason for low predictive value of NT-pro-BNP could be due to the inherited selection bias and the utilisation of low risk patients.

5.3 Summary

Advances in technological and pharmacological approaches to ACS have accelerated and caused rapid changes in the management of patients admitted with chest pain suggestive of ACS. These changes have contributed to the introduction of various cardiovascular specific and non-specific biomarkers designed with different success to aid the clinician in the identification of low risk ACS patients. Many so-called novel biomarkers including IMA are not routinely offered in laboratories across the UK for different reason including inadequate analytical suitability and clinical usability. Common statements such as “can be used for early diagnosis of AMI” and “may contribute to the current ACS patients’ managements”; a different set of question need to be answered before such comment could be made. The “so what?” the introduction of new biomarkers have to convince clinician and health economist to change their current practice. To do so new biomarkers must be sensitive, specific and with good predictive value.

In the present study the AUC of ROC curve for copeptin and H-FABP ranged from 54-58% and from 52-65% on admission and at 90 min after admission respectively. In contrast, the AUC of cTn, myoglobin and CK-MB ranged from

71-90% on admission and from 72-88% at 90 min after admission. Clearly the present study does not support the use of these novel biomarkers as a standalone test in an ED setting. The reason for this low performance of these novel biomarkers can be due to several factors including kinetic release of these biomarkers, specificity, utilisation of low risk patients in this current study and the clinical cut-off utilised. Based on the present study finding IMA testing as a standalone assay is not ready for prime-time use. However, in agreement with a published study; our study also found that the diagnostic efficiency of the combined IMA plus hs-cTn could be used for the diagnosis of AMI (Patil,Banker,Padalkar *et al.*, 2013). The present study also confirmed that the diagnostic efficiency of hs-cTn is superior to the current novel biomarkers. Thus, in conclusion IMA assay could not safely and reliably be used as a prime test for the diagnosis of chest pain suggestive of cardiac ischaemia or ACS. Furthermore, IMA assay is not suitable as prognostic test for the management of ACS patients.

5.4 Case studies

Aim: To discuss the clinical findings of the three patients who were misdiagnosed and subsequently died from AMI.

5.4.1 Case study 1 (3105)

A 62-year-old female presented (23/06/2008) to ED at Edinburgh Royal Infirmary with chest pain suggestive of ACS. She was recruited and entered RATPAC on POCT protocol. On admission she was immediately triaged and an ECG was undertaken. Blood samples were taken for cardiac troponin, myoglobin, creatinine and CK-MB. The spare samples were later analysed for hs-cTnT/I, copeptin, H-FABP, NT-pro-BNP and IMA.

The patient's ECG recording was normal. Blood born biomarkers including cardiac troponins, myoglobin, NT-pro-BNP and CK-MB were normal on admission and 90 min after admission (table 51). The novel biomarkers, copeptin, IMA and H-FABP were below the clinical cut-off on admission and 90 min after admission (table 51). The patient's TIMI score was 0; however, 76 days (07/09/2018) from admission this patient died from an AMI. The final diagnosis was registered as an ACS.

Table 51: Patient results (case study 1)

Tests	Results	Clinical cut-off
Acu TnI 0 min	0.00	42 ng/L
Acu TnI 90 min	0.00	
Cent TnI 0 min	<0.002	40 ng/L
Cent TnI 90 min	<0.002	
H-FABP 0 min	0.76	2.5 mg/L
H-FABP 90 min	0.71	
Myoglobin 0 min	38.86	66 mg/L
Myoglobin 90 min	26.88	
Copeptin 0 min	9.8	17.4 pmol/L
Copeptin 90 min	6.0	
Hs-cTnT 0 min	5.65	14 ng/L
Hs-cTnT 90 min	< 3	
Pro-BNP 0 min	< 20	125 ng/L
Pro-BNP 90 min	< 20	
IMA 0 min	80	127 kU/L
IMA 90 min	82	
CK-MB 0 min	1.1	5 µg/L
CK-MB 90 min	0.9	
Creatinine	116	µmol/L
TIMI	0	
ECG	Normal on admission	
Final diagnosis	ACS	
Days to death	76	

5.4.2 Case study 2 (3021)

A 72-year-old female presented (14/02/2008) to ED at Edinburgh Royal Infirmary with chest pain suggestive of ACS. She was recruited and entered RATPAC on POCT protocol. On admission she was immediately triaged, and

an ECG was undertaken. Blood samples were also taken for cardiac troponin, myoglobin, creatinine and CK-MB and they were found to be normal on admission and 90 min after admission (table 52). Blood samples for the patient were also analysed for other cardiac biomarkers including: copeptin, IMA, hs-cTnT/I and H-FABP were below the clinical cut-off on admission and 90 min after admission (table 52)

A risk assessment was carried out and TIMI score of 1 was recorded; as a result, this patient was considered low risk of CVD. The ED clinicians diagnosed this patient with gastroesophageal reflux and subsequently discharged her back to the community. 76 days from admission this patient died from an AMI. The final diagnosis was registered as an ACS.

Table 52: Patient results (case study 2)

Tests	Results	Clinical cut-off
Acu TnI 0 min	0.00	42 ng/L
Acu TnI 90 min	0.002	
Cent TnI 0 min	< 0.002	40 ng/L
Cent TnI 90 min	< 0.002	
FABP 0 min	2.33	2.5 mg/L
FABP 90 min	1.59	
Myoglobin 0 min	11.14	66 mg/L
Myoglobin 90 min	22.32	
Copeptin 0 min	8.8	17.4 pmol/L
Copeptin 90 min	10.3	
Hs-cTnT 0 min	< 3	14 ng/L
Hs-cTnT 90 min	< 3	
Pro-BNP 0 min	< 20	125 ng/L
Pro-BNP 90 min	< 20	
IMA 0 min	75	127 KU/L
IMA 90 min	74	
CK-MB 0 min	3.36	5 µg/L
CK-MB 90 min	3.2	
Creatinine	114	µmol/L
TIMI	1	
ECG	Normal on admission	
Final diagnosis	Gastro-oesophageal reflux	
Days to death	76	

5.4.3 Case study 3 (6236)

A 60-year-old female presented (06/01/2009) to ED at Leicester Royal Infirmary with chest pain suggestive of ACS. She was recruited and entered RATPAC on POCT protocol. On admission she was immediately triaged and taken to the cardiac department. An ECG was undertaken and was normal. Blood samples were tested for cardiac troponin, myoglobin and CK-MB and they were found to be normal on admission and 90 min after admission (table 53). She was discharged home on the day of admission. Blood samples for the patient were also analyzed for other cardiac biomarkers including: copeptin, IMA, hs-cTn and H-FABP, and were below the clinical cut-off on admission and 90 min after admission (table 53).

A risk assessment was carried out and TIMI score of 0 was recorded; as a result, this patient was considered low risk of CVD. The ED clinicians diagnosed this patient with ACS and discharged her back to the community. 87 days from admission this patient died (03/04/2009) from an AMI. The final diagnosis was registered as an ACS.

Table 53: Patient results (case study 3)

Tests	Results	Clinical cut-off
Acu TnI 0 min	0.03	42 ng/L
Acu TnI 90 min	0.00	
Cent TnI 0 min	0.03	40 ng/L
Cent TnI 90 min	< 0.02	
FABP 0 min	1.05	2.5 mg/L
FABP 90 min	1.48	
Myoglobin 0 min	35.1	66 mg/L
Myoglobin 90 min	44.54	
Copeptin 0 min	9.4	17.4 pmol/L
Copeptin 90 min	6.9	
Hs-cTnT 0 min	8.2	14 ng/L
Hs-cTnT 90 min	5.14	
Pro-BNP 0 min	45	125 ng/L
Pro-BNP 90 min	49	
IMA 0 min	110	127 KU/L
IMA 90 min	115	
CK-MB 0 min	1.3	5 µg/L
CK-MB 90 min	1.3	
Creatinine	NA	µmol/L
TIMI	0	
ECG	Normal on admission	
Final diagnosis	ACS	
Days to death	87	

5.5 Discussion

In most cases, patients presenting to the ED with chest pain suggestive of ACS are considered high-priority triage cases. Evaluation and treatment should follow a predetermined, institution-specific protocol for chest pain. The clinical cut-off for troponin by institution is summarised in table 53. Chest pain can originate from various disorders. However, chest pain of cardiac origin is the most concerning as it is the most life threatening and must be identified or excluded as matter of urgency.

Patients presenting with abnormal vital signs and complaining of chest pain are usually triaged to a critical care area. Intravenous access is established and abnormalities in oxygen saturation treated with oxygen. The objective here is to meet the goal of less than 30-minute door-to-intervention time. A

delay in treating genuine ACS patients is associated with high morbidity and mortality (Goss,Brachmann,Hamm *et al.*, 2017). All patients suspected of ACS require a 12-lead ECG performed within 10 min of arrival. Those cohorts with ECG changes e.g. STEMI are immediately treated. However, if the ECG changes are absent or not conclusive patients are subject to continuous ECG recording or ECG is often repeated after 6 to 24 hours. The reason for this is to compare results (asymptomatic) vs. symptomatic. If ST-segment depression ≥ 0.5 mm (0.05 mV) is recorded in more than one lead, this may suggest NSTEMI (Diercks et al. 2006). NSTEMI is a retrospective diagnosis based on clinical history, troponin levels and ECG finding. The ACC/AHA guidelines recommend that for those patients brought to ED by ambulance to have 12-lead ECG done prior to arrival in order to expedite care (Antman,Anbe,Armstrong *et al.*, 2004).

Table 54: Troponin threshold used by the participating center.

Location	**Troponin assay (Troponin threshold used (µg/L))**	**Laboratory analyser**	**Timing of troponin (hours *)**
Edinburgh (Medical assessment unit)	Siemens Centaur troponin I Ultra (< 0.20)	Siemens Centaur XP	12
Leicester (Inpatients ward)	Siemens Centaur troponin I Ultra (< 0.06)	Siemens Centaur XP	12

- = Timing after onset the worst symptom

The clinician's decision is required to assess chest pain patients' in the ED setting, dependent on the nature of the attending clinician. The ED clinician is usually concerned about early exclusion of AMI (rule-out), whereas, a cardiologist is most concerned with confirmation of AMI (rule-in). Patients not showing characteristic ECG changes are assessed on the basis of ECG and clinical history/features into high, medium or low risk cohort. High risk patients are always admitted for further investigation. On the other hand, medium to low risk patients are those without clinical or ECG evidence of ischaemia and who require cardiac biomarkers measurement to exclude ACS. The

confirmation of AMI in this cohort of patients requires time and serial troponin measurement to confirm or exclude AMI. In these case studies all patients were categorised into low-risk patients with no ECG changes or abnormal troponin finding and appropriately discharged.

While the ECG tends to underestimate the risk of disease in chest pain patients, the clinical history and the ECG findings tends to lead clinicians to overestimate the likelihood of ischaemia (Karlson,Herlitz,Wiklund *et al.*, 1991). A study of 4,500 chest pain patients admitted for rule-out evaluation found that clinician's impression with discharge diagnostic were more than 50% associated with "vague suspicion of AMI'. Clinicians were unable to draw any firm conclusion about the presence or the absence of ischaemia. Thus, the clinicians surveyed in this study felt that the risk was great enough that this cohort of patients should be admitted for extended workup. Within this group 47% of patients were eventually found not to have ischaemic disease (Karlson,Herlitz,Wiklund *et al.*, 1991).

Although, patients with chest pain should not be diagnosed based on symptoms alone; they tend to exhibit wide ranges of presentation and symptoms including prolonged (>20 min) anginal pain, recent destabilisation of previously known SA patients, post-AMI angina, reterosternal pressure or heaviness radiating to the left arm, neck or jaw. This range of symptoms and presentation can also be accompanied by nausea, abdominal pain, dyspnoea, epigastric pain, recent-onset indigestion, stabbing chest pain, and chest pain with some pleuritic features. Moreover, these symptoms appear to be frequently seen in younger adults (25-40 years), > 75 years patients, women and patients with diabetes, dementia and chronic renal failure (Canto,Fincher,Kiefe *et al.*, 2002; Culic,Eterovic,Miric *et al.*, 2002a). Some NSTEMI patients do not exhibit the above symptoms and as a result this leads to under-recognition of the disease (Brieger,Eagle,Goodman *et al.*, 2004). The diagnostic and therapeutic evaluation of patients with NSTEMI becomes extremely difficult when the main diagnostic pointers are masked or

exacerbated by existing condition or abnormal results i.e. intraventricular conduction defect or LV hypertrophy, anaemia, infection, inflammation and absence of atypical symptom (Lev,Battler,Behar *et al.*, 2003).

Chest pain is one of the main symptoms of ACS; however, approximately 25% of patients with non-classical symptoms or atypical presenters have suffered a silent AMI (Khafaji & Suwaidi, 2014). The diagnostic and therapeutic evaluation of an atypical presenter is extremely difficult such as that among patients with diabetes who may not sense pain due to peripheral neuropathy (Nikolaou,Arntz,Bellou *et al.*, 2015). Moreover, chest pain is a common presentation and is associated with a broad spectrum of conditions and has a broad differential diagnosis (table 55).

A greater proportion of women than men with AMI die of sudden cardiac arrest before reaching hospital (Dunlay & Roger, 2012). The prevalence of microvascular angina is higher in women than men (Hochman,Tamis,Thompson *et al.*, 1999). This finding suggests that gender differences may have an influence on cardiovascular disease progression and physiology (Dunlay & Roger, 2012). Women's Ischemia Syndrome Evaluation (WISE) study demonstrated that 1:100 women with normal coronary arteries had in fact approximately 80% defined coronary atherosclerosis that was concealed by positive remodeling. This finding suggests that a better diagnostic tool tailored for women related diseases is required (Khuddus,Pepine,Handberg *et al.*, 2010).

The diagnostic of CAD in women presents a challenge not observed in men. Epidemiological studies of CAD in women suggest that women are generally of low risk until they reach the seventh decade of life (Berger,Elliott,Gallup *et al.*, 2009). In symptomatic low risk women, non-invasive diagnostic studies such as cardiac imaging are recommended (Fass & Navarro-Rodriguez, 2008). Thus, until adequate evidence is available; women with chest pain should be screened for CVD risk factors and treated using evidence-based protocols (Mishra,Ray,Dalal *et al.*, 2016). In all the above study cases other

diagnostic modalities such as resting echocardiography were not requested or carried out.

Table 55: Chest pain-common differential diagnosis

AMI
Stable angina
Unstable angina
Oesophageal reflux
Pulmonary embolic disease
Musculoskeletal
Cervical root compression
Aortic dissection
Chest wall pain
Cholecystitis
Pancreatitis
Anxiety

5.5.1 Sudden cardiac death and sudden cardiac arrest

Sudden cardiac death (SCD) is defined as *"death from an unexpected circulatory arrest, usually due to cardiac arrhythmia occurring within 1 h of the onset of symptoms"*. On the other hand, ACC/AHA/ESC 2006 describes and defines sudden cardiac arrest as *"death from an unexpected circulatory arrest, usually due to a cardiac arrhythmia accruing within 1 h of the onset of symptom, in whom medical intervention (e.g. defibrillation) reverses the events"* (Priori,Wilde,Horie *et al.*, 2013).

In the USA, cardiovascular disease was responsible of every 2.9 deaths in all fatalities in 2006. 50% of the overall death was classified as sudden. Similar incidents are also observed in Europe, SCD is defined as death due to cardiac causes occurring within 1 h of the onset of symptom.

The most common cause of SCD is ischaemic heart disease (Modi & Krahn, 2011). Autopsy finding shows that such patients may have an occlusive recent thrombosis in a major artery but the largest group has no recent occlusion (Modi & Krahn, 2011). Out-of-hospital SCD occurs among patients in whom cardiac disease has previously been identified but classified as low risk or in whom SCD is the first clinical expression. The annual incidence of SCD increases as a function of advancing age (Myerburg & Junttila, 2012).

Sudden cardiac arrest (SCA) can also be caused by coronary spasm with minimal or no pre-existing CAD. Females tend to present with mitral valve prolapse with ECG repolarization abnormalities. In general, SCA can also be triggered by poor air quality (pollution), pre-existing chronic diseases such as AIDS, lower socioeconomic groups, depression, anxiety, social isolation and acute emotional stress (Basso,Perazzolo Marra,Rizzo *et al.*, 2015; Ensor,Raun & Persse, 2013; Modi & Krahn, 2011).

Case study one and two could be the result of either SCD or SCA; it is difficult to establish the exact cause of death without an autopsy and some degree of speculation. Moreover, the inability to obtain the patient's notes makes an evidence-based judgment difficult (Lloyd-Jones,Hong,Labarthe *et al.*, 2010). Nevertheless, it is likely that all these case study patients suffer from cardiac ischaemia. To date there is no reliable cardiac biomarker for ischaemia.

5.5.2 Can gastroesophageal reflux provoke or worsen myocardial ischaemia?

Patient's history and characteristics do not reliably distinguish between cardiac and oesophageal causes of chest pain (Locke,Talley,Fett *et al.*, 1997). Gastroesophageal reflux disease (GERD) is characterised by symptoms and complications such as oesophageal stricture, Barrett oesophagus and esophagitis and is caused by the reflux of gastric contents (Hong, 2010). Non-

cardiac chest pain is a term that encompasses all causes of chest pain that are not of cardiac origin and after excluding cardiovascular disease. The term includes aetiologies such as inflammatory disorders, musculoskeletal, pulmonary, neurological, and oesophageal diseases. In clinical practice, esophageal chest pain (ECP) is a common problem whose diagnosis and treatment remain challenging (Galmiche, 2006). The Rome III criteria define ECP as an episode of unexplained chest pain of visceral quality and usually midline in location. The exact prevalence of ECP is difficult to estimate; because its diagnosis requires the use of multiple diagnostic tests to exclude other conditions (Remes-Troche,Maher,Mudipalli *et al.*, 2007). However, some studies showed that ECP is diagnosed based on symptoms (Rao, 2011). A population-based survey in USA and Australia found that the annual prevalence was estimated to be between 23% and 33% respectively, with an equal sex distribution (Locke,Talley,Fett *et al.*, 1997). There is little consensus on how to diagnose a patient with ECP.

Chest pain is a common complaint and cardiac and esophageal pains are often similar. It has been suggested that esophageal dysfunction could itself trigger myocardial ischaemia (Liu,He,Chen *et al.*, 2013). Making differential diagnosis of chest pain and evaluating the symptoms is often difficult and not sufficient to predict the underlying disease in this cohort of patients. This difficulties lies in the fact that the distal oesophagus and heart share common afferent vagal supply; thus mechanical and/or chemical stimulation of the oesophagus can provoke myocardial ischaemia leading to chest pain (Johansson,Wallander,Ruigomez *et al.*, 2003). Furthermore, non-cardiac chest pain gastroesophageal reflux occurs at the same frequency in patients with normal and pathological coronary angiographies (Fenster, 2004). It is often difficult to distinguish between non-cardiac and cardiac chest pain based upon symptom at presentation alone because the nerves that supply the heart also supply the esophagus (Mudipalli,Remes-Troche,Andersen *et al.*, 2007). It is possible that in case three the chest pain was in fact due gastroesophageal reflux and it is also possible that gastroesophageal reflux could provoke

myocardial ischaemia and subsequently AMI. Without an autopsy it is impossible to determine the exact cause of AMI and death in this patient.

5.5.3 Conclusion

Unfortunate overreliance on negative hs-cTn, patient's history, TIMI score and normal ECG is common reason for misdiagnosing AMI patients. It is critical to remember that cardiovascular biomarkers are useful for risk stratification, but no test is capable of stratifying patients risk to zero. Evaluation of patients with chest pain in an ED setting is expensive, time consuming and may results in uncertain diagnosis (Fox, 2005). It is also a common misconception that the prognosis from NSTEMI is better than that for STEMI. Hospital mortality is greater for STEMI than for any other ACS condition and this is largely due to the fact that STEMI is always associated with myocardium damage and cell death (Scholz,Maier,Maier *et al.*, 2018). However, mortality rate following discharges with NSTEMI is higher and approximately one third of these patients will suffer a further AMI, death or readmission with an ACS year later (Saaby,Poulsen,Hosbond *et al.*, 2013; Scholz,Maier,Maier *et al.*, 2018). The simplest approach to reduce mortality with NSTEMI cohort is to offer angiography in all patients admitted. A study looking at the cost of caring for 69 patients in the 12 months before and after coronary angiography; showed the cost of the procedure would offset after 18 months if this cohort of patients were successfully treated. This approach is implemented in some centers (Newby,Fox,Flint *et al.*, 1998).

A study conducted by *Bandstein et al.,* found in a large cohort (n = 14,636) of patients with the first hs-cTnT of < 5 ng/L combined with no sign of ischaemia on ECG is safe to rule-out AMI with nearly 100% NPV of AMI and a 100% NPV for death within 30 days, irrespective of sex, age or other cardiovascular risk factors (Bandstein,Ljung,Johansson *et al.*, 2014). Although, *Bandstein et al.,* work is true in all these case studies, however, a reliable test which detected

ischaemia is still warranted. In addition, in all three cases, novel biomarkers were all below the clinical cut-off on admission and 90 min after admission and if used at the time of admission they would not change the care pathway for these patients.

Women and men differ in their genetic map by a single chromosome. However, this single chromosome out of the 46 in human affects both the expression of disease and behavioral characteristics; thus, may reduce or increase susceptibility to cardiovascular disease. In future, gender specific cardiovascular biomarker, reference ranges, diagnostic algorithm, and treatment of cardiovascular disease may be required.

5.6 Cost-effectiveness-analysis of ischemia modified-albumin

The National Health Service (NHS) is burdened with significant costs when patients present to the ED with chest pain suggestive of ACS/AMI. Biochemical testing is not a major expenditure in hospitals; however, hospital bed occupancy and intervention contribute significantly the NHS budget. To reduce expenditure, these institutions have formulated strategies to rule-in or rule-out AMI in the shortest period of time (Chang,Shofer,Weiner *et al.*, 2008). The use of hs-cTn is the main driver for this strategy because most patients can be discharged or admitted for investigation and treatments within 3 h, in alignment with the current ESC recommendations.

In the scenario were a hospital uses multiple cardiac biomarker strategy, the combination of IMA plus hs-cTnT may be cost effective. However, this strategy assumes that the diagnostic utility provided with IMA is equal to that of hs-cTnT. IMA study clearly demonstrates that this is not the case; moreover, IMA is a biomarker for ischaemia whereas hs-cTn indicates myocardial injury. The outcomes are therefore not equivalent. In this instance cost benefit analysis is the appropriate method to use because it ignores the clinical situation when the IMA will be used in the current clinical practice. As demonstrated in the present study, IMA test alone is not diagnostically superior to hs-cTn; however, when combined with hs-cTnT or hs-cTnI it may be useful.

The cost of a single IMA test (£27.00 per test) is five-fold more expensive than a single hs-cTnT (£4.00 per test). However, despite the initial higher cost per test of IMA, the overall cost could be mitigated when we take into consideration serial hs-cTnT requirement to achieve the desired optimum diagnostic for AMI and average daily bed cost (£300.00 per day), the average cost of a day case (£696.00), the average cost of an ED day attendance (£114.00), the average cost of a non-elective inpatient short and long stay (£1,489.00), imaging cost, litigation and value of knowing (Chang,Shofer,Weiner *et al.*, 2008; England,

2012-2013; Goodacre, 2003). "Value of knowing" focus on the value of a diagnostic testing in resolving patients' uncertainties about their medical condition (Lee, Neumann, & Rizzo 2010b). The value of knowing is not usually fully integrated in the cost-effectiveness analysis.

The objective of the health cost section in this study is to firstly demonstrate the hypothetical scenarios whereby IMA assay alone is introduced to the ED setting and secondly, when combine diagnostic efficiency panel of IMA plus hs-cTnT are also introduced to the main stream laboratory diagnostic in an ED setting. Although, the cost benefit of IMA in combination with H-FABP, NT-pro-BNP and copeptin is not warranted due low diagnostic efficiency. In this thesis an overall cost benefit will also be calculated.

It is well documented hs-cTn testing over a period of time reaches 100% sensitive and specificity (Keller,Zeller,Ojeda *et al.*, 2011; Reichlin,Hochholzer,Bassetti *et al.*, 2009). Therefore, in order for IMA, H-FABP, copeptin and NT-pro-BNP testing to contribute to ACS patient's managements; their diagnostic efficiency must be assessed on admission and should be based on a single sampling i.e. only one blood sample collect on admission. The incremental value of IMA combined with other biomarkers can only be adopted by healthcare professional in an ED setting if it demonstrates that low risk patients with chest pain suggestive of IMA can be discharged safely or admitted for further investigation at presentation.

The present study identified three patients with AMI as confirmed by the final diagnosis. In the scenario were IMA assay alone is introduced, in order to save one high risk patients out of 174 who present to ED with chest pain suggestive of AMI a total of £1,334.00 per high risk patients is required. The cost could also be expressed as NNT, the current project finding suggests a total of 50 patients (£ 380,00 per high risk patients) needed to be screened in the view of detecting one correct ACS patient. The introduction of IMA assay

testing to the main stream healthcare system would account for an increase of £1102.00 per high risk patient. In contrast, the current cost is only £232.00 per high risk patients when using hs-cTnT alone on admission and £464.00 when measured on admission and 90 min. This scenario is unlikely to be adopted in an ED setting because in this study IMA assay is not sensitive or specific enough for the diagnosis of AMI.

When implementing the scenario of the combined diagnostic efficiency of IMA assay plus hs-cTnT; in order to save one high risk patients out of 174 patients presented to ED with chest pain suggestive of AMI a total of £ 1,566.00 is required. The NHS will have to contribute an additional cost of £1,334.00 per high risk patient or an extra £31.00 per screened patients compared to £ 8.00 when using hs-cTnT on admission and 90 min. It is evident that even with a serial hs-cTnT measurement up to 3 h the latter would be cheaper than a single IMA testing. Identifying high risk patients in a timely fashion preferably on admission may save the trust additional cost including AMI related complications, bed occupancy, imaging investigation, loss of earning and unnecessary stress to patients. Correct identification of ACS patients on admission could contribute with a projected saving of £889.00 per patient successfully prevented from being admitted (Byrne, 2014). In real terms the introduction of IMA plus hs-cTnT to healthcare systems may be cost neutral or beneficial, as the extra expenses will be offset by the cost of admitting this cohort of patients. The true cost of the introducing both IMA and hs-cTnT in combination to the healthcare system requires further investigation and costing that take into consideration the cost of possible litigation, AMI related complications, bed occupancy and overhead expenditure. Thus, future health economic evaluation is required.

5.6.1 Implementation of the combined diagnostic efficiency of ischemia modified-albumin plus highs sensitivity cardiac troponin for the diagnosis of acute myocardial infarction

AMI is associated with poor prognosis so immediate and accurate diagnosis is important to allow appropriate treatment and care as soon as possible. In some cases, the treatment for medium and high-risk patients with suspected ACS is usually started before a diagnosis is confirmed. However, patients presenting to ED with chest pain suggestive of AMI at St George's Healthcare NHS Trust will undergo ECG as first line of investigation, if ECG abnormalities is recorded patients will be transferred to a specialized cardiac unit for urgent treatment. However, if ECG is inconclusive and AMI is still suspected; the patients will be asked to provide blood and stay for observation for up to 3 h. Moreover, if suspicion is still present, the patients may be required to stay in observation up to 12 h. The triage algorithm used at St George's Healthcare is as follow (Figure 55).

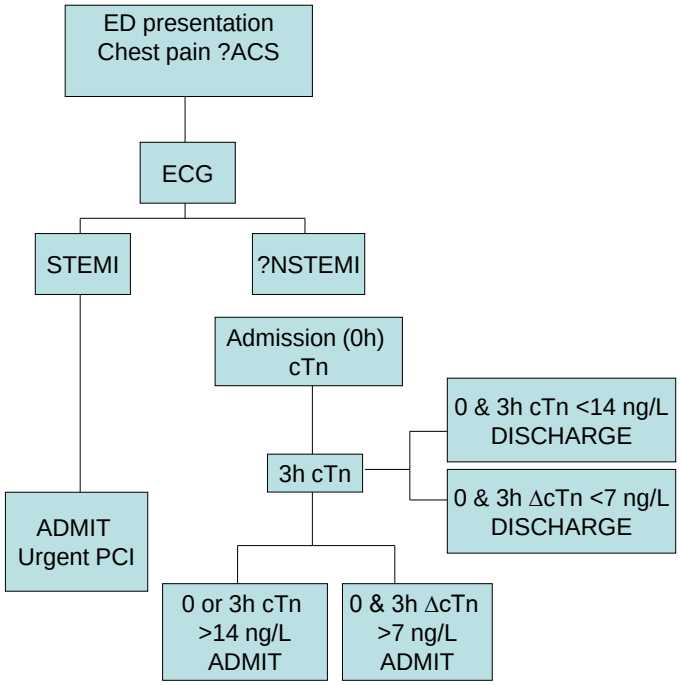


Figure 55: Positive ECG patients are immediately admitted to cardiac unit treatment and PCI. Low risk patients with abnormal, normal or inconclusive ECG are required to stay for observation up to 3 h. In this cohort blood sample is obtained on admission and 3 h after admission. Patients with the absolute or delta changes of hs-cTn concentration on admission and 3 h are less than 14 ng/L and 7 ng/L respectively are discharged or AMI is rule-out. However, patients with the absolute or delta changes of hs-cTn concentration on admission and 3 h is greater than 14 ng/L and 7 ng/L respectively are admitted and referred for urgent treatment and PCI. (St George's Healthcare NHS Trust, London).

The current algorithm utilised at St George's Healthcare NHS Trust was adopted in order to improve the sensitivity and specificity of hs-cTn in the diagnosis of ACS, triage and to comply with the current guidelines (ESC, NICE and ACC) which require serial blood sampling. As recommended by the current guidelines ideally blood should be measured for hs-cTn concentration at the first sign of chest symptom and 3 h after admission (Hamm et al., 2011). Repeated hs-cTn measurement in the second time maximizes the safety and certainty to rule-in or rule-out an AMI (Chenevier-Gobeaux et al., 2015, Thygesen et al., 2012c).

The lack of clear definition of a rise and fall (delta changes) of hs-cTn concentrations has led many clinicians to adopt a change of ≥ 20% as a practical cut-off. Conversely, a study has showed that cut-off value of ≥ 20% needed to be reassessed and increased in patients with hs-cTn in a lower range, older patient, and in-patients with existing co-morbidities such as renal failure (Apple, 2009; Giannitsis,Becker,Kurz *et al.*, 2010). The introduction of ≥ 20% delta rules over 2 h period has increased specificity to 92% (95% CI: 90-94%) but reduced sensitivity to 56% (95% CI, 48.0-63%) (Aldous,Pemberton,Richards *et al.*, 2012). The absolute value changes of hs-cTn assays (Roche and Siemens) were diagnostically (2 h) superior to relative value changes (Reichlin et al., 2011); this finding were also supported and confirmed by other studies (Mueller et al., 2012; Wildi et al., 2013). The implementation of delta value over 2 h is supported by the observation that early presenters (<2 h from chest pain) tend to have hs-cTn values at admission below the 99th percentile cut off compared to patient presenting with chest pain > 3 h (Patil,Banker,Padalkar *et al.*, 2013; Rubini Gimenez,Twerenbold,Reichlin *et al.*, 2014). This finding may suggest a reduced diagnostic efficiency of hs-cTn if measured only on admission and 2 h. Thus, the introduction of 2 h measurement is not warranted as early presenters are underrepresented and further studies are required (Vorlat,Van Hoof,Hammami *et al.*, 2015). The above algorithm is the best strategy available; however, patients who do not fit the criteria of a classical ACS may

be kept in ED for observation up to 12 h. Thus, admitting patients for observation will result in an increased cost to the health system.

5.6.2 Proposed algorithm for triaging low risk patients presenting with chest pain suggestive of acute coronary syndrome

Tools to help in risk stratification of patients presenting to ED with chest pain and ACS exist but none is adequately equipped to help clinicians to determine which patients can safely be discharged (Christenson et al., 2004; Antman et al., 2000; Goldman et al., 1988; Tatum et al., 1997). The NICE guidelines require clinicians to offer PCI treatments to restore blood flow within 72 h only to medium and high-risk patients; whereas, low risk patients are excluded from these life-saving interventions. Clinical presentation of low risk patients suspected of NSTEMI is challenging and requires a carful assessment of each patient from presentation, diagnosis, risk stratification, therapeutic strategy and response to treatment. Long term follow-up of patients who survived and reached medical assistance showed a higher mortality rate of two-fold difference at 4 years of NSTEMI compared with STEMI (Terkelsen,Lassen,Norgaard *et al.*, 2005). One probable reason for this is that NSTEMI patients tend to be older with existing co-morbidities (Ren,Ye,Wang *et al.*, 2014; Rosengren,Wallentin,A *et al.*, 2004).

The European Society of Cardiology (ESC) guidelines for the management of ACS patients; requires that all patients admitted to the ED with chest pain suggestive of ACS should have a resting 12-lead ECG carried out and interpreted by a qualified person within 10 min of arrival. The problem with using ECG is that a completely normal ECG does not exclude ACS/AMI (50% sensitivity). Several studies found that approximately 5% of patients, who were sent home because of normal ECG, were subsequently readmitted with UA or AMI (Goodacre,Cross,Arnold *et al.*, 2005; Schreck & Fishberg, 2014). In addition, up to 50% of patients presenting to the ED with chest pain do not

show STEMI in their ECG assessment (Apple,Wu,Mair *et al.*, 2005). To compensate for the poor sensitivity of the ECG and the uncertainty associated with it; the ESC guidelines for the management of ACS and in particular patients with suspected NSTEMI requires the measurement of a biomarker of myocardial injury such as hs-cTnI or hs-cTnT. The presence of hs-cTn is not considered an early biomarker of cardiac ischaemia, because hs-cTn is only detected after myocardial injury.

Although, hs-cTn is highly specific for myocardial injury, it is also known to be elevated in non-cardiac related diseases such as sepsis, endocarditis, pulmonary embolism, cocaine abuse, chemotherapy, and renal disease. Low hs-cTn specificity combined with detectable concentrations of hs-cTn in non-cardiac diseases and healthy populations makes the interpretation of AMI difficult (Gamble,Carlton,Orr *et al.*, 2013). Currently there is no blood borne accepted “gold standard” test for cardiac ischaemia; although, blood lactate was previously used (Attana,Lazzeri,Picariello *et al.*, 2012; Henning,Weil & Weiner, 1982). Despite the introduction of hs-cTn with an increased sensitivity, the current opinion suggests that it is still unable to detect acute ischaemia before myocardial damage. Controversially, there are various proposed mechanisms of cTn release that are apparently not related to cardiomyocyte injury including apoptosis, increased cell permeability with stress and the production of the membranous blebs that contain cTn (Piper,Schwartz,Spahr *et al.*, 1984; White, 2011). Moreover, the exact mechanism and the kinetic release of cTn is unknown, one suggestion is that the molecular weight difference between of cTnT (35 kDa) and cTnI (24 kDa) may be responsible (Wu,Feng,Moore *et al.*, 1998). The possibility of detecting cTn in ischaemic patients using the current analytical methods is still debatable.

In the present study the combined sensitivity and specificity of IMA plus hs-cTnT on admission was 71% (95% CI, 56-82%) and 100% (95% CI, 98-100%)

respectively and an NPV of 94%. The proposed algorithm (Figure 56) appears to identify high risk patients before substantial, permanent myocardial damage occurs, and when potential for myocardial salvage is optimal (Burke & Virmani, 2007). The present study identified 3 out of 174 low risk patients, who had a final diagnosis of AMI including one fatality as confirmed by follow up RATPAC study. This cohort of patients was used to estimate health cost and to develop an algorithm for the triage of low risk patients presenting with chest pain suggestive of AMI.

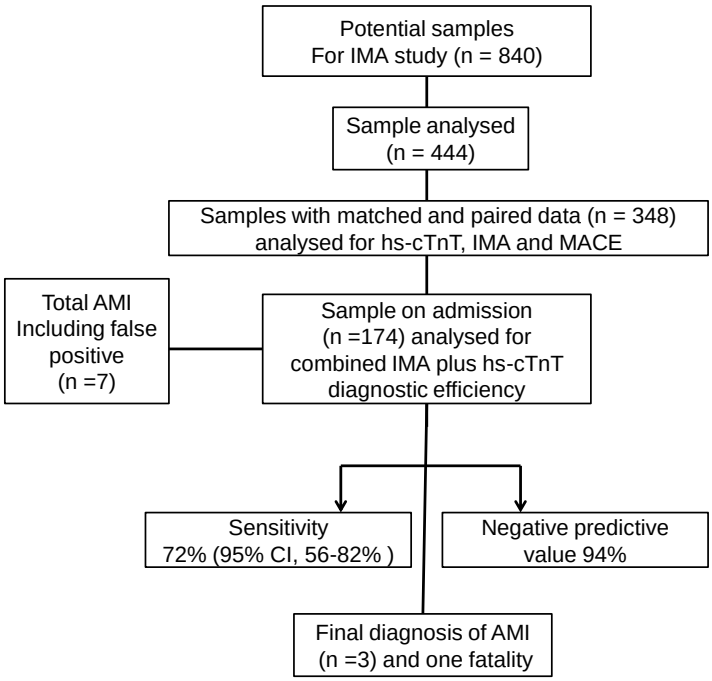


Figure 56: A total of 444 out of 840 unmatched samples were available for the present study, from which n = 174 samples were analysed for the combined diagnostic efficiency of IMA plus hs-cTnT. The sensitivity was 72% (95% CI, 56-82%), NPV of 94% and n = 7 patients were diagnosed with AMI using this protocol. The n = 174 out of the 444 available samples had a complete matched and paired data including concentration values for hs-cTnT, hs-cTnI, copeptin, H-FABP, myoglobin, CK-MB, IMA and final outcome of MACE at 30 days. The final diagnosis of AMI as established by the RATPAC was n=3 AMI and one fatality.

The combined diagnostic efficiency of IMA plus hs-cTnT identified 7 AMI patients including the three correct patients as established by the final diagnosis. Clearly the combined diagnostic efficiency of IMA plus hs-cTnT could be used to rule-in and rule-out AMI. In terms of percentage the combined diagnostic efficiency of IMA plus hs-cTnT misclassified 4 patients as false positive out of 174 (< 3%). This cohort of misclassified patients would benefit from further investigations as clinicians would be unable to draw any firm conclusion about the presence of the absence of cardiac ischaemia or AMI and they will have no choice but to admit these patients for extended investigation as per current protocol (Figure 56). Based on the above evidence, the proposed algorithm using the combined diagnostic efficiency of IMA plus hs-cTnT could be as follows (Figure 57).

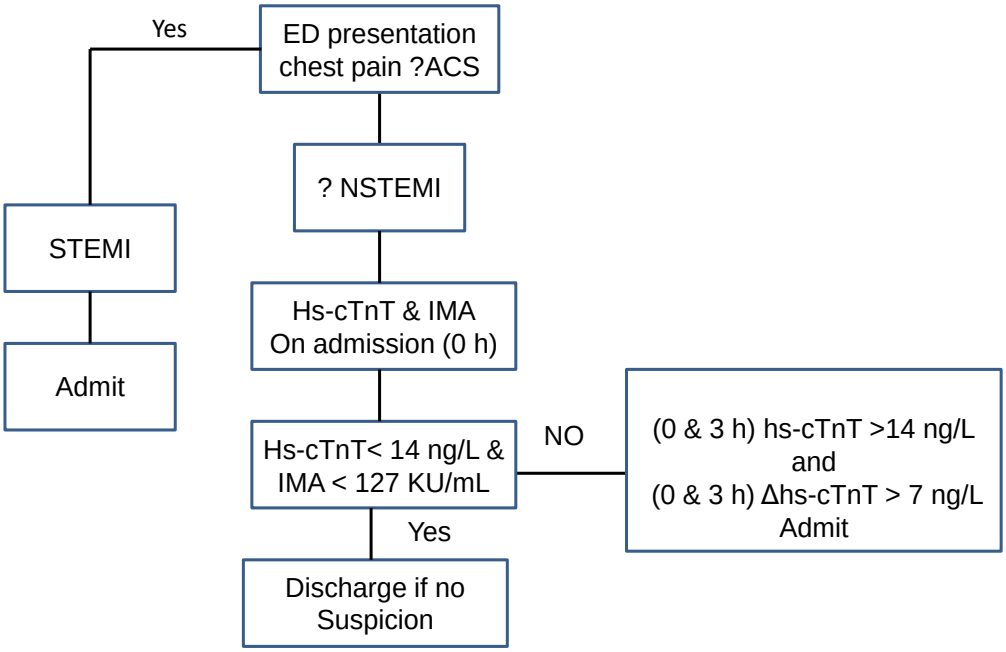


Figure 57: Positive ECG patients are immediately admitted to cardiac unit treatment and PCI. Low risk patients with abnormal, normal or inconclusive ECG will be required to stay for observation up to 3 h. In this cohort blood sample is obtained on admission for hs-cTnT and IMA analysis. Patients with hs-cTnT and IMA bellow the clinical cut-off will be discharged. Patients with either IMA or hs-cTnT or both above the clinical cut-off are retested for hs-cTnT after 3 h. Patients with the absolute or delta changes of hs-cTn concentration on admission and 3 h is greater than 14 ng/L and 7 ng/L respectively are admitted and referred for urgent treatment.

This proposed algorithm allows standardization of the clinical routine protocol and thereby improves workflow and quality of care. However, bearing in mind the heterogeneous spectrum of ACS and patients with different risk in term of MACE, death, AMI and recurrent AMI. Thus, it is important that in each patient, the clinician must make an individual decision regarding patients' management, taking into account patients existing co-morbidity; thereby clinician may deviate from the proposed algorithm.

5.7 Advantages and disadvantages of IMA study

The present study has adopted guidelines as stated by the AHA. The AHA has produced these guidelines and recommendations for evaluating and researching the diagnostic and prognostic value of cardiovascular biomarkers (Moons, 2010). These guidelines were produced in an attempt to regulate and control an early introduction of these potentially unnecessary and incomplete new biomarkers to the clinical profession. Recently, Hlatky and colleagues issued a statement on behalf of the AHA outlining the criteria for evaluating a novel biomarker for cardiovascular risk assessment (Hlatky et al., 2009). They suggested that follow-up studies, randomized or non-randomized studies are required for the assessment of cardiovascular biomarkers that will be used for screening and predicting treatment response. Also the randomised control trial model is regarded as the "gold standard" method for testing the efficiency of medical interventions (Chow & Chang, 2008).

The present study was a retrospective observational study generated from a prospective randomised controlled trial of point of care testing of cardiac biomarkers. The advantages of this study within a larger RCT are twofold. Firstly, the surplus blood samples provided by the RATPAC study represent an opportunity to extend the findings of the RCT in a cost effective way compared to a further independent study (Biesheuvel et al., 2008b). Secondly, the present study shares the same limitations as the RATPAC RCT trial including patients selection and bias (Chow & Chang, 2008).

Although, patient's enrolment is prospective, the present study including the cardiac biomarkers evaluation is retrospective. Not only are the samples selected from RATPAC study are from well characterised patients, but these patients have also been followed up for MACE. The RATPAC trial population is ideal for the present study, because patients were selected on the basis of a low probability of cardiac risk rather than high probability, representing a more realistic typical ED chest pain population (Bhandari et al., 2006). The

samples collected for the present study were taken as part of routine management and therefore subjected to possible pre-analytical errors. These include variations in time from blood draw to storage and variations in storage temperatures between different sites. Following the RATPAC study, many samples were insufficient to allow for the measurement of albumin. Thus, the ability to correct IMA concentrations by IMA/albumin ratio could not be performed.

An adequately powered RCT, will detect important differences between IMA assay alone and compared with other ranges of cardiovascular biomarkers, including hs-cTn, H-FABP, NT-pro-BNP and copeptin (Whitsel, Boyko, & Siscovick, 2000). However, the present study did not reach the desire power due to missing samples. Although, the blood samples were collected on admission in nearly all the patients; the 90 min after admission samples were only collected in patients with lower likelihood of developing AMI. Thus, there is a selection bias, as patients with a diagnosis of AMI may be under represented; however, this unintended bias reflects the current chest pain population experienced daily in the ED and this bias is also reflected in the results in present study.

To mitigate the introduction of systematic errors, the RATPAC study was designed to include the participation of different hospitals thus ensuring that the overall clinical utility of IMA was generalised across a range of settings. In addition, the exclusion of participants due to non-compliance in the RATPAC study and in particular in the standard care group was less than 1.1%, thus minimising the bias of the estimate of the true value. Sampling error in the present study could have been reduced if a very large group of participants were used as originally planned.

Prognostic information obtained from the present study may be liable to restricted generalisation because of strict eligibility criteria, low participation and large numbers of missing samples (Bhandari et al., 2006).

In a clinical setting when low risk patients present with chest pain suggestive of AMI using hs-cTn to rule-out (sensitivity and NPV) is not entirely satisfactory, because low level hs-cTn is detectable in healthy population. Therefore, it may be logical to put the emphasis on specificity to rule-in AMI patients. Thus, the combined diagnostic efficiency of IMA, H-FABP, NT-pro-BNP and copeptin may be useful.

5.8 Implication for practice and future research

According to the myocardial ischaemia national audit 2012; 80,000 NSTEMI patients were admitted annually compared to 30,000 STEMI (Gavalova & Weston, 2012). The annual incidence of STEMI is 13 per 1000 habitants exceeding that of NSTEMI at 7 per 1000 habitants (McManus,Gore,Yarzebski *et al.*, 2011). One reason for this disparity is thought to be due to improved cardiac management and the introduction of hs-cTn (Melki,Lugnegard,Alfredsson *et al.*, 2015). The Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART) study of 48,594 patients found that the introduction of hs-cTnT into clinical practice had resulted in the identification and reclassification of a large cohort (21.6%) of previously undetected AMI patients (hs-cTnT concentration of 14 ng/L and 49 ng/L) (Melki,Lugnegard,Alfredsson *et al.*, 2015). Very low concentration of hs-cTnT can now be detected in patients with stable angina (SA) (Omland,de Lemos,Sabatine *et al.*, 2009). However, detectable hs-cTnT in the SA cohort was significantly associated with the incidence of cardiovascular death and heart failure but not with AMI (Omland,de Lemos,Sabatine *et al.*, 2009). Moreover, reclassifying these low risk patients with AMI resulted in better outcomes because these patients benefited from early treatment (Mills,Churchhouse,Lee *et al.*, 2011). Other studies suggested that since the introduction of hs-cTn across the UK, AMI diagnosis increased at an estimated rate between 10% and 47% (Aldous,Florkowski,Crozier *et al.*, 2012;

Mills,Lee,McAllister *et al.*, 2012). It clear that hs-cTn is beneficial to ACS patient.

The International Liaison Committee on Resuscitation support the use of biomarker of ACS only if they achieve sensitivity > 95% or specificity > 92% and a combined cardiac biomarker sensitivity of > 90% (Hazinski,Nolan,Billi *et al.*, 2010; Nolan,Hazinski,Billi *et al.*, 2010). Ideally early biomarkers characterised by high sensitivity, specificity is essential to rule-out or rule-in AMI. However, the majority of cardiac biomarkers utilised currently do not entirely subscribe to this (Lippi & Guidi,2008).

Cardiac biomarkers for myocardial cell damage such as hs-cTn are an important tool for helping to differential AMI from non-AMI in most cases. Although these biomarkers are important, they are not ideal for detecting patients who are suffering from cardiac ischaemia. Theoretically, the perfect ischaemic biomarker should be 100% sensitive and 100% specific for predicting AMI. The ideal candidate biomarker should be released from disrupted cardiomyocyte prior to necrosis of the cell.

The presences of biomarkers of cardiac necrosis such as hs-cTn are not beneficial to cardiac ischaemic patients as they are linked to poor prognosis. A surrogate cardiac biomarker such as IMA is ideal for this role. However, the introduction of cardiac biomarkers into prime use in hospitals, need to achieve certain criteria which take into consideration evidence-based medicine. In addition, the method should be available on large automated chemistry or immunoassay platforms to assist in laboratory workflow management. The concentration of a preferred biomarker should prior to initiation of necrotic mechanisms indicate an AMI. These criteria can be categorised into analytical suitability and clinical usability. Although, IMA was not characterised in terms of protein structure or even purified for assay optimisation; a patent application for a method for IMA purification by high performance liquid chromatography (HPLC) has been granted in the USA (Patent No: WO00/20840). There is also a commercially available ELISA kit for the determination of IMA, although not available for routine diagnostic use. It is clear that further research into

characterisation of the IMA entity is required before new methods are appropriate for prime-time use.

5.8.1 Future application for ischemia modified-albumin assay

Cardiovascular biomarkers for cardiac cells injury or necrosis such as troponin T & I are gold standard for the diagnosis of AMI. However, the only traditional diagnostic tools used for diagnosing cardiac ischaemia are physical examination, patient's history, ECG; imaging techniques, which are less specific, less sensitive, not routinely available, and requires expertise. The clinicians are aware of the fact that after the onset of myocardial ischaemia, there is time frame of approximately 1 h before the myocardium start to die and least takes 3 h before cTn could rule-in or rule-out AMI in most cases. The challenge is to correctly identify these vulnerable patients and treat them using a range of drugs including thrombus-busting drugs such as streptokinase and tissue plasminogen activator (tPA). A further challenge is time to access specialised cardiac healthcare. Access in rural areas is often limited and involves a step-wise approach via district general hospitals followed by transfer to specialised cardiac units (Watt,Franks & Sheldon, 1994).

The ideal placement of a combined IMA and hs-cTn testing strategy is as a POCT device ideally placed on board emergency vehicles. Rapid pre-hospital access to such a device can generate biomarker results which would be available to ED clinicians on patient arrival. Not only POCT is beneficial to critically ill patients, POCT could also be used in primary care setting. Identifying pre-AMI or cardiac ischaemic patients could improve patients' management by shortening the need for waiting for life saving treatments and reduce cost to the healthcare system. The case for the introduction of IMA at POCT is supported by the observation that low cTn concentration release in NSTEMI is related to a chronic and prolonged ischaemia and micro-embolisation of atherothrombotic material (Hamm,Heeschen,Goldmann *et al.*, 1999).

The implementation of POCT within the emergency staff and in the community is needed to prevent ACS related co-morbidity and to reduce cost to the healthcare system. Further development of POCT in the community and in primary care with the involvement of biomedical scientist community would allow wider service to the end users and might be advantageous in suspected low risk patients. Future research which aims at introducing POCT must engage clinicians in the process of the redesign of the service distribution and workforce planning. A successful introduction of POCT requires the service users to be less constrained by existing local conventions and institutional boundaries. The stake holders need to shift focus on patients care and clinical management, in order to ensure high-quality effective service.

Any novel cardiac biomarkers should demonstrate its clinical usefulness and must be cost effective, with a clear aim to reduce mortality and help patient's managements. Hs-cTn and NT-pro-BNP are the biomarker that changed the way we manage ACS and heart failure patients. Currently, a reliable cardiac biomarker of ischaemia is still required and is subject to future research. In addition, our study challenge investigator to perform additional basic science regarding IMA structure. As J K Rowling said in Harry Potter and the Chamber of Secrets "Never trust anything that can think for itself if you can't see where it keeps its brain?"

Chapter 6

Personal reflection

6.1 Introduction; a brief history of my career

After 8 years of travelling around the word I met my lovely wife Louisa in Turkey, nearly 22 years ago. My academic journey started when I decided that biomedical sciences was my chosen profession. The journey from medical laboratory assistance (MLA), without a degree, to embarking on a Doctorate in Biomedical Science (DBMS) has been exciting, fascinating, hard work, and life changing.

6.2 Education, training and professional development

I started my career at the Royal Surrey County hospital NHS trust in 1999 as an MLA, and then year later I secured a trainee position in the Immunology Department at St Thomas' Hospital NHS Trust London. While training I was allowed to study part time for a degree in Biomedical Sciences at the University of Portsmouth.

I developed a highly analytical mind because of my work place training and my education at the University of Portsmouth. Studying and working at the same time allowed me to see the 'bigger picture' to a strategic level and prepared me throughout my career to work well within teams and autonomously.
Embarking on a part time Masters in Biomedical Sciences (MSc) was very challenging because I was not supported at work and by then I had my lovely boy Noah now nearly 11 years old. Family commitment meant that I had to study only when my son was asleep. MSc studies meant no holiday for two years as I had to use my holiday entitlement to study. I remember when I was awarded my MSc degree my son cried because he was overwhelmed with pride and emotion. It was a proud moment for my family.

Currently I am integral to the day-to-day running of manual biochemistry laboratory. My duties include running tests, advising clinicians regarding the

appropriate use of diagnostic tests, results authorisation, supervising new members of staff, providing formal training, troubleshooting, writing SOPs (to current ISO standard) and liaising with service users at all levels as required.

Embarking on a professional doctorate in Biomedical Sciences increased my confidence in my ability to scientifically and systematically appraise and tackle a wide range of work-related issues. Including method development and formal staff training. Following a merger of three NHS Trusts, St George's Healthcare NHS Trust has successfully become the South West Pathology Hub. In order to harmonise pathology services new equipment has been purchased from Roche. To comply with ISO standards, I was asked to work in collaboration with the Pathology Quality Manager, General Managers and Lead biomedical scientists to design and implement the quality assurance and analytical evaluation of the new equipment. Integral to the change management program I am also delivering a program of training to members of staff including Clinical Scientists, BMS (all grades) and Associate Practitioners. I have also presented at department level at St George's Healthcare NHS Trust.

6.3 Reflection on the doctorate in biomedical sciences taught element part one

The purpose for this unit is to expose student to research requirement at doctoral level. This is achieved through the following modules:

1. Advanced research techniques
2. Publication and dissemination
3. Professional review and development

6.3.1 Advanced research techniques

This module removed the ambiguities surrounding statistic whether qualitative or qualitative. This taught module allowed me to manipulate data with

confidence. The successful completion of this module requires a submission of three assignments:

1) Data analysis
2) Critical appraisal of literature
3) Applying theory to practice

The successful completion of these three assignments and the skill gained, contributed directly to my thesis and to my professional work. Critical appraisal of literature unit taught me the skill required in vetting scientific research papers and to be able to utilise only good quality information; whereas, data analysis allowed me to use statistic in action. The final assignment was critical in understanding my research mythology.

6.3.2 Publication and dissemination

This unit encouraged me to consider publishing my work in a professional capacity. I also learned how to avoid many pitfalls and traps for the unwary. Moreover, during my doctorate study I was invited to contribute, as a co-author, to an international collaboration resulting in the publication of a professional reference book (Human Serum Albumin, New insight on its structural dynamics, functional impact and pharmaceutical applications (2013)) for the biotechnology and pharmaceutical industry (Appendix 6). I have also provided expert opinion and peer reviewed for Science World, a journal with an international readership.

6.3.3 Professional review and development

Reflective practice is a multi-layered process involving reflection-on-experience, reflection-in-action, reflection-within-the-moment, and mindfulness practice. As famously quoted by Donald Schon, *"reflection and reflexivity is a swamp that can unearth strong feeling of self-doubt, uncertainty and inconclusive outcomes".* This module made me aware of the fact that I am always reflecting to some capacity just not aware of it in the formal way. Moreover, reflection is an essential part of biomedical scientist and a requirement for continuous professional development.

6.3.4 Reflection on the doctorate in biomedical science taught element part two

Advanced research techniques including statistical analysis (quantitative) lectures and assignment; made me appreciate medical statistics fully. Becoming competent in quantitative data analysis help me progress through my project and also improve my research skills. This task was far the best, as for the first time I have actually started to understand what number in statistics means. Learning how to use SPSS® before I could do my assignment was another challenge that I really enjoyed. The confidence gained in this session allowed me to use my statistical skill to plan and write my thesis.

6.4 Summary

Since embarking on the DBMS course, I have been overwhelmed with the richness of it content and I have also enjoyed learning and debating with my peers and tutors. Although, studying at doctorate level has made me question my learning style, which I previously never contemplated. Studying at this level has made me aware of the current academic thinking and the very high degree of skill and autonomy required at this level. It has become apparent that the tutorials, workshops, and lectures were only an introduction to a particular

subject, and the hard work is left to me. The challenge is real and a great deal of organisational skill was required in order to achieve my goal to successfully finish my professional doctorate course and beyond.

References

Abboud, H., Labreuche, J., Meseguer, E., Lavallee, P.C., Simon, O., Olivot, J.M.*, et al.* (2007). Ischemia-modified albumin in acute stroke. *Cerebrovasc Dis*, 23(2-3), 216-220.

Acher, R., Chauvet, J., & Rouille, Y. (2002). Dynamic processing of neuropeptides: sequential conformation shaping of neurohypophysial preprohormones during intraneuronal secretory transport. *J Mol Neurosci*, 18(3), 223-228.

Aldous, S., Pemberton, C., Richards, A.M., Troughton, R., & Than, M. (2012). High-sensitivity troponin T for early rule-out of myocardial infarction in recent onset chest pain. *Emerg Med J*, 29(10), 805-810.

Aldous, S.J., Florkowski, C.M., Crozier, I.G., & Than, M.P. (2012). The performance of high sensitivity troponin for the diagnosis of acute myocardial infarction is underestimated. *Clin Chem Lab Med*, 50(4), 727-729.

Alpert, J.S., Thygesen, K., Antman, E., & Bassand, J.P. (2000). Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol*, 36(3), 959-969.

American College of Cardiology Foundation Appropriate Use Criteria Task, F., American Society of, E., American Heart, A., American Society of Nuclear, C., Heart Failure Society of, A., Heart Rhythm, S.*, et al.* (2011). ACCF/ASE/AHA/ASNC/HFSA/HRS/SCAI/SCCM/SCCT/SCMR 2011 Appropriate Use Criteria for Echocardiography. A Report of the American College of Cardiology Foundation Appropriate Use Criteria Task Force, American Society of Echocardiography, American Heart Association, American Society of Nuclear Cardiology, Heart Failure Society of America, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, Society of Critical Care Medicine, Society of Cardiovascular Computed Tomography, and Society for Cardiovascular Magnetic Resonance Endorsed by the American College of Chest Physicians. *J Am Coll Cardiol*, 57(9), 1126-1166.

Amsterdam, E.A., Kirk, J.D., Bluemke, D.A., Diercks, D., Farkouh, M.E., Garvey, J.L.*, et al.* (2010). Testing of low-risk patients presenting to the emergency department with chest pain: a scientific statement from the American Heart Association. *Circulation*, 122(17), 1756-1776.

Amsterdam, E.A., Wenger, N.K., Brindis, R.G., Casey, D.E., Jr., Ganiats, T.G., Holmes, D.R., Jr.*, et al.* (2014). 2014 AHA/ACC Guideline for the Management of Patients with Non-ST-Elevation Acute Coronary Syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*, 64(24), e139-228.

Anderson, J.L., Adams, C.D., Antman, E.M., Bridges, C.R., Califf, R.M., Casey, D.E., Jr., *et al.* (2007). ACC/AHA 2007 guidelines for the management of patients with unstable

angina/non-ST-Elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction) developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine. *J Am Coll Cardiol*, 50(7), e1-e157.

Anderson, P.A., Greig, A., Mark, T.M., Malouf, N.N., Oakeley, A.E., Ungerleider, R.M., Allen, P.D., & Kay, B.K. (1995). Molecular basis of human cardiac troponin T isoforms expressed in the developing, adult, and failing heart. *Circ Res*, 76(4), 681-686.

Andersson, C., Shilane, D., Go, A.S., Chang, T.I., Kazi, D., Solomon, M.D., Boothroyd, D.B., & Hlatky, M.A. (2014). beta-blocker therapy and cardiac events among patients with newly diagnosed coronary heart disease. *J Am Coll Cardiol*, 64(3), 247-252.

Antman, E.M., Anbe, D.T., Armstrong, P.W., Bates, E.R., Green, L.A., Hand, M.*, et al.* (2004). ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction; A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Management of patients with acute myocardial infarction). *J Am Coll Cardiol*, 44(3), E1-E211.

Antman, E.M., Cohen, M., Bernink, P. J., McCabe, C. H., Horacek, T., Papuchis, G. . (2000). The TIMI risk score for unstable angina/non-ST elevation MI: A method for prognostication and therapeutic decision making. *JAMA,*, 284, 835-842.

Apple, F.S. (2009). A new season for cardiac troponin assays: it's time to keep a scorecard. *Clin Chem*, 55(7), 1303-1306.

Apple, F.S. (2011). High-sensitivity cardiac troponin for screening large populations of healthy people: is there risk? *Clin Chem*, 57(4), 537-539.

Apple, F.S., Collinson, P.O., & Biomarkers, I.T.F.o.C.A.o.C. (2012). Analytical characteristics of high-sensitivity cardiac troponin assays. *Clin Chem*, 58(1), 54-61.

Apple, F.S., Greenspan, N.S., & Dietzler, D.N. (1982). Elevation of creatine kinase BB CK in hospitalized patients. Importance of distinguishing BB CK from MB CK. *Ann Clin Lab Sci*, 12(5), 398-402.

Apple, F.S., & Jaffe, A.S. (2001). Bedside multimarker testing for risk stratification in chest pain units: The chest pain evaluation by creatine kinase-MB, myoglobin, and troponin I (CHECKMATE) study. *Circulation*, 104(22), E125-126.

Apple, F.S., Jesse, R.L., Newby, L.K., Wu, A.H., Christenson, R.H., Cannon, C.P.*, et al.* (2007). National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice

Guidelines: analytical issues for biochemical markers of acute coronary syndromes. *Clin Chem*, 53(4), 547-551.

Apple, F.S., & Murakami, M.M. (2005). The diagnostic utility of cardiac biomarkers in detecting myocardial infarction. *Clin Cornerstone*, 7 Suppl 1, S25-30.

Apple, F.S., Murakami, M.M., Quist, H.H., Pearce, L.A., Wieczorek, S., & Wu, A.H. (2003). Prognostic value of the Ortho Vitros cardiac troponin I assay in patients with symptoms of myocardial ischemia. Risk stratification using European Society of Cardiology/American College of Cardiology recommended cutoff values. *Am. J. Clin. Pathol*, 120(1), 114-120.

Apple, F.S., Pearce, L.A., Chung, A., Ler, R., & Murakami, M.M. (2007). Multiple biomarker use for detection of adverse events in patients presenting with symptoms suggestive of acute coronary syndrome. *Clin Chem*, 53(5), 874-881.

Apple, F.S., Quist, H.E., Otto, A.P., Mathews, W.E., & Murakami, M.M. (2002). Release characteristics of cardiac biomarkers and ischemia-modified albumin as measured by the albumin cobalt-binding test after a marathon race. *Clin Chem*, 48(7), 1097-1100.

Apple, F.S., Smith, S.W., Pearce, L.A., & Murakami, M.M. (2009). Assessment of the multiple-biomarker approach for diagnosis of myocardial infarction in patients presenting with symptoms suggestive of acute coronary syndrome. *Clin Chem*, 55(1), 93-100.

Apple, F.S., Wu, A.H., Jaffe, A.S., Panteghini, M., Christenson, R.H., Committee, N., & Ifcc, C.S. (2008). National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: analytical issues for biomarkers of heart failure. *Clin Biochem*, 41(4-5), 222-226.

Apple, F.S., Wu, A.H., Mair, J., Ravkilde, J., Panteghini, M., Tate, J., *et al.* (2005). Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. *Clin Chem*, 51(5), 810-824.

Appleby, J. (2013). *Spending on health and social care over the next 50 years*. Retrieved from www.kingsfund.org.uk

Arakawa, N., Nakamura, M., Aoki, H., & Hiramori, K. (1996). Plasma brain natriuretic peptide concentrations predict survival after acute myocardial infarction. *J Am Coll Cardiol*, 27(7), 1656-1661.

Aroney, C.N., Dunlevie, H. L., & Bett, J. H. . (2003). Use of an accelerated chest pain assessment protocol in patients at intermediate risk of adverse cardiac events. . *Med J Aust.,*, 178, 370-374.

Arumugam, S., Sreedhar, R., Thandavarayan, R.A., Karuppagounder, V., & Watanabe, K. (2016). Targeting fatty acid metabolism in heart failure: is it a suitable therapeutic approach? *Drug Discov Today*.

Athanassoulis, G.T., IC. (2015). Beyond the Markovian assumption: response–excitation probabilistic solution to random nonlinear differential equations in the long time. *Porceedings of the Royal Society*.

Attana, P., Lazzeri, C., Picariello, C., Dini, C.S., Gensini, G.F., & Valente, S. (2012). Lactate and lactate clearance in acute cardiac care patients. *Eur Heart J Acute Cardiovasc Care*, 1(2), 115-121.

Baigent, C., Keech, A., Kearney, P.M., Blackwell, L., Buck, G., Pollicino, C.*, et al.* (2005). Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*, 366(9493), 1267-1278.

Bajaj, A., Rathor, P., Sehgal, V., Shetty, A., Kabak, B., & Hosur, S. (2015). Risk stratification in acute pulmonary embolism with heart-type fatty acid-binding protein: A meta-analysis. *J Crit Care*, 30(5), 1151 e1151-1157.

Balanescu, S., Kopp, P., Gaskill, M.B., Morgenthaler, N.G., Schindler, C., & Rutishauser, J. (2011). Correlation of plasma copeptin and vasopressin concentrations in hypo-, iso-, and hyperosmolar States. *J Clin Endocrinol Metab*, 96(4), 1046-1052.

Bali, L., Cuisset, T., Giorgi, R., Monserrat, C., Quilici, J., Carrega, L.*, et al.* (2008). Prognostic value of ischaemia-modified albumin in patients with non-ST-segment elevation acute coronary syndromes. *Arch Cardiovasc Dis*, 101(10), 645-651.

Balk, E.M., Ioannidis, J.P., Salem, D., Chew, P.W., & Lau, J. (2001). Accuracy of biomarkers to diagnose acute cardiac ischemia in the emergency department: a meta-analysis. *Ann Emerg Med*, 37(5), 478-494.

Bandstein, N., Ljung, R., Johansson, M., & Holzmann, M.J. (2014). Undetectable high-sensitivity cardiac troponin T level in the emergency department and risk of myocardial infarction. *J Am Coll Cardiol*, 63(23), 2569-2578.

Bangalore, S., Parkar, S., & Messerli, F.H. (2009). Long-acting calcium antagonists in patients with coronary artery disease: a meta-analysis. *Am J Med*, 122(4), 356-365.

Bar-or D, W.J. (2001). Reduced albumin-cobalt binding with transient myocardial ischemia after elected percutaneous transluminal angioplasty: a preliminary comparison to creatine kinase-MB, myoglobin and troponin I. . *Am Heart J*, 141, 985-991.

Bassan, R., Potsch, A., Maisel, A., Tura, B., Villacorta, H., Nogueira, M.V.*, et al.* (2005). B-type natriuretic peptide: a novel early blood marker of acute myocardial infarction in patients with chest pain and no ST-segment elevation. *Eur Heart J*, 26(3), 234-240.

Bassan, R., Tura, B.R., & Maisel, A.S. (2009). B-type natriuretic peptide: a strong predictor of early and late mortality in patients with acute chest pain without ST-segment elevation in the emergency department. *Coron Artery Dis*, 20(2), 143-149.

Basso, C., Perazzolo Marra, M., Rizzo, S., De Lazzari, M., Giorgi, B., Cipriani, A.*, et al.* (2015). Arrhythmic Mitral Valve Prolapse and Sudden Cardiac Death. *Circulation*, 132(7), 556-566.

Bathia, D.P., Carless, D.R., Viswanathan, K., Hall, A.S., & Barth, J.H. (2009). Serum 99th centile values for two heart-type fatty acid binding protein assays. *Ann Clin Biochem*, 46(Pt 6), 464-467.

Beetham, R., Monk, C., Keating, L., Benger, J.R., & Kendall, J. (2006). Effects of storage at -20 degrees C on ischaemia-modified albumin results. *Ann Clin Biochem*, 43(Pt 6), 500-502.

Begg, C.B., & Mazumdar, M. (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics*, 50(4), 1088-1101.

Berger, J.S., Elliott, L., Gallup, D., Roe, M., Granger, C.B., Armstrong, P.W.*, et al.* (2009). Sex differences in mortality following acute coronary syndromes. *JAMA*, 302(8), 874-882.

Bhagavan, N.V., Lai, E.M., Rios, P.A., Yang, J., Ortega-Lopez, A.M., Shinoda, H.*, et al.* (2003). Evaluation of human serum albumin cobalt binding assay for the assessment of myocardial ischemia and myocardial infarction. *Clin Chem*, 49(4), 581-585.

Bhandari, M., Pape, H. C., & Giannoudis, P. V. . (2006). Issues in the planning and conduct of randomised trials. . *Injury,Injury, 37, 349-354.*, 37, 349-354.

Bhardwaj, A., Truong, Q.A., Peacock, W.F., Yeo, K.T., Storrow, A., Thomas, S.*, et al.* (2011). A multicenter comparison of established and emerging cardiac biomarkers for the diagnostic evaluation of chest pain in the emergency department. *Am Heart J*, 162(2), 276-282 e271.

Bhatnagar, P. (2015). The epidemiology of cardiovascular disease in the UK 2014. *Heart*, 101(15), 1182-1189.

Bidmead, T. (2015). Factors influencing unspecified chest pain admission rates in England. *Emerg Med J*, 32(6), 439-443.

Boden, W.E., Padala, S.K., Cabral, K.P., Buschmann, I.R., & Sidhu, M.S. (2015). Role of short-acting nitroglycerin in the management of ischemic heart disease. *Drug Des Devel Ther*, 9, 4793-4805.

Bodi, V., Sanchis, J., Llacer, A., Facila, L., Nunez, J., Pellicer, M., Bertomeu, V., Ruiz, V., & Chorro, F.J. (2005). Multimarker risk strategy for predicting 1-month and 1-year major events in non-ST-elevation acute coronary syndromes. *Am Heart J*, 149(2), 268-274.

Body, R., McDowell, G., Carley, S., Wibberley, C., Ferguson, J., & Mackway-Jones, K. (2011). A FABP-ulous 'rule out' strategy? Heart fatty acid binding protein and troponin for rapid exclusion of acute myocardial infarction. *Resuscitation*, 82(8), 1041-1046.

Borenstein M, H.L., Higgins JPT. (2009). Introduction to meta-analysis. *Wiley*.

Bowling, A., Bond, M., McKee, D., McClay, M., Banning, A.P., Dudley, N., Elder, A., Martin, A., & Blackman, I. (2001). Equity in access to exercise tolerance testing, coronary angiography, and coronary artery bypass grafting by age, sex and clinical indications. *Heart*, 85(6), 680-686.

Braunwald, E., Antman, E.M., Beasley, J.W., Califf, R.M., Cheitlin, M.D., Hochman, J.S.*, et al.* (2002). ACC/AHA guideline update for the management of patients with unstable

angina and non-ST-segment elevation myocardial infarction--2002: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina). *Circulation*, 106(14), 1893-1900.

Braunwald, E., Maroko, P.R., & Libby, P. (1974). Reduction of infarct size following coronary occlusion. *Circ Res*, 35 Suppl 3, 192-201.

Breitkreutz, R., Price, S., Steiger, H.V., Seeger, F.H., Ilper, H., Ackermann, H., *et al.* (2010). Focused echocardiographic evaluation in life support and peri-resuscitation of emergency patients: a prospective trial. *Resuscitation*, 81(11), 1527-1533.

Brieger, D., Eagle, K.A., Goodman, S.G., Steg, P.G., Budaj, A., White, K., Montalescot, G., & Investigators, G. (2004). Acute coronary syndromes without chest pain, an underdiagnosed and undertreated high-risk group: insights from the Global Registry of Acute Coronary Events. *Chest*, 126(2), 461-469.

Brown, A.M., Sease, K.L., Robey, J.L., Shofer, F.S., & Hollander, J.E. (2007). The impact of B-type natriuretic peptide in addition to troponin I, creatine kinase-MB, and myoglobin on the risk stratification of emergency department chest pain patients with potential acute coronary syndrome. *Ann Emerg Med*, 49(2), 153-163.

Bruins Slot, M.H., Reitsma, J.B., Rutten, F.H., Hoes, A.W., & van der Heijden, G.J. (2010). Heart-type fatty acid-binding protein in the early diagnosis of acute myocardial infarction: a systematic review and meta-analysis. *Heart*, 96(24), 1957-1963.

Buckley, M.G., Marcus, N.J., Yacoub, M.H., & Singer, D.R. (1998). Prolonged stability of brain natriuretic peptide: importance for non-invasive assessment of cardiac function in clinical practice. *Clin Sci (Lond)*, 95(3), 235-239.

Burke, A.P., & Virmani, R. (2007). Pathophysiology of acute myocardial infarction. *Med Clin North Am*, 91(4), 553-572; ix.

Byrne, J. (2014). Introducing a chest pain pathway in the emergency department to improve quality of care for patients with possible cardiac chest pain. *BMJ Qual Improv Rep*, 3(1).

Caliendo, A.M., Gilbert, D.N., Ginocchio, C.C., Hanson, K.E., May, L., Quinn, T.C., *et al.* (2013). Better tests, better care: improved diagnostics for infectious diseases. *Clin Infect Dis*, 57 Suppl 3, S139-170.

Cannon, C.P., Steinberg, B.A., Murphy, S.A., Mega, J.L., & Braunwald, E. (2006). Meta-analysis of cardiovascular outcomes trials comparing intensive versus moderate statin therapy. *J Am Coll Cardiol*, 48(3), 438-445.

Canto, J.G., Fincher, C., Kiefe, C.I., Allison, J.J., Li, Q., Funkhouser, E., Centor, R.M., Selker, H.P., & Weissman, N.W. (2002). Atypical presentations among Medicare beneficiaries with unstable angina pectoris. *Am J Cardiol*, 90(3), 248-253.

Carlton, E.W., Gamble, J.H., & Greaves, K. (2013). Testing times: we are still some way from getting the best out of sensitive troponin assays. *JAMA Intern Med*, 173(6), 477-478.

Carroll, C., Al Khalaf, M., Stevens, J.W., Leaviss, J., Goodacre, S., Collinson, P.O., & Wang, J. (2013). Heart-type fatty acid binding protein as an early marker for myocardial infarction: systematic review and meta-analysis. *Emerg Med J*, 30(4), 280-286.

Cassidy, J.D. (2000). Effect of eliminating compensation for pain and suffering on the outcome of insurance claims for whiplash injury. *N Engl J Med*, 342(16), 1179-1186.

Cassidy, J.D., Carroll, L.J., Cote, P., Lemstra, M., Berglund, A., & Nygren, A. (2000). Effect of eliminating compensation for pain and suffering on the outcome of insurance claims for whiplash injury. *N Engl J Med*, 342(16), 1179-1186.

Chan, C.P., Sanderson, J.E., Glatz, J.F., Cheng, W.S., Hempel, A., & Renneberg, R. (2004). A superior early myocardial infarction marker. Human heart-type fatty acid-binding protein. *Z Kardiol*, 93(5), 388-397.

Chang, A.M., Shofer, F.S., Weiner, M.G., Synnestvedt, M.B., Litt, H.I., Baxt, W.G., & Hollander, J.E. (2008). Actual financial comparison of four strategies to evaluate patients with potential acute coronary syndromes. *Acad Emerg Med*, 15(7), 649-655.

Charpentier, S., & Chenevier-Gobeaux, C. (2016). [2015 ESC guidelines: 1-hour rule-out and rule-in of acute myocardial infarction with high-sensitive troponin T]. *Presse Med*, 45(10), 859-864.

Charpentier, S., Ducasse, J.L., Cournot, M., Maupas-Schwalm, F., Elbaz, M., Baixas, C., Juchet, H., Lang, T., & Lauque, D. (2010). Clinical assessment of ischemia-modified albumin and heart fatty acid-binding protein in the early diagnosis of non-ST-elevation acute coronary syndrome in the emergency department. *Acad Emerg Med*, 17(1), 27-35.

Charpentier, S., Maupas-Schwalm, F., Cournot, M., Elbaz, M., Botella, J.M., & Lauque, D. (2012). Combination of copeptin and troponin assays to rapidly rule out non-ST elevation myocardial infarction in the emergency department. *Acad Emerg Med*, 19(5), 517-524.

Cheitlin, M.D., Armstrong, W.F., Aurigemma, G.P., Beller, G.A., Bierman, F.Z., Davis, J.L.*, et al.* (2003). ACC/AHA/ASE 2003 Guideline Update for the Clinical Application of Echocardiography: summary article. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/ASE Committee to Update the 1997 Guidelines for the Clinical Application of Echocardiography). *J Am Soc Echocardiogr*, 16(10), 1091-1110.

Chenevier-Gobeaux, C., Bonnefoy-Cudraz, E., Charpentier, S., Dehoux, M., Lefevre, G., Meune, C., Ray, P., & Sfbc, S.F.C.S.T.w. (2015). High-sensitivity cardiac troponin assays: answers to frequently asked questions. *Arch Cardiovasc Dis*, 108(2), 132-149.

Cho, D.K., Choi, J.O., Kim, S.H., Choi, J., Rhee, I., Ki, C.S., Lee, S.C., & Gwon, H.C. (2007). Ischemia-modified albumin is a highly sensitive serum marker of transient myocardial ischemia induced by coronary vasospasm. *Coron Artery Dis*, 18(2), 83-87.

Chopra, S., Cherian, D., Verghese, P.P., & Jacob, J.J. (2013). Physiology and clinical significance of natriuretic hormones. *Indian J Endocrinol Metab*, 17(1), 83-90.

Christeinsen, D. (2005). Fast algorithms for the calcualation of Kendall's T. . *Computational Statistics*, 20, 51-62.

Christenson, J. (2004). Safety and efficiency of emergency department assessment of chest discomfort. *CMAJ*, 170(12), 1803-1807.

Christenson, R.H., Duh, S.H., Sanhai, W.R., Wu, A.H., Holtman, V., Painter, P.*, et al.* (2001). Characteristics of an Albumin Cobalt Binding Test for assessment of acute coronary syndrome patients: a multicenter study. *Clin Chem*, 47(3), 464-470.

Christenson, R.H., & Tang, W.H. (2006). Institute for Quality in Laboratory Medicine series--controversies in laboratory medicine: insights into B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide measurements. *MedGenMed*, 8(2), 62.

Christenson, R.H., Vaidya, H., Landt, Y., Bauer, R.S., Green, S.F., Apple, F.A.*, et al.* (1999). Standardization of creatine kinase-MB (CK-MB) mass assays: the use of recombinant CK-MB as a reference material. *Clin Chem*, 45(9), 1414-1423.

Clerico, A., Fortunato, A., Ripoli, A., Prontera, C., Zucchelli, G.C., & Emdin, M. (2008). Distribution of plasma cardiac troponin I values in healthy subjects: pathophysiological considerations. *Clin Chem Lab Med*, 46(6), 804-808.

Cohen, A., Assyag, P., Boyer-Chatenet, L., Cohen-Solal, A., Perdrix, C., Dalichampt, M.*, et al.* (2014). An education program for risk factor management after an acute coronary syndrome: a randomized clinical trial. *JAMA Intern Med*, 174(1), 40-48.

Collinson, P.O. (1998). Troponin T or troponin I or CK-MB (or none?). . *Eur.Heart J.,*, 19 Suppl, N16-N24.

Collinson, P.O. (2000a). Prospective audit of incidence of prognostically important myocardial damage in patients discharged from emergency department. *BMJ*, 320(7251), 1702-1705.

Collinson, P.O., Gaze, D.C., Bainbridge, K., Morris, F., Morris, B., Price, A., & Goodacre, S. (2006). Utility of admission cardiac troponin and "Ischemia Modified Albumin" measurements for rapid evaluation and rule out of suspected acute myocardial infarction in the emergency department. *Emerg Med J*, 23(4), 256-261.

Collinson, P.O., Gaze, D.C., Thokala, P., & Goodacre, S. (2013). Randomised Assessment of Treatment using Panel Assay of Cardiac markers--Contemporary Biomarker Evaluation (RATPAC CBE). *Health Technol Assess*, 17(15), v-vi, 1-122.

Collinson, P.O., Gaze, D. C., Bainbridge, K., Morris, F., Morris, B., Price, A. et al. . (2006). Utility of admission cardiac troponin and "Ischemia Modified Albumin" measurements for rapid evaluation and rule out of suspected acute myocardial infarction in the emergency department. . *Emerg.Med.J.,*, 23, 256-261.

Collinson, P.O., Premachandram, S., & Hashemi, K. . (2000b). Prospective audit of incidence of prognostically important myocardial damage in patients discharged from emergency department. . *BMJ,*, 320, 1702-1705.

Collinson, P.O., van Dieijen-Visser, M.P., Pulkki, K., Hammerer-Lercher, A., Suvisaari, J., Ravkilde, J., Stavljenic-Rukavina, A., Baum, H., & Laitinen, P. (2012). Evidence-based laboratory medicine: how well do laboratories follow recommendations and guidelines? The Cardiac Marker Guideline Uptake in Europe (CARMAGUE) study. *Clin Chem*, 58(1), 305-306.

Cooper, R.S., & Ford, E. (1992). Comparability of risk factors for coronary heart disease among blacks and whites in the NHANES-I Epidemiologic Follow-up Study. *Ann Epidemiol*, 2(5), 637-645.

Culic, V., Eterovic, D., Miric, D., & Silic, N. (2002a). Symptom presentation of acute myocardial infarction: influence of sex, age, and risk factors. *American Heart Journal*, 144(6), 1012-1017.

Culic, V., Eterovic, D., Miric, D., & Silic, N. (2002b). Symptom presentation of acute myocardial infarction: influence of sex, age, and risk factors. *Am Heart J*, 144(6), 1012-1017.

Davey Smith, G., Egger, M., & Phillips, A.N. (1997). Meta-analysis. Beyond the grand mean? *BMJ*, 315(7122), 1610-1614.

Depre, C., Wijns, W., Robert, A.M., Renkin, J.P., & Havaux, X. (1997). Pathology of unstable plaque: correlation with the clinical severity of acute coronary syndromes. *J Am Coll Cardiol*, 30(3), 694-702.

DerSimonian, R., & Laird, N. (1986). Meta-analysis in clinical trials. *Control Clin Trials*, 7(3), 177-188.

Deshpande, A., & Birnbaum, Y. (2014). ST-segment elevation: Distinguishing ST elevation myocardial infarction from ST elevation secondary to nonischemic etiologies. *World J Cardiol*, 6(10), 1067-1079.

Devlin N, P.D. (2004). (2004) Does NICE have a cost effectiveness threshold and what other factors influence its decisions? A binary choice analysis. 52. . *Health Economics*(13(5)), 437.

DeWood, M.A., Spores, J., Notske, R., Mouser, L.T., Burroughs, R., Golden, M.S., & Lang, H.T. (1980). Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. *N Engl J Med*, 303(16), 897-902.

Di Marca, S., Rando, A., Cataudella, E., Pulvirenti, A., Alaimo, S., Terranova, V.*, et al.* (2018). B-type natriuretic peptide may predict prognosis in older adults admitted with a diagnosis other than heart failure. *Nutr Metab Cardiovasc Dis*.

Dobsa, L., & Edozien, K.C. (2013). Copeptin and its potential role in diagnosis and prognosis of various diseases. *Biochem Med (Zagreb)*, 23(2), 172-190.

Dorresteijn, J.A., Boekholdt, S.M., van der Graaf, Y., Kastelein, J.J., LaRosa, J.C., Pedersen, T.R.*, et al.* (2013). High-dose statin therapy in patients with stable coronary artery disease: treating the right patients based on individualized prediction of treatment effect. *Circulation*, 127(25), 2485-2493.

Douglas, P.S., Khandheria, B., Stainback, R.F., Weissman, N.J., Brindis, R.G., Patel, M.R.*, et al.* (2007). ACCF/ASE/ACEP/ASNC/SCAI/SCCT/SCMR 2007 appropriateness criteria for transthoracic and transesophageal echocardiography: a report of the American College of Cardiology Foundation Quality Strategic Directions Committee Appropriateness Criteria Working Group, American Society of Echocardiography, American College of Emergency Physicians, American Society of Nuclear Cardiology, Society for Cardiovascular Angiography and Interventions, Society of Cardiovascular Computed Tomography, and the Society for Cardiovascular Magnetic Resonance endorsed by the American College of Chest Physicians and the Society of Critical Care Medicine. *J Am Coll Cardiol*, 50(2), 187-204.

Dunlay, S.M., & Roger, V.L. (2012). Gender differences in the pathophysiology, clinical presentation, and outcomes of ischemic heart failure. *Curr Heart Fail Rep*, 9(4), 267-276.

Durak-Nalbantic, A., Dzubur, A., Dilic, M., Pozderac, Z., Mujanovic-Narancic, A., Kulic, M.*, et al.* (2012). Brain natriuretic peptide release in acute myocardial infarction. *Bosn J Basic Med Sci*, 12(3), 164-168.

Dusek, J., St'asek, J., Tichy, M., Bis, J., Gregor, J., Vojacek, J.*, et al.* (2006). Prognostic significance of ischemia modified albumin after percutaneous coronary intervention. *Clin Chim Acta*, 367(1-2), 77-80.

Egger, M., Davey Smith, G., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, 315(7109), 629-634.

Eggers, K.M., Oldgren, J., Nordenskjold, A., & Lindahl, B. (2004). Diagnostic value of serial measurement of cardiac markers in patients with chest pain: limited value of adding myoglobin to troponin I for exclusion of myocardial infarction. *Am Heart J*, 148(4), 574-581.

Ellis, A.K. (1991). Serum protein measurements and the diagnosis of acute myocardial infarction. *Circulation*, 83(3), 1107-1109.

England, T.N.T.P.S.i.E.f.t.D.o.H.t.M.a.N. (2012-2013). *Reference cost 2012-2013*. Retrieved from

Ensor, K.B., Raun, L.H., & Persse, D. (2013). A case-crossover analysis of out-of-hospital cardiac arrest and air pollution. *Circulation*, 127(11), 1192-1199.

Escolar, E., Lamas, G.A., Mark, D.B., Boineau, R., Goertz, C., Rosenberg, Y.*, et al.* (2014). The effect of an EDTA-based chelation regimen on patients with diabetes mellitus and prior myocardial infarction in the Trial to Assess Chelation Therapy (TACT). *Circ Cardiovasc Qual Outcomes*, 7(1), 15-24.

European Association for Cardiovascular, P., Rehabilitation, Reiner, Z., Catapano, A.L., De Backer, G., Graham, I.*, et al.* (2011). ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J*, 32(14), 1769-1818.

Falk, E. (2006). Pathogenesis of atherosclerosis. *J Am Coll Cardiol*, 47(8 Suppl), C7-12.

Falkensammer, J., Stojakovic, T., Huber, K., Hammerer-Lercher, A., Gruber, I., Scharnagl, H., *et al.* (2007). Serum levels of ischemia-modified albumin in healthy volunteers after exercise-induced calf-muscle ischemia. *Clin Chem Lab Med*, 45(4), 535-540.

Farkouh, M.E., Aneja, A., Reeder, G. S., Smars, P. A., Bansilal, S., Lennon, R. J. . (2009). Clinical risk stratification in the emergency department predicts long-term cardiovascular outcomes in a population-based cohort presenting with acute chest pain: primary results of the Olmsted county chest pain study. Medicine (Baltimore) *Medicine*, 88, 307-313.

Farooq, V., Serruys, P.W., Zhang, Y., Mack, M., Stahle, E., Holmes, D.R., *et al.* (2013). Short-term and long-term clinical impact of stent thrombosis and graft occlusion in the SYNTAX trial at 5 years: Synergy Between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery trial. *J Am Coll Cardiol*, 62(25), 2360-2369.

Fass, R., & Achem, S.R. (2011). Noncardiac chest pain: epidemiology, natural course and pathogenesis. *J Neurogastroenterol Motil*, 17(2), 110-123.

Fass, R., & Navarro-Rodriguez, T. (2008). Noncardiac chest pain. *J Clin Gastroenterol*, 42(5), 636-646.

Fenster, P.E. (2004). Evaluation of chest pain: a cardiology perspective for gastroenterologists. *Gastroenterol Clin North Am*, 33(1), 35-40.

Finn, A.V., Nakano, M., Narula, J., Kolodgie, F. D., & Virmani, R. . (2010). Concept of vulnerable/unstable plaque. . *Thromb.Vasc.Biol.,*, 30.

Fishbein, M.C., Wang, T., Matijasevic, M., Hong, L., & Apple, F.S. (2003). Myocardial tissue troponins T and I. An immunohistochemical study in experimental models of myocardial ischemia. *Cardiovasc Pathol*, 12(2), 65-71.

Fonarow, G.C., Peacock, W.F., Horwich, T.B., Phillips, C.O., Givertz, M.M., Lopatin, M., Wynne, J., Committee, A.S.A., & Investigators. (2008). Usefulness of B-type natriuretic peptide and cardiac troponin levels to predict in-hospital mortality from ADHERE. *Am J Cardiol*, 101(2), 231-237.

Ford, E.S.C., S. . (2007). Coronary heart disease mortality among young adults in the U.S. from 1980 through 2002: concealed leveling of mortality rates. . *J.Am.Coll.Cardiol.,*, 20, 2128-2132.

Fournier, N.C., & Richard, M.A. (1990). Role of fatty acid-binding protein in cardiac fatty acid oxidation. *Mol Cell Biochem*, 98(1-2), 149-159.

Fox, K.A., Steg, P.G., Eagle, K.A., Goodman, S.G., Anderson, F.A., Jr., Granger, C.B., *et al.* (2007). Decline in rates of death and heart failure in acute coronary syndromes, 1999-2006. *JAMA*, 297(17), 1892-1900.

Fox, K.F. (2005). Investigation and management of chest pain. *Heart*, 91(1), 105-110.

Furberg, C.D., Psaty, B.M., & Meyer, J.V. (1995). Nifedipine. Dose-related increase in mortality in patients with coronary heart disease. *Circulation*, 92(5), 1326-1331.

Fuster, V., Badimon, L., Badimon, J. J., & Chesebro, J. H. . (1992). The pathogenesis of coronary artery disease and the acute coronary syndromes (2). . *N.Engl.J.Med.,*, 326, 310-318.

Gafni A, B.S. (1993). Guidelines for the adoption of new technologies: a prescription for uncontrolled growth in expenditures and how to avoid the prob-lem. . *Can Med Assoc J*(148), 913–917.

Galmiche, J.P. (2006). Non-erosive reflux disease and atypical gastro-oesophageal reflux disease manifestations: treatment results. *Drugs*, 66 Suppl 1, 7-13; discussion 29-33.

Galper, B.Z., Wang, Y.C., & Einstein, A.J. (2015). Strategies for Primary Prevention of Coronary Heart Disease Based on Risk Stratification by the ACC/AHA Lipid Guidelines, ATP III Guidelines, Coronary Calcium Scoring, and C-Reactive Protein, and a Global Treat-All Strategy: A Comparative--Effectiveness Modeling Study. *PLoS One*, 10(9), e0138092.

Gamble, J.H., Carlton, E.W., Orr, W.P., & Greaves, K. (2013). High-sensitivity cardiac troponins: no more 'negatives'. *Expert Rev Cardiovasc Ther*, 11(9), 1129-1139.

Garcia-Fernandez, M.A., Macchioli, R.O., Moreno, P.M., Yanguela, M.M., Thomas, J.B., Sendon, J.L., Lopez de Sa, E., & Abdou, Y.H. (2001). Use of contrast echocardiography in the diagnosis of subacute myocardial rupture after myocardial infarction. *J Am Soc Echocardiogr*, 14(9), 945-947.

Gavalova, L.a.W., C. . (2012). Myocardial Ischaemia National Audit

George B, H.A., Mitchell A. (2001). Cost-effectiveness analysis and the consistency of decision making. Evidence from pharmaceutical reimbursement in Australia (1991–1996). . *Pharmacoeconomics*19, 1103-1109.

George, J., Rapsomaniki, E., Pujades-Rodriguez, M., Shah, A.D., Denaxas, S., Herrett, E., Smeeth, L., Timmis, A., & Hemingway, H. (2015). How Does Cardiovascular Disease First Present in Women and Men? Incidence of 12 Cardiovascular Diseases in a Contemporary Cohort of 1,937,360 People. *Circulation*, 132(14), 1320-1328.

Giannitsis, E., Becker, M., Kurz, K., Hess, G., Zdunek, D., & Katus, H.A. (2010). High-sensitivity cardiac troponin T for early prediction of evolving non-ST-segment elevation myocardial infarction in patients with suspected acute coronary syndrome and negative troponin results on admission. *Clin Chem*, 56(4), 642-650.

Golias, C., Charalabopoulos, A., Stagikas, D., Charalabopoulos, K., & Batistatou, A. (2007). The kinin system--bradykinin: biological effects and clinical implications. Multiple role of the kinin system--bradykinin. *Hippokratia*, 11(3), 124-128.

Goodacre, S., Cross, E., Arnold, J., Angelini, K., Capewell, S., & Nicholl, J. (2005). The health care burden of acute chest pain. *Heart*, 91(2), 229-230.

Goodacre, S., Mason, S., Arnold, J., & Angelini, K. (2001). Psychologic morbidity and health-related quality of life of patients assessed in a chest pain observation unit. *Ann Emerg Med*, 38(4), 369-376.

Goodacre, S., Nicholl, J., Dixon, S., Cross, E., Angelini, K., Arnold, J*., et al.* (2004). Randomised controlled trial and economic evaluation of a chest pain observation unit compared with routine care. *BMJ*, 328(7434), 254.

Goodacre, S.C., N. . (2003). Goodacre, S. & Calvert, N. (2003). Cost effectiveness of diagnostic strategies for patients with acute, undifferentiated chest pain. . *Emerg.Med.J.,*, 20(429-433.).

Goodacre, S.W., Bradburn, M., Cross, E., Collinson, P., Gray, A., Hall, A.S., & Team, R.R. (2011). The Randomised Assessment of Treatment using Panel Assay of Cardiac Markers (RATPAC) trial: a randomised controlled trial of point-of-care cardiac markers in the emergency department. *Heart*, 97(3), 190-196.

Goodacre, S.W., Bradburn, M., Cross, E., Collinson, P., Gray, A., & Hall, A. S. . (2011). The Randomised Assessment of Treatment using Panel Assay of Cardiac Markers (RATPAC) trial: a randomised controlled trial of point-of-care cardiac markers in the emergency department. . *Heart,*, 97, 190-196.

Goss, F., Brachmann, J., Hamm, C.W., Haerer, W., Reifart, N., & Levenson, B. (2017). High adherence to therapy and low cardiac mortality and morbidity in patients after acute coronary syndrome systematically managed by office-based cardiologists in Germany: 1-year outcomes of the ProAcor Study. *Vasc Health Risk Manag*, 13, 127-137.

Gotoh, T., Endo, M., & Oike, Y. (2011). Endoplasmic reticulum stress-related inflammation and cardiovascular diseases. *Int J Inflam*, 2011, 259462.

Gotoh, T., & Mori, M. (2006). Nitric oxide and endoplasmic reticulum stress. *Arterioscler Thromb Vasc Biol*, 26(7), 1439-1446.

Govender, R., De Greef, J., Delport, R., Becker, P.J., & Vermaak, W.J. (2008). Biological variation of ischaemia-modified albumin in healthy subjects. *Cardiovasc J Afr*, 19(3), 141-144.

Graham, M.M., Westerhout, C.M., Kaul, P., Norris, C.M., & Armstrong, P.W. (2008). Sex differences in patients seeking medical attention for prodromal symptoms before an acute coronary event. *Am Heart J*, 156(6), 1210-1216 e1211.

Grenadier, E., Keidar, S., Kahana, L., Alpan, G., Marmur, A., & Palant, A. (1983). The roles of serum myoglobin, total CPK, and CK-MB isoenzyme in the acute phase of myocardial infarction. *Am Heart J*, 105(3), 408-416.

Gu, Y.L., Voors, A.A., Zijlstra, F., Hillege, H.L., Struck, J., Masson, S*., et al.* (2011). Comparison of the temporal release pattern of copeptin with conventional biomarkers in acute myocardial infarction. *Clin Res Cardiol*, 100(12), 1069-1076.

Guo, X.B., Ma, W.L., Liu, L.J., Huang, Y.L., Wang, J., Huang, L.H*., et al.* (2017). Effects of gene polymorphisms in the endoplasmic reticulum stress pathway on clinical outcomes of

chemoradiotherapy in Chinese patients with nasopharyngeal carcinoma. *Acta Pharmacol Sin*, 38(4), 571-580.

Gupta, A., Wang, Y., Spertus, J.A., Geda, M., Lorenze, N., Nkonde-Price, C., D'Onofrio, G., Lichtman, J.H., & Krumholz, H.M. (2014). Trends in acute myocardial infarction in young patients and differences by sex and race, 2001 to 2010. *J Am Coll Cardiol*, 64(4), 337-345.

Gururajan, P., Gurumurthy, P., Nayar, P., Srinivasa Nageswara Rao, G., Babu, S., & Cherian, K.M. (2010). Heart fatty acid binding protein (H-FABP) as a diagnostic biomarker in patients with acute coronary syndrome. *Heart Lung Circ*, 19(11), 660-664.

Haaf, P., Reichlin, T., Corson, N., Twerenbold, R., Reiter, M., Steuer, S.*, et al.* (2011). B-type natriuretic peptide in the early diagnosis and risk stratification of acute chest pain. *Am J Med*, 124(5), 444-452.

Haltern, G., Peiniger, S., Bufe, A., Reiss, G., Gulker, H., & Scheffold, T. (2010). Comparison of usefulness of heart-type fatty acid binding protein versus cardiac troponin T for diagnosis of acute myocardial infarction. *Am J Cardiol*, 105(1), 1-9.

Hama, N., Itoh, H., Shirakami, G., Nakagawa, O., Suga, S., Ogawa, Y.*, et al.* (1995). Rapid ventricular induction of brain natriuretic peptide gene expression in experimental acute myocardial infarction. *Circulation*, 92(6), 1558-1564.

Hamm, C.W., Bassand, J. P., Agewall, S., Bax, J., Boersma, E., Bueno, H. . (2011). ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur.Heart J.,* , 32(32), 2999-3054.

Hamm, C.W., Heeschen, C., Goldmann, B., Vahanian, A., Adgey, J., Miguel, C.M.*, et al.* (1999). Benefit of abciximab in patients with refractory unstable angina in relation to serum troponin T levels. c7E3 Fab Antiplatelet Therapy in Unstable Refractory Angina (CAPTURE) Study Investigators. *N Engl J Med*, 340(21), 1623-1629.

Hans-Georg, E.S., X. . (2004). Use of Cost-Effectiveness Analysis in Health-Care Resource Allocation Decision-Making: How Are Cost-Effectiveness Thresholds Expected to Emerge? *VALUE IN HEALTH*, 7(5), 518 – 552.

Hascoet, S., Bongard, V., Chabbert, V., Marachet, M.A., Rousseau, H., Charpentier, S.*, et al.* (2012). Early triage of emergency department patients with acute coronary syndrome: contribution of 64-slice computed tomography angiography. *Arch Cardiovasc Dis*, 105(6-7), 338-346.

Hazinski, M.F., Nolan, J.P., Billi, J.E., Bottiger, B.W., Bossaert, L., de Caen, A.R.*, et al.* (2010). Part 1: Executive summary: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. *Circulation*, 122(16 Suppl 2), S250-275.

Health, D.o. (2012-2013). Confirmation of Payment by Results arrangments. http://www.dh.gov.uk.

Heart Outcomes Prevention Evaluation Study, I., Yusuf, S., Sleight, P., Pogue, J., Bosch, J., Davies, R., & Dagenais, G. (2000). Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med*, 342(3), 145-153.

Heidenreich, P.A., McDonald, K.M., Hastie, T., Fadel, B., Hagan, V., Lee, B.K., & Hlatky, M.A. (1999a). An evaluation of beta-blockers, calcium antagonists, nitrates, and alternative therapies for stable angina. *Evid Rep Technol Assess (Summ)*(10), 1-2.

Heidenreich, P.A., McDonald, K.M., Hastie, T., Fadel, B., Hagan, V., Lee, B.K., & Hlatky, M.A. (1999b). Meta-analysis of trials comparing beta-blockers, calcium antagonists, and nitrates for stable angina. *JAMA*, 281(20), 1927-1936.

Henning, R.J., Weil, M.H., & Weiner, F. (1982). Blood lactate as prognostic indicator of survival in patients with acute myocardial infarction. *Circ Shock*, 9(3), 307-315.

Higgins, J.P., Thompson, S.G., Deeks, J.J., & Altman, D.G. (2003). Measuring inconsistency in meta-analyses. *BMJ*, 327(7414), 557-560.

Hjortshoj, S., Dethlefsen, C., Kristensen, S.R., & Ravkilde, J. (2009). Kinetics of ischaemia modified albumin during ongoing severe myocardial ischaemia. *Clin Chim Acta*, 403(1-2), 114-120.

Hjortshoj, S., Kristensen, S.R., & Ravkilde, J. (2010). Diagnostic value of ischemia-modified albumin in patients with suspected acute coronary syndrome. *Am J Emerg Med*, 28(2), 170-176.

Hochman, J.S., Tamis, J.E., Thompson, T.D., Weaver, W.D., White, H.D., Van de Werf, F., Aylward, P., Topol, E.J., & Califf, R.M. (1999). Sex, clinical presentation, and outcome in patients with acute coronary syndromes. Global Use of Strategies to Open Occluded Coronary Arteries in Acute Coronary Syndromes IIb Investigators. *N Engl J Med*, 341(4), 226-232.

Holwerda, D.A. (1972). A glycopeptide from the posterior lobe of pig pituitaries. I. Isolation and characterization. *Eur J Biochem*, 28(3), 334-339.

Hong, S.J. (2010). [Diagnosis and management of esophageal chest pain]. *Korean J Gastroenterol*, 55(4), 217-224.Inverness. (2005). *Ischemia modified albumin test package insert*

Irwig, L., Tosteson, A.N., Gatsonis, C., Lau, J., Colditz, G., Chalmers, T.C., & Mosteller, F. (1994). Guidelines for meta-analyses evaluating diagnostic tests. *Ann Intern Med*, 120(8), 667-676.

Ishii, J., Ozaki, Y., Lu, J., Kitagawa, F., Kuno, T., Nakano, T.*, et al.* (2005). Prognostic value of serum concentration of heart-type fatty acid-binding protein relative to cardiac troponin T on admission in the early hours of acute coronary syndrome. *Clin Chem*, 51(8), 1397-1404.

Jaffe, A.S., Babuin, L., & Apple, F. S. (2006). Biomarkers in acute cardiac disease: the present and the future. . *J.Am.Coll.Cardiol.,*, 48, 1-11.Johannesson M, J.B., Karlsson G. (1996). Outcome measurement in economic evaluation. . *Health Econ*, 5, 279–296.

Johansson, S., Wallander, M.A., Ruigomez, A., & Garcia Rodriguez, L.A. (2003). Is there any association between myocardial infarction, gastro-oesophageal reflux disease and acid-suppressing drugs? *Aliment Pharmacol Ther*, 18(10), 973-978.

Jones, R.M., Prasad, M.R., & Das, D.K. (1990). Modulation of fatty acid-binding capacity of heart fatty acid-binding protein by oxygen-derived free radicals. *Mol Cell Biochem*, 98(1-2), 161-166.

Kagen, L., Scheidt, S., Roberts, L., Porter, A., & Paul, H. (1975). Myoglobinemia following acute myocardial infarction. *Am J Med*, 58(2), 177-182.

Karlson, B.W., Herlitz, J., Wiklund, O., Richter, A., & Hjalmarson, A. (1991). Early prediction of acute myocardial infarction from clinical history, examination and electrocardiogram in the emergency room. *Am J Cardiol*, 68(2), 171-175.

Katan, M., & Christ-Crain, M. (2010). The stress hormone copeptin: a new prognostic biomarker in acute illness. *Swiss Med Wkly*, 140, w13101.

Katus, H.A., Remppis, A., Scheffold, T., Diederich, K.W., & Kuebler, W. (1991). Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction. *Am J Cardiol*, 67(16), 1360-1367.

Katz, P.O., & Castell, D.O. (2000). Approach to the patient with unexplained chest pain. *Am J Gastroenterol*, 95(8 Suppl), S4-8.

Kavsak, P.A., MacRae, A.R., Lustig, V., Bhargava, R., Vandersluis, R., Palomaki, G.E., Yerna, M.J., & Jaffe, A.S. (2006). The impact of the ESC/ACC redefinition of myocardial infarction and new sensitive troponin assays on the frequency of acute myocardial infarction. *Am Heart J*, 152(1), 118-125.

Keating, L., Benger, J.R., Beetham, R., Bateman, S., Veysey, S., Kendall, J., & Pullinger, R. (2006). The PRIMA study: presentation ischaemia-modified albumin in the emergency department. *Emerg Med J*, 23(10), 764-768.

Keller, T., Ojeda, F., Zeller, T., Wild, P.S., Tzikas, S., Sinning, C.R*., et al.* (2013). Defining a reference population to determine the 99th percentile of a contemporary sensitive cardiac troponin I assay. *Int J Cardiol*, 167(4), 1423-1429.

Keller, T., Tzikas, S., Zeller, T., Czyz, E., Lillpopp, L., Ojeda, F.M*., et al.* (2010). Copeptin improves early diagnosis of acute myocardial infarction. *J Am Coll Cardiol*, 55(19), 2096-2106.

Keller, T., Zeller, T., Ojeda, F., Tzikas, S., Lillpopp, L., Sinning, C*., et al.* (2011). Serial changes in highly sensitive troponin I assay and early diagnosis of myocardial infarction. *JAMA*, 306(24), 2684-2693.

Keller, T., Zeller, T., Peetz, D., Tzikas, S., Roth, A., Czyz, E*., et al.* (2009). Sensitive troponin I assay in early diagnosis of acute myocardial infarction. *N Engl J Med*, 361(9), 868-877.

Kendall, M. (1938). A New Measure of Rank Correlation. . *Biometrika*(30), 81-89.

Ketchum, E.S., & Levy, W.C. (2011). Establishing prognosis in heart failure: a multimarker approach. *Prog Cardiovasc Dis*, 54(2), 86-96.

Khafaji, H.A., & Suwaidi, J.M. (2014). Atypical presentation of acute and chronic coronary artery disease in diabetics. *World J Cardiol*, 6(8), 802-813.

Khuddus, M.A., Pepine, C.J., Handberg, E.M., Bairey Merz, C.N., Sopko, G., Bavry, A.A*., et al.* (2010). An intravascular ultrasound analysis in women experiencing chest pain in the absence of obstructive coronary artery disease: a substudy from the National Heart, Lung and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *J Interv Cardiol*, 23(6), 511-519.

Kilcullen, N., Viswanathan, K., Das, R., Morrell, C., Farrin, A., Barth, J.H., Hall, A.S., & Investigators, E.-. (2007). Heart-type fatty acid-binding protein predicts long-term mortality after acute coronary syndrome and identifies high-risk patients across the range of troponin values. *J Am Coll Cardiol*, 50(21), 2061-2067.

Kim, J., & Hashim, I.A. (2016). The clinical utility of CK-MB measurement in patients suspected of acute coronary syndrome. *Clin Chim Acta*, 456, 89-92.

Kleine, A.H., Glatz, J.F., Van Nieuwenhoven, F.A., & Van der Vusse, G.J. (1992). Release of heart fatty acid-binding protein into plasma after acute myocardial infarction in man. *Mol Cell Biochem*, 116(1-2), 155-162.

Kloner, R.A., Shook, T., Przyklenk, K., Davis, V. G., Junio, L., Matthews, R. V. . (1995). Previous angina alters in-hospital outcome in TIMI 4. A clinical correlate to preconditioning? . *Circulation,*, 91, 37-45.

Kones, R., & Rumana, U. (2016). Stable Ischemic Heart Disease. *Heart Fail Clin*, 12(1), 11-29.

Kontos, M.C., Diercks, D.B., & Kirk, J.D. (2010). Emergency department and office-based evaluation of patients with chest pain. *Mayo Clin Proc*, 85(3), 284-299.

Kubo, T., Maehara, A., Mintz, G.S., Doi, H., Tsujita, K., Choi, S.Y*., et al.* (2010). The dynamic nature of coronary artery lesion morphology assessed by serial virtual histology intravascular ultrasound tissue characterization. *J Am Coll Cardiol*, 55(15), 1590-1597.

Lamas, G.A., Goertz, C., Boineau, R., Mark, D.B., Rozema, T., Nahin, R.L., Drisko, J.A., & Lee, K.L. (2012). Design of the Trial to Assess Chelation Therapy (TACT). *Am Heart J*, 163(1), 7-12.

Lamas, G.A., Goertz, C., Boineau, R., Mark, D.B., Rozema, T., Nahin, R.L*., et al.* (2013). Effect of disodium EDTA chelation regimen on cardiovascular events in patients with previous myocardial infarction: the TACT randomized trial. *JAMA*, 309(12), 1241-1250.

Lampe, F.C., Whincup, P. H., Wannamethee, S. G., Ebrahim, S., Walker, M., & Shaper, A. G. . (1998). Chest pain on questionnaire and prediction of major ischaemic heart disease events in men. . *Eur.Heart J.,*, 19, 63-73.

Lancellotti, P., Price, S., Edvardsen, T., Cosyns, B., Neskovic, A.N., Dulgheru, R*., et al.* (2015). The use of echocardiography in acute cardiovascular care: recommendations of the European Association of Cardiovascular Imaging and the Acute Cardiovascular Care Association. *Eur Heart J Cardiovasc Imaging*, 16(2), 119-146.

Landmesser, U., & Drexler, H. (2006). Effect of angiotensin II type 1 receptor antagonism on endothelial function: role of bradykinin and nitric oxide. *J Hypertens Suppl*, 24(1), S39-43.

Latini, R., Tognoni, G., Maggioni, A.P., Baigent, C., Braunwald, E., Chen, Z.M.*, et al.* (2000). Clinical effects of early angiotensin-converting enzyme inhibitor treatment for acute myocardial infarction are similar in the presence and absence of aspirin: systematic overview of individual data from 96,712 randomized patients. Angiotensin-converting Enzyme Inhibitor Myocardial Infarction Collaborative Group. *J Am Coll Cardiol*, 35(7), 1801-1807.

Lavie, C.J., Milani, R.V., Mehra, M.R., & Ventura, H.O. (2009). Omega-3 polyunsaturated fatty acids and cardiovascular diseases. *J Am Coll Cardiol*, 54(7), 585-594.

Lee, T.H., Cook, E. F., Weisberg, M., Sargent, R. K., Wilson, C., & Goldman, L. . (1985). Acute chest pain in the emergency room. Identification and examination of low-risk patients. . *Arch.Intern.Med.,*, 145, , 65-69.

Lee, T.H., Rouan, G.W., Weisberg, M.C., Brand, D.A., Cook, E.F., Acampora, D., & Goldman, L. (1987). Sensitivity of routine clinical criteria for diagnosing myocardial infarction within 24 hours of hospitalization. *Ann Intern Med*, 106(2), 181-186.

Lev, E.I., Battler, A., Behar, S., Porter, A., Haim, M., Boyko, V., & Hasdai, D. (2003). Frequency, characteristics, and outcome of patients hospitalized with acute coronary syndromes with undetermined electrocardiographic patterns. *Am J Cardiol*, 91(2), 224-227.

Levin, E.R., Gardner, D.G., & Samson, W.K. (1998). Natriuretic peptides. *N Engl J Med*, 339(5), 321-328.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gotzsche, P.C., Ioannidis, J.P.*, et al.* (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Ann Intern Med*, 151(4), W65-94.

Liebetrau, C., Nef, H., Szardien, S., Dorr, O., Willmer, M., Voss, S.*, et al.* (2013). Release kinetics of copeptin in patients undergoing transcoronary ablation of septal hypertrophy. *Clin Chem*, 59(3), 566-569.

Lin, S., Yokoyama, H., Rac, V.E., & Brooks, S.C. (2012). Novel biomarkers in diagnosing cardiac ischemia in the emergency department: a systematic review. *Resuscitation*, 83(6), 684-691.

Lindsay, A., Carr, S., Draper, N., & Gieseg, S.P. (2015). Urinary myoglobin quantification by high-performance liquid chromatography: An alternative measurement for exercise-induced muscle damage. *Anal Biochem*, 491, 37-42.

Linton, M.F., Yancey, P.G., Davies, S.S., Jerome, W.G.J., Linton, E.F., & Vickers, K.C. (2000). The Role of Lipids and Lipoproteins in Atherosclerosis. In De Groot, L. J., Chrousos, G., Dungan, K., Feingold, K. R., Grossman, A., Hershman, J. M., Koch, C., Korbonits, M., McLachlan, R., New, M., Purnell, J., Rebar, R., Singer, F., & Vinik, A. (Eds.), *Endotext*. South Dartmouth (MA).

Lipinski, M.J., Baker, N.C., Escarcega, R.O., Torguson, R., Chen, F., Aldous, S.J.*, et al.* (2015). Comparison of conventional and high-sensitivity troponin in patients with chest pain: a collaborative meta-analysis. *Am Heart J*, 169(1), 6-16 e16.

Lippi, G., Mattiuzzi, C., & Cervellin, G. (2013). Critical review and meta-analysis on the combination of heart-type fatty acid binding protein (H-FABP) and troponin for early diagnosis of acute myocardial infarction. *Clin Biochem*, 46(1-2), 26-30.

Liu, Y., He, S., Chen, Y., Xu, J., Tang, C., Tang, Y., & Luo, G. (2013). Acid reflux in patients with coronary artery disease and refractory chest pain. *Intern Med*, 52(11), 1165-1171.

Liuzzo, J.P., & Ambrose, J.A. (2005). Chest pain from gastroesophageal reflux disease in patients with coronary artery disease. *Cardiol Rev*, 13(4), 167-173.

Lloyd-Jones, D.M., Hong, Y., Labarthe, D., Mozaffarian, D., Appel, L.J., Van Horn, L.*, et al.* (2010). Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation*, 121(4), 586-613.

Locke, G.R., 3rd, Talley, N.J., Fett, S.L., Zinsmeister, A.R., & Melton, L.J., 3rd. (1997). Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. *Gastroenterology*, 112(5), 1448-1456.

Lollgen, H., Bockenhoff, A., & Knapp, G. (2009). Physical activity and all-cause mortality: an updated meta-analysis with different intensity categories. *Int J Sports Med*, 30(3), 213-224.

Lomaestro, B.M. (1994). More on the GUSTO Trial. *Ann Intern Med*, 121(10), 818-819.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V.*, et al.* (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 380(9859), 2095-2128.

Lusis, A.J. (2000). Atherosclerosis. . *Nature,*, 4074, 233-241.

Lythall, D.A., Gibson, D.G., Kushwaha, S.S., Norell, M.S., Mitchell, A.G., & Ilsley, C.J. (1992). Changes in myocardial echo amplitude during reversible ischaemia in humans. *Br Heart J*, 67(5), 368-376.

Mack, M.J., Head, S.J., Holmes, D.R., Jr., Stahle, E., Feldman, T.E., Colombo, A.*, et al.* (2013). Analysis of stroke occurring in the SYNTAX trial comparing coronary artery bypass surgery and percutaneous coronary intervention in the treatment of complex coronary artery disease. *JACC Cardiovasc Interv*, 6(4), 344-354.

Maguire, O.C., O'Sullivan, J., Ryan, J., & Cunningham, S.K. (2006). Evaluation of the albumin cobalt binding (ACB) assay for measurement of ischaemia-modified albumin (IMA) on the Beckman Coulter LX-20. *Ann Clin Biochem*, 43(Pt 6), 494-499.

Mair, J., Artner-Dworzak, E., Dienstl, A., Lechleitner, P., Morass, B., Smidt, J., Wagner, I., Wettach, C., & Puschendorf, B. (1991). Early detection of acute myocardial infarction by measurement of mass concentration of creatine kinase-MB. *Am J Cardiol*, 68(17), 1545-1550.

Maisel, A., Mueller, C., Neath, S.X., Christenson, R.H., Morgenthaler, N.G., McCord, J.*, et al.* (2013). Copeptin helps in the early detection of patients with acute myocardial infarction: primary results of the CHOPIN trial (Copeptin Helps in the early detection Of Patients with acute myocardial INfarction). *J Am Coll Cardiol*, 62(2), 150-160.

Marumoto, K., Hamada, M., & Hiwada, K. (1995). Increased secretion of atrial and brain natriuretic peptides during acute myocardial ischaemia induced by dynamic exercise in patients with angina pectoris. *Clin Sci (Lond)*, 88(5), 551-556.

Mathers, C.D., & Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*, 3(11), e442.

Maynard, C., Fisher, L.D., Passamani, E.R., & Pullum, T. (1986). Blacks in the Coronary Artery Surgery Study: risk factors and coronary artery disease. *Circulation*, 74(1), 64-71.

McCann, C.J., Glover, B.M., Menown, I.B., Moore, M.J., McEneny, J., Owens, C.G.*, et al.* (2008). Novel biomarkers in early diagnosis of acute myocardial infarction compared with cardiac troponin T. *Eur Heart J*, 29(23), 2843-2850.

McCullough, P.A., Duc, P., Omland, T., McCord, J., Nowak, R.M., Hollander, J.E.*, et al.* (2003). B-type natriuretic peptide and renal function in the diagnosis of heart failure: an analysis from the Breathing Not Properly Multinational Study. *Am J Kidney Dis*, 41(3), 571-579.

McLean, A.S., & Huang, S.J. (2012). Cardiac biomarkers in the intensive care unit. *Ann Intensive Care*, 2, 8.

McMahon, C.G., Lamont, J.V., Curtin, E., McConnell, R.I., Crockard, M., Kurth, M.J., Crean, P., & Fitzgerald, S.P. (2012). Diagnostic accuracy of heart-type fatty acid-binding protein for the early diagnosis of acute myocardial infarction. *Am J Emerg Med*, 30(2), 267-274.

McManus, D.D., Gore, J., Yarzebski, J., Spencer, F., Lessard, D., & Goldberg, R.J. (2011). Recent trends in the incidence, treatment, and outcomes of patients with STEMI and NSTEMI. *Am J Med*, 124(1), 40-47.

McSweeney, J.C., Cody, M., O'Sullivan, P., Elberson, K., Moser, D.K., & Garvin, B.J. (2003). Women's early warning symptoms of acute myocardial infarction. *Circulation*, 108(21), 2619-2623.

McSweeney, J.C., O'Sullivan, P., Cody, M., & Crane, P.B. (2004). Development of the McSweeney Acute and Prodromal Myocardial Infarction Symptom Survey. *J Cardiovasc Nurs*, 19(1), 58-67.

Mehta, M.D., Marwah, S.A., Ghosh, S., Shah, H.N., Trivedi, A.P., & Haridas, N. (2015). A synergistic role of ischemia modified albumin and high-sensitivity troponin T in the early diagnosis of acute coronary syndrome. *J Family Med Prim Care*, 4(4), 570-575.

Melki, D., Lugnegard, J., Alfredsson, J., Lind, S., Eggers, K.M., Lindahl, B., & Jernberg, T. (2015). Implications of Introducing High-Sensitivity Cardiac Troponin T Into Clinical Practice: Data From the SWEDEHEART Registry. *J Am Coll Cardiol*, 65(16), 1655-1664.

Melse JM, E.-B.M., Kramers PGN, Hoey-mans N. . (2000). A national burden of disease calculation: Dutch disability-adjusted life years. . *Am J Public Health*, 90, 1241-1247.

Micha, R., Kalantarian, S., Wirojratana, P., Byers, T., Danaei, G., Elmadfa, I.*, et al.* (2012). Estimating the global and regional burden of suboptimal nutrition on chronic disease: methods and inputs to the analysis. *Eur J Clin Nutr*, 66(1), 119-129.

Michielsen, E.C., Diris, J.H., Kleijnen, V.W., Wodzig, W.K., & Van Dieijen-Visser, M.P. (2007). Investigation of release and degradation of cardiac troponin T in patients with acute myocardial infarction. *Clin Biochem*, 40(12), 851-855.

Middleton, N., Shave, R., George, K., Whyte, G., Forster, J., Oxborough, D., Gaze, D., & Collinson, P. (2006). Novel application of flow propagation velocity and ischaemia-modified albumin in analysis of postexercise cardiac function in man. *Exp Physiol*, 91(3), 511-519.

Mills, E.J., Rachlis, B., Wu, P., Devereaux, P.J., Arora, P., & Perri, D. (2008). Primary prevention of cardiovascular mortality and events with statin treatments: a network meta-analysis involving more than 65,000 patients. *J Am Coll Cardiol*, 52(22), 1769-1781.

Mills, N.L., Churchhouse, A.M., Lee, K.K., Anand, A., Gamble, D., Shah, A.S.*, et al.* (2011). Implementation of a sensitive troponin I assay and risk of recurrent myocardial infarction and death in patients with suspected acute coronary syndrome. *JAMA*, 305(12), 1210-1216.

Mills, N.L., Lee, K.K., McAllister, D.A., Churchhouse, A.M., MacLeod, M., Stoddart, M.*, et al.* (2012). Implications of lowering threshold of plasma troponin concentration in diagnosis of myocardial infarction: cohort study. *BMJ*, 344, e1533.

Mishra, S. (2016). Does modern medicine increase life-expectancy: Quest for the Moon Rabbit? *Indian Heart J*, 68(1), 19-27.

Mishra, S., Ray, S., Dalal, J.J., Sawhney, J.P., Ramakrishnan, S., Nair, T., Iyengar, S.S., & Bahl, V.K. (2016). Management standards for stable coronary artery disease in India. *Indian Heart J*, 68 Suppl 3, S31-S49.

Mockel, M., Searle, J., Hamm, C., Slagman, A., Blankenberg, S., Huber, K.*, et al.* (2015). Early discharge using single cardiac troponin and copeptin testing in patients with suspected acute coronary syndrome (ACS): a randomized, controlled clinical process study. *Eur Heart J*, 36(6), 369-376.

Modi, S., & Krahn, A.D. (2011). Sudden cardiac arrest without overt heart disease. *Circulation*, 123(25), 2994-3008.

Mohr, F.W., Morice, M.C., Kappetein, A.P., Feldman, T.E., Stahle, E., Colombo, A.*, et al.* (2013). Coronary artery bypass graft surgery versus percutaneous coronary intervention in patients with three-vessel disease and left main coronary disease: 5-year follow-up of the randomised, clinical SYNTAX trial. *Lancet*, 381(9867), 629-638.

Morgenthaler, N.G., Struck, J., Jochberger, S., & Dunser, M.W. (2008). Copeptin: clinical use of a new biomarker. *Trends Endocrinol Metab*, 19(2), 43-49.

Morrow, D.A., & Braunwald, E. (2003). Future of biomarkers in acute coronary syndromes: moving toward a multimarker strategy. *Circulation*, 108(3), 250-252.

Mozaffarian, D., Appel, L.J., & Van Horn, L. (2011). Components of a cardioprotective diet: new insights. *Circulation*, 123(24), 2870-2891.

Mudipalli, R.S., Remes-Troche, J.M., Andersen, L., & Rao, S.S. (2007). Functional chest pain: esophageal or overlapping functional disorder. *J Clin Gastroenterol*, 41(3), 264-269.

Munzel, T., Daiber, A., & Gori, T. (2011). Nitrate therapy: new aspects concerning molecular action and tolerance. *Circulation*, 123(19), 2132-2144.

Myerburg, R.J., & Junttila, M.J. (2012). Sudden cardiac death caused by coronary heart disease. *Circulation*, 125(8), 1043-1052.

Ndrepepa, G., Braun, S., Schomig, A., & Kastrati, A. (2007). Accuracy of N-terminal pro-brain natriuretic peptide to predict mortality in various subsets of patients with coronary artery disease. *Am J Cardiol*, 100(4), 575-578.

Nemec, M., Koller, M.T., Nickel, C.H., Maile, S., Winterhalder, C., Karrer, C., Laifer, G., & Bingisser, R. (2010). Patients presenting to the emergency department with non-specific complaints: the Basel Non-specific Complaints (BANC) study. *Acad Emerg Med*, 17(3), 284-292.

Neskovic, A.N., Hagendorff, A., Lancellotti, P., Guarracino, F., Varga, A., Cosyns, B.*, et al.* (2013). Emergency echocardiography: the European Association of Cardiovascular Imaging recommendations. *Eur Heart J Cardiovasc Imaging*, 14(1), 1-11.

Newby, D.E., Fox, K.A., Flint, L.L., & Boon, N.A. (1998). A 'same day' direct-access chest pain clinic: improved management and reduced hospitalization. *QJM*, 91(5), 333-337.

Newby, L.K., Jesse, R.L., Babb, J.D., Christenson, R.H., De Fer, T.M., Diamond, G.A.*, et al.* (2012). ACCF 2012 expert consensus document on practical clinical considerations in the interpretation of troponin elevations: a report of the American College of Cardiology Foundation task force on Clinical Expert Consensus Documents. *J Am Coll Cardiol*, 60(23), 2427-2463.

NICE. (2008). *Guide to the methods of technological appraisal.*

. (ref: N1618).

Nickel, C.H., Bingisser, R., & Morgenthaler, N.G. (2012). The role of copeptin as a diagnostic and prognostic biomarker for risk stratification in the emergency department. *BMC Med*, 10, 7.

Nikolaou, N.I., Arntz, H.R., Bellou, A., Beygui, F., Bossaert, L.L., Cariou, A., & Initial management of acute coronary syndromes section, C. (2015). European Resuscitation Council Guidelines for Resuscitation 2015 Section 8. Initial management of acute coronary syndromes. *Resuscitation*, 95, 264-277.

Nolan, J.P., Hazinski, M.F., Billi, J.E., Boettiger, B.W., Bossaert, L., de Caen, A.R.*, et al.* (2010). Part 1: Executive summary: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. *Resuscitation*, 81 Suppl 1, e1-25.

Nunes, J.P. (2017). Statins in primary prevention: impact on mortality. A meta-analysis study. *Minerva Cardioangiol*, 65(5), 531-538.

Omland, T., Aakvaag, A., Bonarjee, V.V., Caidahl, K., Lie, R.T., Nilsen, D.W., Sundsfjord, J.A., & Dickstein, K. (1996). Plasma brain natriuretic peptide as an indicator of left ventricular systolic function and long-term survival after acute myocardial infarction. Comparison with plasma atrial

natriuretic peptide and N-terminal proatrial natriuretic peptide. *Circulation*, 93(11), 1963-1969.

Omland, T., de Lemos, J.A., Sabatine, M.S., Christophi, C.A., Rice, M.M., Jablonski, K.A*., et al.* (2009). A sensitive cardiac troponin T assay in stable coronary artery disease. *N Engl J Med*, 361(26), 2538-2547.

Pai, J.K., Pischon, T., Ma, J., Manson, J.E., Hankinson, S.E., Joshipura, K*., et al.* (2004). Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med*, 351(25), 2599-2610.

Pan, S.M., Tong, C.Y., Lin, Q., Yao, C.L., Zhao, J., & Deng, Z. (2010). Ischemia-modified albumin measured with ultra-filtration assay in early diagnosis of acute coronary syndrome. *World J Emerg Med*, 1(1), 37-40.

Panteghini, M. (2009). Assay-related issues in the measurement of cardiac troponins. *Clin Chim Acta*, 402(1-2), 88-93.

Patil, S.M., Banker, M.P., Padalkar, R.K., Pathak, A.P., Bhagat, S.S., Ghone, R.A., & Phatake, A.S. (2013). The clinical assessment of ischaemia modified albumin and troponin I in the early diagnosis of the acute coronary syndrome. *J Clin Diagn Res*, 7(5), 804-808.

Peacock, F., Morris, D.L., Anwaruddin, S., Christenson, R.H., Collinson, P.O., Goodacre, S.W*., et al.* (2006). Meta-analysis of ischemia-modified albumin to rule out acute coronary syndromes in the emergency department. *Am Heart J*, 152(2), 253-262.

Pelsers, M.M., & Glatz, J.F. (2005). Detection of brain injury by fatty acid-binding proteins. *Clin Chem Lab Med*, 43(8), 802-809.

Petrie, A., Bulman, J.S., & Osborn, J.F. (2003). Further statistics in dentistry Part 8: Systematic reviews and meta-analyses. *Br Dent J*, 194(2), 73-78.

Piper, H.M., Schwartz, P., Spahr, R., Hutter, J.F., & Spieckermann, P.G. (1984). Early enzyme release from myocardial cells is not due to irreversible cell damage. *J Mol Cell Cardiol*, 16(4), 385-388.

Poole-Wilson, P.A., Lubsen, J., Kirwan, B.A., van Dalen, F.J., Wagener, G., Danchin, N*., et al.* (2004). Effect of long-acting nifedipine on mortality and cardiovascular morbidity in patients with stable angina requiring treatment (ACTION trial): randomised controlled trial. *Lancet*, 364(9437), 849-857.

Pope, J.H., Aufderheide, T. P., Ruthazer, R., Woolard, R. H., Feldman, J. A., Beshansky, J. R. et al. . (2000). Missed diagnoses of acute cardiac ischemia in the emergency department. . *N.Engl.J.Med.,*, 342, 1163-1170.

Pour-Ghaz, I., Bob-Manuel, T., Marella, H.K., Kelly, J., Nanda, A., Skelton, W.P.t., & Khouzam, R.N. (2018). Incidence and predictors of acute coronary syndrome within a year following a negative stress test-a false sense of security: is routine screening any useful? *Ann Transl Med*, 6(1), 13.

Pourafkari, L., Tajlil, A., Ghaffari, S., Parvizi, R., Chavoshi, M., Kolahdouzan, K.*, et al.* (2017). The frequency of initial misdiagnosis of acute aortic dissection in the emergency department and its impact on outcome. *Intern Emerg Med*, 12(8), 1185-1195.

Price, C.P. (2008). Evaluating new diagnostic technologies: perspectives in the UK and US. *Clin Chem*, 54(9), 1421-1423.

Priori, S.G., Wilde, A.A., Horie, M., Cho, Y., Behr, E.R., Berul, C.*, et al.* (2013). HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm*, 10(12), 1932-1963.

Pryor, D.B., Shaw, L., McCants, C.B., Lee, K.L., Mark, D.B., Harrell, F.E., Jr., Muhlbaier, L.H., & Califf, R.M. (1993). Value of the history and physical in identifying patients at increased risk for coronary artery disease. *Ann Intern Med*, 118(2), 81-90.

Puleo, P.R., Meyer, D., Wathen, C., Tawa, C. B., Wheeler, S., Hamburg, R. J. et al. (1994). Use of a rapid assay of subforms of creatine kinase-MB to diagnose or rule out acute myocardial infarction. . *N.Engl.J.Med.,*, 331, 561-566.

Pursnani, S., Korley, F., Gopaul, R., Kanade, P., Chandra, N., Shaw, R.E., & Bangalore, S. (2012). Percutaneous coronary intervention versus optimal medical therapy in stable coronary artery disease: a systematic review and meta-analysis of randomized clinical trials. *Circ Cardiovasc Interv*, 5(4), 476-490.

Qi, W., Mathisen, P., Kjekshus, J., Simonsen, S., Bjornerheim, R., Endresen, K., & Hall, C. (2001). Natriuretic peptides in patients with aortic stenosis. *Am Heart J*, 142(4), 725-732.

Quiles, J., Roy, D., Gaze, D., Garrido, I.P., Avanzas, P., Sinha, M., & Kaski, J.C. (2003). Relation of ischemia-modified albumin (IMA) levels following elective angioplasty for stable angina pectoris to duration of balloon-induced myocardial ischemia. *Am J Cardiol*, 92(3), 322-324.

Raisch, D. (2000). Understanding quality-adjusted life years and their application to pharmacoeconomic research. . *Ann Pharmacother*, 34, 906–914.

Ramakrishnan, S., Bhatt, K., Dubey, A.K., Roy, A., Singh, S., Naik, N., Seth, S., & Bhargava, B. (2013). Acute electrocardiographic changes during smoking: an observational study. *BMJ Open*, 3(4).

Rao, S.S. (2011). Diagnosis and management of esophageal chest pain. *Gastroenterol Hepatol (N Y)*, 7(1), 50-52.

Rapezzi, C., Biagini, E., & Branzi, A. (2008). Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes: the task force for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes of the European Society of Cardiology. *Eur Heart J*, 29(2), 277-278.

Rawles, J. (1994). Halving of mortality at 1 year by domiciliary thrombolysis in the Grampian Region Early Anistreplase Trial (GREAT). *J Am Coll Cardiol*, 23(1), 1-5.

Reddy, V.S., Bukke, S., Mahato, K., Kumar, V., Reddy, N.V., Munikumar, M., & Vodelu, B. (2017). A meta-analysis of the association of serum ischaemia-modified albumin levels with human hypothyroidism and hyperthyroidism. *Biosci Rep*, 37(1).

Redfield, M.M., Rodeheffer, R.J., Jacobsen, S.J., Mahoney, D.W., Bailey, K.R., & Burnett, J.C., Jr. (2002). Plasma brain natriuretic peptide concentration: impact of age and gender. *J Am Coll Cardiol*, 40(5), 976-982.

Reichlin, T., Hochholzer, W., Bassetti, S., Steuer, S., Stelzig, C., Hartwiger, S.*, et al.* (2009). Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med*, 361(9), 858-867.

Reichlin, T., Hochholzer, W., Stelzig, C., Laule, K., Freidank, H., Morgenthaler, N.G.*, et al.* (2009). Incremental value of copeptin for rapid rule out of acute myocardial infarction. *J Am Coll Cardiol*, 54(1), 60-68.

Remes-Troche, J.M., Maher, J., Mudipalli, R., & Rao, S.S. (2007). Altered esophageal sensory-motor function in patients with persistent symptoms after Nissen fundoplication. *Am J Surg*, 193(2), 200-205.

Ren, L., Ye, H., Wang, P., Cui, Y., Cao, S., & Lv, S. (2014). Comparison of long-term mortality of acute ST-segment elevation myocardial infarction and non-ST-segment elevation acute coronary syndrome patients after percutaneous coronary intervention. *Int J Clin Exp Med*, 7(12), 5588-5592.

Research, C.f.E.a.B. (2014). The economic cost of cardiovascular disease from 2014-2020 in six European Economies.

Richards, A.M., Nicholls, M.G., Yandle, T.G., Frampton, C., Espiner, E.A., Turner, J.G.*, et al.* (1998). Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. *Circulation*, 97(19), 1921-1929.

Ridker, P.M. (2002). On evolutionary biology, inflammation, infection, and the causes of atherosclerosis. *Circulation*, 105(1), 2-4.

Rifai, N., & Ridker, P.M. (2002). Inflammatory markers and coronary heart disease. *Curr Opin Lipidol*, 13(4), 383-389.

Robbins, M.J., Epstein, E.M., & Shah, S. (1997). Creatine kinase subform analysis in hemodialysis patients without acute coronary syndromes. *Nephron*, 76(3), 296-299.

Roberts, E., Ludman, A.J., Dworzynski, K., Al-Mohammad, A., Cowie, M.R., McMurray, J.J., Mant, J., & Failure, N.G.D.G.f.A.H. (2015). The diagnostic accuracy of the natriuretic peptides in heart failure: systematic review and diagnostic meta-analysis in the acute care setting. *BMJ*, 350, h910.

Roberts, R. (1998). Early diagnosis of myocardial infarction with MB CK isoforms. *Clin Chim Acta*, 272(1), 33-45.

Romano, S., Dagianti, A., Penco, M., Varveri, A., Biffani, E., Fedele, F., & Dagianti, A. (2000). Usefulness of echocardiography in the prognostic evaluation of non-Q-wave myocardial infarction. *Am J Cardiol*, 86(4A), 43G-45G.

Rosengren, A., Wallentin, L., A, K.G., Behar, S., Battler, A., & Hasdai, D. (2004). Sex, age, and clinical presentation of acute coronary syndromes. *Eur Heart J*, 25(8), 663-670.

Ross, R. (1999). Atherosclerosis--an inflammatory disease. *N Engl J Med*, 340(2), 115-126.

Roy, D., Quiles, J., Aldama, G., Sinha, M., Avanzas, P., Arroyo-Espliguero, R., Gaze, D., Collinson, P., & Carlos Kaski, J. (2004). Ischemia Modified Albumin for the assessment of patients presenting to the emergency department with acute chest pain but normal or non-diagnostic 12-lead electrocardiograms and negative cardiac troponin T. *Int J Cardiol*, 97(2), 297-301.

Rubini Gimenez, M., Twerenbold, R., Reichlin, T., Wildi, K., Haaf, P., Schaefer, M.*, et al.* (2014). Direct comparison of high-sensitivity-cardiac troponin I vs. T for the early diagnosis of acute myocardial infarction. *Eur Heart J*, 35(34), 2303-2311.

Ruck, A., Gustafsson, T., Norrbom, J., Nowak, J., Kallner, G., Soderberg, M., Sylven, C., & Drvota, V. (2004). ANP and BNP but not VEGF are regionally overexpressed in ischemic human myocardium. *Biochem Biophys Res Commun*, 322(1), 287-291.

Saaby, L., Poulsen, T.S., Hosbond, S., Larsen, T.B., Pyndt Diederichsen, A.C., Hallas, J., Thygesen, K., & Mickley, H. (2013). Classification of myocardial infarction: frequency and features of type 2 myocardial infarction. *Am J Med*, 126(9), 789-797.

Sabatine, M.S., Morrow, D.A., de Lemos, J.A., Gibson, C.M., Murphy, S.A., Rifai, N.*, et al.* (2002). Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. *Circulation*, 105(15), 1760-1763.

Sabia, P., Afrookteh, A., Touchstone, D.A., Keller, M.W., Esquivel, L., & Kaul, S. (1991). Value of regional wall motion abnormality in the emergency room diagnosis of acute myocardial infarction. A prospective study using two-dimensional echocardiography. *Circulation*, 84(3 Suppl), I85-92.

Sadanandan, S., Cannon, C.P., Chekuri, K., Murphy, S.A., Dibattiste, P.M., Morrow, D.A., de Lemos, J.A., Braunwald, E., & Gibson, C.M. (2004). Association of elevated B-type natriuretic peptide levels with angiographic findings among patients with unstable angina and non-ST-segment elevation myocardial infarction. *J Am Coll Cardiol*, 44(3), 564-568.

Saenger, A.K., & Jaffe, A.S. (2008). Requiem for a heavyweight: the demise of creatine kinase-MB. *Circulation*, 118(21), 2200-2206.

Sakakura, K., Nakano, M., Otsuka, F., Ladich, E., Kolodgie, F.D., & Virmani, R. (2013). Pathophysiology of atherosclerosis plaque progression. *Heart Lung Circ*, 22(6), 399-411.

Salama, R., El-Moniem, A., El-Hefney, N., & Samor, T. (2011a). N-terminal pro-BNP in acute coronary syndrome patients with ST elevation (STE-ACS) versus non ST elevation (NSTE-ACS). *Int J Health Sci (Qassim)*, 5(2 Suppl 1), 27-29.

Salama, R.H., El-Moniem, A.E., El-Hefney, N., & Samor, T. (2011b). N-TerminaL PRO-BNP in Acute Coronary Syndrome Patients with ST Elevation Versus Non ST Elevation in Qassim Region of Saudi Arabia. *Int J Health Sci (Qassim)*, 5(2), 136-145.

Saraste, A., Pulkki, K., Kallajoki, M., Henriksen, K., Parvinen, M., & Voipio-Pulkki, L. M. . (1997). Apoptosis in human acute myocardial infarction. . *Circulation,*, 95, 320-323.

Sbarouni, E., Georgiadou, P., Panagiotakos, D., Kyrzopoulos, S., Tsiapras, D., Voudris, V., & Kremastinos, D.T. (2008). Ischemia modified albumin in relation to pharmacologic stress testing in coronary artery disease. *Clin Chim Acta*, 396(1-2), 58-61.

Schnabel, R.B., Schulz, A., Messow, C.M., Lubos, E., Wild, P.S., Zeller, T.*, et al.* (2010). Multiple marker approach to risk stratification in patients with stable coronary artery disease. *Eur Heart J*, 31(24), 3024-3031.

Scholz, K.H., Maier, S.K.G., Maier, L.S., Lengenfelder, B., Jacobshagen, C., Jung, J.*, et al.* (2018). Impact of treatment delay on mortality in ST-segment elevation myocardial infarction (STEMI) patients presenting with and without haemodynamic instability: results from the German prospective, multicentre FITT-STEMI trial. *Eur Heart J*, 39(13), 1065-1074.

Schreck, D.M., & Fishberg, R.D. (2014). Diagnostic accuracy of a new cardiac electrical biomarker for detection of electrocardiogram changes suggestive of acute myocardial ischemic injury. *Ann Noninvasive Electrocardiol*, 19(2), 129-144.

Servonnet, A., Delacour, H., Dehan, C., & Gardet, V. (2006). [Heart fatty-acid binding protein (h-FABP): a new cardiac marker]. *Ann Biol Clin (Paris)*, 64(3), 209-217.

Shave, R., George, K., & Gaze, D. (2007). The influence of exercise upon cardiac biomarkers: a practical guide for clinicians and scientists. *Curr Med Chem*, 14(13), 1427-1436.

Sherman, C.T., Litvack, F., Grundfest, W., Lee, M., Hickey, A., Chaux, A.*, et al.* (1986). Coronary angioscopy in patients with unstable angina pectoris. *N Engl J Med*, 315(15), 913-919.

Shiran, A., Blondheim, D.S., Shimoni, S., Jabarren, M., Rosenmann, D., Sagie, A.*, et al.* (2017). Two-dimensional strain echocardiography for diagnosing chest pain in the emergency room: a multicentre prospective study by the Israeli echo research group. *Eur Heart J Cardiovasc Imaging*, 18(9), 1016-1024.

Sinha, M.K., Gaze, D.C., Tippins, J.R., Collinson, P.O., & Kaski, J.C. (2003). Ischemia modified albumin is a sensitive marker of myocardial ischemia after percutaneous coronary intervention. *Circulation*, 107(19), 2403-2405.

Sinha, M.K., Roy, D., Gaze, D.C., Collinson, P.O., & Kaski, J.C. (2004). Role of "Ischemia modified albumin", a new biochemical marker of myocardial ischaemia, in the early diagnosis of acute coronary syndromes. *Emerg Med J*, 21(1), 29-34.

Sinha, M.K., Vazquez, J.M., Calvino, R., Gaze, D.C., Collinson, P.O., & Kaski, J.C. (2006). Effects of balloon occlusion during percutaneous coronary intervention on circulating Ischemia Modified Albumin and transmyocardial lactate extraction. *Heart*, 92(12), 1852-1853.

Slawson, D. (2018). High-Sensitivity Troponin I of Less Than 5 ng per L Has Negative Predictive Value of 99.9% for Cardiac Death at One Year. *Am Fam Physician*, 97(6), 410.

Smyth, A., Dehghan, M., O'Donnell, M., Anderson, C., Teo, K., Gao, P.*, et al.* (2015). Healthy eating and reduced risk of cognitive decline: A cohort from 40 countries. *Neurology*, 84(22), 2258-2265.

Staub, D., Morgenthaler, N.G., Buser, C., Breidthardt, T., Potocki, M., Noveanu, M., Reichlin, T., Bergmann, A., & Mueller, C. (2009). Use of copeptin in the detection of myocardial ischemia. *Clin Chim Acta*, 399(1-2), 69-73.

Stergiopoulos, K., Boden, W.E., Hartigan, P., Mobius-Winkler, S., Hambrecht, R., Hueb, W., Hardison, R.M., Abbott, J.D., & Brown, D.L. (2014). Percutaneous coronary intervention outcomes in patients with stable obstructive coronary artery disease and myocardial ischemia: a collaborative meta-analysis of contemporary randomized clinical trials. *JAMA Intern Med*, 174(2), 232-240.

Sterne, J.A., & Egger, M. (2001). Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *J Clin Epidemiol*, 54(10), 1046-1055.

Sterne, J.A., Sutton, A.J., Ioannidis, J.P., Terrin, N., Jones, D.R., Lau, J.*, et al.* (2011). Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ*, 343, d4002.

Straface, A.L., Myers, J.H., Kirchick, H.J., & Blick, K.E. (2008). A rapid point-of-care cardiac marker testing strategy facilitates the rapid diagnosis and management of chest pain patients in the emergency department. *Am J Clin Pathol*, 129(5), 788-795.

Taghaddosi, M., Dianati, M., Fath Gharib Bidgoli, J., & Bahonaran, J. (2010). Delay and its related factors in seeking treatment in patients with acute myocardial infarction. *ARYA Atheroscler*, 6(1), 35-41.

Talwalkar, S.S., Bon Homme, M., Miller, J.J., & Elin, R.J. (2008). Ischemia modified albumin, a marker of acute ischemic events: a pilot study. *Ann Clin Lab Sci*, 38(2), 132-137.

Tamargo, J., Caballero, R., Gomez, R., Valenzuela, C., & Delpon, E. (2004). Pharmacology of cardiac potassium channels. *Cardiovasc Res*, 62(1), 9-33.

Tanaka, T., Hirota, Y., Sohmiya, K., Nishimura, S., & Kawamura, K. (1991). Serum and urinary human heart fatty acid-binding protein in acute myocardial infarction. *Clin Biochem*, 24(2), 195-201.

Task Force, M., Montalescot, G., Sechtem, U., Achenbach, S., Andreotti, F., Arden, C.*, et al.* (2013). 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. *Eur Heart J*, 34(38), 2949-3003.

Taylor, F.C., Huffman, M., & Ebrahim, S. (2013). Statin therapy for primary prevention of cardiovascular disease. *JAMA*, 310(22), 2451-2452.

Terada, T., Johnson, J.A., Norris, C., Padwal, R., Qiu, W., Sharma, A.M., Janzen, W., & Forhan, M. (2016). Severe Obesity Is Associated With Increased Risk of Early Complications and Extended Length of Stay Following Coronary Artery Bypass Grafting Surgery. *J Am Heart Assoc*, 5(6).

Terkelsen, C.J., Lassen, J.F., Norgaard, B.L., Gerdes, J.C., Jensen, T., Gotzsche, L.B., Nielsen, T.T., & Andersen, H.R. (2005). Mortality rates in patients with ST-elevation vs. non-ST-elevation acute myocardial infarction: observations from an unselected cohort. *Eur Heart J*, 26(1), 18-26.

Thokala, P., Goodacre, S.W., Collinson, P.O., Stevens, J.W., Mills, N.L., Newby, D.E., Morris, F., Kendall, J., & Stevenson, M.D. (2012). Cost-effectiveness of presentation versus delayed troponin testing for acute myocardial infarction. *Heart*, 98(20), 1498-1503.

Thygesen, K., Alpert, J.S., Jaffe, A.S., Simoons, M.L., Chaitman, B.R., White, H.D*., et al.* (2012). Third universal definition of myocardial infarction. *Eur Heart J*, 33(20), 2551-2567.

Thygesen, K., Alpert, J.S., White, H.D., Joint, E.S.C.A.A.H.A.W.H.F.T.F.f.t.R.o.M.I., Jaffe, A.S., Apple, F.S*., et al.* (2007). Universal definition of myocardial infarction. *Circulation*, 116(22), 2634-2653.

Thygesen, K., Mair, J., Giannitsis, E., Mueller, C., Lindahl, B., Blankenberg, S*., et al.* (2012). How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J*, 33(18), 2252-2257.

Thygesen, K., Mair, J., Katus, H., Plebani, M., Venge, P., Collinson, P*., et al.* (2010). Recommendations for the use of cardiac troponin measurement in acute cardiac care. *Eur Heart J*, 31(18), 2197-2204.

Ting, H.H., Lee, T. H., Soukup, J. R., Cook, E. F., Tosteson, A. N., Brand, D. A. et al. . (1991). Impact of physician experience on triage of emergency room patients with acute chest pain at three teaching hospitals. . *Am.J.Med.,*, 91, 401-408.

Trikalinos, T.A., Alsheikh-Ali, A.A., Tatsioni, A., Nallamothu, B.K., & Kent, D.M. (2009). Percutaneous coronary interventions for non-acute coronary artery disease: a quantitative 20-year synopsis and a network meta-analysis. *Lancet*, 373(9667), 911-918.

Trogdon, J.G., Finkelstein, E.A., Nwaise, I.A., Tangka, F.K., & Orenstein, D. (2007). The economic burden of chronic cardiovascular disease for major insurers. *Health Promot Pract*, 8(3), 234-242.

Tucker, J.F., Collins, R.A., Anderson, A.J., Hauser, J., Kalas, J., & Apple, F.S. (1997). Early diagnostic efficiency of cardiac troponin I and Troponin T for acute myocardial infarction. *Acad Emerg Med*, 4(1), 13-21.

Van Nieuwenhoven, F.A., Kleine, A.H., Wodzig, W.H., Hermens, W.T., Kragten, H.A., Maessen, J.G*., et al.* (1995). Discrimination between myocardial and skeletal muscle injury by assessment of the plasma ratio of myoglobin over fatty acid-binding protein. *Circulation*, 92(10), 2848-2854.

Vasan, R.S. (2006). Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation*, 113(19), 2335-2362.

Villarreal-Levy, G., Ma, T.S., Kerner, S.A., Roberts, R., & Perryman, M.B. (1987). Human creatine kinase: isolation and sequence analysis of cDNA clones for the B subunit, development of subunit specific probes and determination of gene copy number. *Biochem Biophys Res Commun*, 144(3), 1116-1127.

Virmani, R., Kolodgie, F.D., Burke, A.P., Finn, A.V., Gold, H.K., Tulenko, T.N., Wrenn, S.P., & Narula, J. (2005). Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol*, 25(10), 2054-2061.

Viswanathan, K., Kilcullen, N., Morrell, C., Thistlethwaite, S.J., Sivananthan, M.U., Hassan, T.B., Barth, J.H., & Hall, A.S. (2010). Heart-type fatty acid-binding protein predicts long-term mortality and re-infarction in consecutive patients with suspected acute coronary syndrome who are troponin-negative. *J Am Coll Cardiol*, 55(23), 2590-2598.

Vorlat, A., Van Hoof, V.O., Hammami, R., van Kerckhoven, S., Van der Heijden, C.M., Coenen, D.*, et al.* (2015). Usefulness of early rule-in and rule-out biomarker protocols to estimate ischemia-induced myocardial injury in early chest pain presenters. *Am J Cardiol*, 115(12), 1667-1671.

Wald, N.J.L., M. R. . (2003). A strategy to reduce cardiovascular disease by more than 80%. . *BMJ,.* 326., 1419.

Wang, Z.J., Zhou, Y.J., Galper, B.Z., Gao, F., Yeh, R.W., & Mauri, L. (2015). Association of body mass index with mortality and cardiovascular events for patients with coronary artery disease: a systematic review and meta-analysis. *Heart*, 101(20), 1631-1638.

Warren, T.Y., Barry, V., Hooker, S.P., Sui, X., Church, T.S., & Blair, S.N. (2010). Sedentary behaviors increase risk of cardiovascular disease mortality in men. *Med Sci Sports Exerc*, 42(5), 879-885.

Watt, I.S., Franks, A.J., & Sheldon, T.A. (1994). Health and health care of rural populations in the UK: is it better or worse? *J Epidemiol Community Health*, 48(1), 16-21.

Weinstein M, Z.R. (1973). Critical ratios and efficient allocation. . *J Public Econ*(2), 147–157.

Weinstein, M.C., & Stason, W.B. (1977). Foundations of cost-effectiveness analysis for health and medical practices. *N Engl J Med*, 296(13), 716-721.

Wenger, N.K., Hellerstein, H.K., Blackburn, H., & Castranova, S.J. (1982). Physician practice in the management of patients with uncomplicated myocardial infarction: changes in the past decade. *Circulation*, 65(3), 421-427.

White, H.D. (2011). Pathobiology of troponin elevations: do elevations occur with myocardial ischemia as well as necrosis? *J Am Coll Cardiol*, 57(24), 2406-2408.

Wildi, K., Zellweger, C., Twerenbold, R., Jaeger, C., Reichlin, T., Haaf, P.*, et al.* (2015). Incremental value of copeptin to highly sensitive cardiac Troponin I for rapid rule-out of myocardial infarction. *Int J Cardiol*, 190, 170-176.

Windecker, S., Stortecky, S., Stefanini, G.G., da Costa, B.R., Rutjes, A.W., Di Nisio, M.*, et al.* (2014). Revascularisation versus medical treatment in patients with stable coronary artery disease: network meta-analysis. *BMJ*, 348, g3859.

Wiviott, S.D., de Lemos, J.A., & Morrow, D.A. (2004). Pathophysiology, prognostic significance and clinical utility of B-type natriuretic peptide in acute coronary syndromes. *Clin Chim Acta*, 346(2), 119-128.

Wu, A.H., Apple, F.S., Gibler, W.B., Jesse, R.L., Warshaw, M.M., & Valdes, R., Jr. (1999). National Academy of Clinical Biochemistry Standards of Laboratory Practice: recommendations for the use of cardiac markers in coronary artery diseases. *Clin Chem*, 45(7), 1104-1121.

Wu, A.H., Feng, Y.J., Moore, R., Apple, F.S., McPherson, P.H., Buechler, K.F., & Bodor, G. (1998). Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. American Association for Clinical Chemistry Subcommittee on cTnI Standardization. *Clin Chem*, 44(6 Pt 1), 1198-1208.

Xing, Z., Pei, J., Tang, L., & Hu, X. (2018). Traditional cardiovascular risk factors and coronary collateral circulation: Protocol for a systematic review and meta-analysis of case-control studies. *Medicine (Baltimore)*, 97(17), e0417.

Yamamoto, T., Sato, N., Yasutake, M., Takagi, H., Morita, N., Akutsu, K.*, et al.* (2006). B-type natriuretic peptide as an integrated risk marker in non-ST elevation acute coronary syndromes. *Int J Cardiol*, 111(2), 224-230.

Yoshimoto, K., Tanaka, T., Somiya, K., Tsuji, R., Okamoto, F., Kawamura, K., Ohkaru, Y., Asayama, K., & Ishii, H. (1995). Human heart-type cytoplasmic fatty acid-binding protein as an indicator of acute myocardial infarction. *Heart Vessels*, 10(6), 304-309.

Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F. et al. . (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. . *Lancet,*, 364, 937-952.

Zaman, A.G., Helft, G., Worthley, S.G., & Badimon, J.J. (2000). The role of plaque rupture and thrombosis in coronary artery disease. *Atherosclerosis*, 149(2), 251-266.

Zapico-Muniz, E., Santalo-Bel, M., Merce-Muntanola, J., Montiel, J.A., Martinez-Rubio, A., & Ordonez-Llanos, J. (2004). Ischemia-modified albumin during skeletal muscle ischemia. *Clin Chem*, 50(6), 1063-1065.

Appendices

Appendix 1: Wandsworth Local Research Ethics Committee (the use of redundant samples for method evaluation and work up).

Wandsworth Local Research Ethics Committee
Room 1.027, 1st Floor Grosvenor Wing.
St George's Healthcare NHS Trust
Blackshaw Road, Tooting, London, SW17 0QT

Direct Line: 020 8725 3398
Direct Fax: 020 8725 1221

Our Ref: CH.MF.0128.11

18th November 2011

Dr David C Gaze
Cardiac Research Scientist
Chemical Pathology 2nd Floor Jenner Wing
St George's Hospital
Tooting, London, SW17 0QT

Dear Dr Gaze

Re: The use of redundant samples for method evaluation and work up

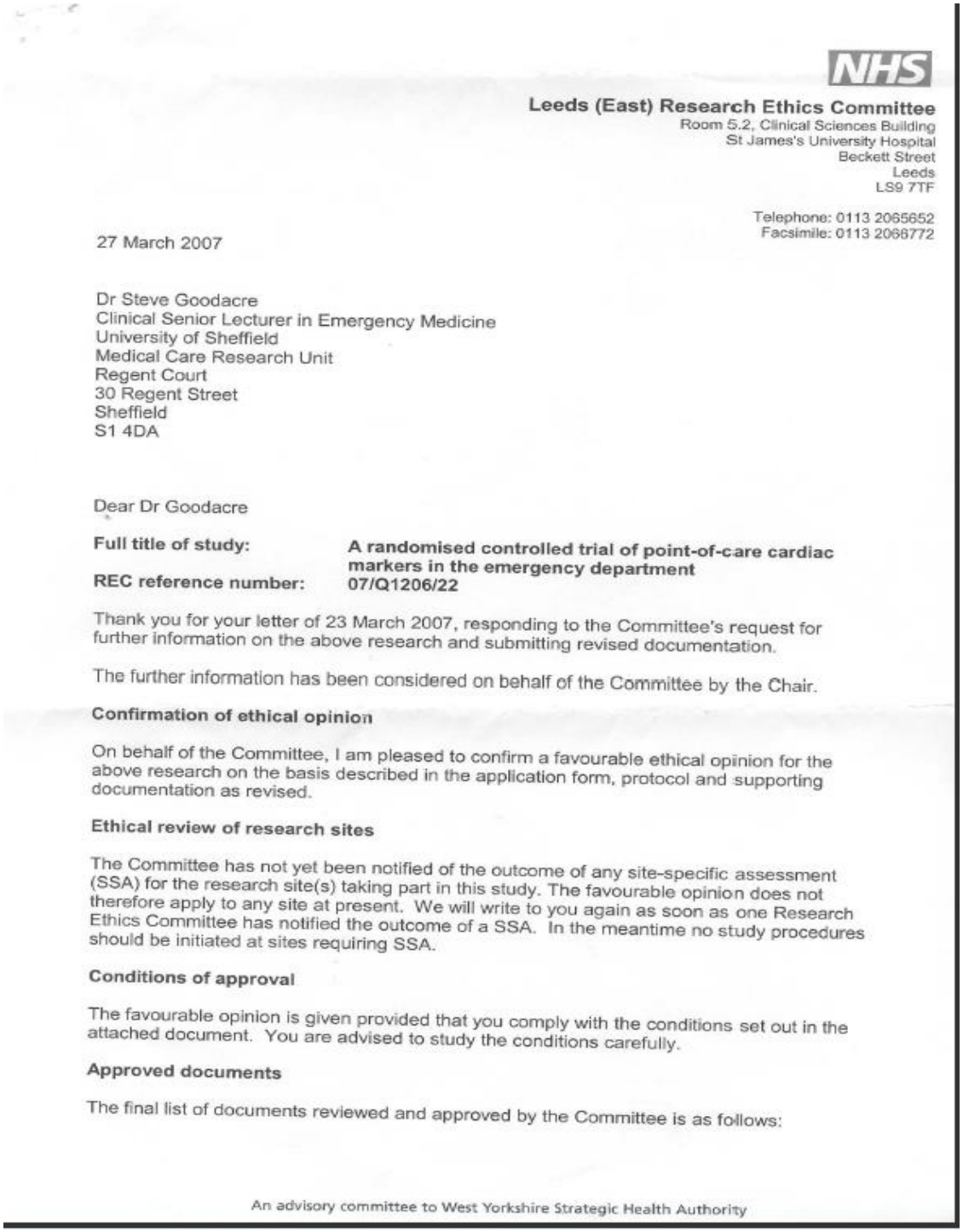
Thank you for your letter dated 7th November addressed to the Wandsworth LREC. On behalf of the committee, I am happy to clarify that there is no formal requirement for ethical approval for the use of redundant serum or plasma samples, surplus to clinical investigation in method evaluation work, if there is adherence to the current MRC guidelines. This applies to both redundant individual samples and those that are used to construct serum or plasma pools. Any studies requiring clinical and demographic data would obviously require formal ethical approval and patient consent as per the norm, a process that I am well aware that you are familiar with.

The Wandsworth LREC is more than happy that the current protocols in place within your department meet these guidelines and that anonomysed samples sent outside of the trust meet the Caldecott guidelines. If you are unfamiliar with these please seek advice from the Caldecott guardian of the Trust.

With every best wish
Yours sincerely,

Dr Christine Heron
Vice Chair/Clinical Secretary

Appendix 2: Confirmation of ethical opinion



NHS

Leeds (East) Research Ethics Committee
Room 5.2, Clinical Sciences Building
St James's University Hospital
Beckett Street
Leeds
LS9 7TF

Telephone: 0113 2065652
Facsimile: 0113 2068772

27 March 2007

Dr Steve Goodacre
Clinical Senior Lecturer in Emergency Medicine
University of Sheffield
Medical Care Research Unit
Regent Court
30 Regent Street
Sheffield
S1 4DA

Dear Dr Goodacre

Full title of study: **A randomised controlled trial of point-of-care cardiac markers in the emergency department**

REC reference number: **07/Q1206/22**

Thank you for your letter of 23 March 2007, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the research site(s) taking part in this study. The favourable opinion does not therefore apply to any site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at sites requiring SSA.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Appendix 3: Supplementary data

Two by two tables (sample 1)

Combined ischaemia modified-albumin and heart fatty-acid-binding protein (h-FABP) on admission (Sample 1).

N=169

		AMI (Sample 1)		
		+	-	
IMA+h-FABP	+	2	27	29
	-	5	135	140
		7	162	169

Sample 1	%	Confidence interval (95%)
Sensitivity	29	(0.08-0.64)
Specificity	83.3	(0.77-0.88)
Positive likelihood ratio	1.714	(0.51-5.81)
Negative likelihood ratio	0.857	(0.53-1.38)
Diagnostic odd ratio	2.0	-
Relative risk	1.93	(1.07-2.79)

Combined ischaemia modified albumin and copeptin (Cop) (Sample 1).

N=173

		AMI (Sample 1)		
		+	-	
IMA+Cop	+	5	89	94
	-	2	77	79
		7	166	173

Sample 1	%	Confidence interval (95%)
Sensitivity	71.4	(0.36-0.92)
Specificity	46.4	(0.39-0.54)
Positive likelihood ratio	1.332	(0.82-2.17)
Negative likelihood ratio	0.616	(0.19-2.0)
Diagnostic odd ratio	2.163	-
Relative risk	2.10	(1.25-2.95)

Combined ischaemia modified albumin and troponin T (hs-cTnT) (Sample 1).

N=173

		AMI (Sample 1)		
		+	-	
IMA + hs-TnT	+	3	20	23
	-	4	146	150
		7	166	173

Sample 1	%	Confidence interval (95%)
Sensitivity	42.9	(0.16-0.75)
Specificity	88	(0.82-0.92)
Positive likelihood ratio	3.557	(1.38-9.19)
Negative likelihood ratio	0.650	(0.34-1.24)
Diagnostic odd ratio	5.475	-
Relative risk	4.89	(4.09-5.69)

Combined ischaemia modified albumin and Troponin I (TnI) (Sample 1).

N=143

		AMI (Sample 1)		
		+	-	
IMA+TnI	+	0	11	11
	-	5	127	132

5	138	143

Sample 1	**%**	**Confidence interval (95%)**
Sensitivity	0.0	(0.0-0.43)
Specificity	92	(0.86-0.95)
Positive likelihood ratio	0.0	(1.38-9.19)
Negative likelihood ratio	1.087	(1.03-1.14)
Diagnostic odd ratio	5.475	-
Relative risk	0.0	-

Combined Ischaemia modified albumin, h-FAB and copeptin (Sample 1).

N=169

		AMI (Sample 1)		
		+	-	
IMA+fab+cop	+	6	91	97
	-	1	71	72
		7	162	169

Sample 1	**%**	**Confidence interval (95%)**
Sensitivity	86	(0.49-0.97)
Specificity	43.8	(0.36-0.52)
Positive likelihood ratio	1.526	(1.10-2.13)
Negative likelihood ratio	0.326	(0.05-2.02)
Diagnostic odd ratio	4.681	-
Relative risk	4.45	(3.36-5.54)

Two by two tables (sample 2)

Combined ischaemia modified albumin and cardiac troponin T (Sample 2).

N=173

		AMI (Sample 2)		
		+	-	
IMA + hs-TnT	+	7	40	47
	-	0	126	126
		7	166	173

Sample 2	%	Confidence interval (95%)
Sensitivity	1.0	(0.06-1.0)
Specificity	76	(0.69-0.82)
Positive likelihood ratio	4.15	(3.1-5.4)
Negative likelihood ratio	-	-
Diagnostic odd ratio	-	-
Relative risk	-	-

Combined ischaemia modified albumin and cardiac troponin I (Sample 2).

N=142

		AMI (Sample 2)		
		+	-	
IMA+TnI	+	2	11	13
	-	3	126	129
		5	137	142

Sample 2	%	Confidence interval (95%)
Sensitivity	40	(0.11-0.77)
Specificity	92	(0.86-0.95)
Positive likelihood ratio	4.98	(1.47-16.77)
Negative likelihood ratio	0.65	(0.31-1.34)
Diagnostic odd ratio	7.63	-
Relative risk	6.62	(5.65-7.58)

Combined ischaemia modified albumin and copeptin (Sample 2).

N=173

		AMI (Sample 2)		
		+	-	
IMA + Copeptin	+	5	74	79
	-	2	92	94
		7	166	173

Sample 2	%	Confidence interval (95%)
Sensitivity	71.4	(0.36-0.92)
Specificity	55.4	(0.48-0.63)
Positive likelihood ratio	1.60	(0.97-2.63)
Negative likelihood ratio	0.52	(0.15-1.67)
Diagnostic odd ratio	3.12	(2.12-3.83)
Relative risk	2.97	-

Combined ischaemia modified albumin and heart fatty-acid binding protein (h-FABP) (Sample 2).

N=165

		AMI (Sample 2)		
		+	-	
IMA + h-FABP	+	4	20	24
	-	3	138	141
		7	166	165

Sample 2	%	Confidence interval (95%)
Sensitivity	57.1	(0.25-0.84)
Specificity	87.3	(0.82-0.92)
Positive likelihood ratio	4.51	(2.11-9.66)
Negative likelihood ratio	0.49	(0.21-1.16)
Diagnostic odd ratio	9.2	-
Relative risk	7.83	(7.0-8.6)

Combined ischaemia modified albumin, TnT, TnI, h-FAB and copeptin (Sample 2).

N=574

		AMI (Sample 2)		
		+	-	
Tests	+	6	81	87
	-	1	85	86
		7	166	173

Sample 2	%	Confidence interval (95%)
Sensitivity	86	(0.49-0.97)
Specificity	51.2	(0.43-0.59)
Positive likelihood ratio	1.76	(1.25-2.47)
Negative likelihood ratio	0.28	(0.04-1.72)
Diagnostic odd ratio	6.30	-
Relative risk	5.93	(4.84-7.02)

Correlation of biomarkers concentrations on admission and at 90 min after admission

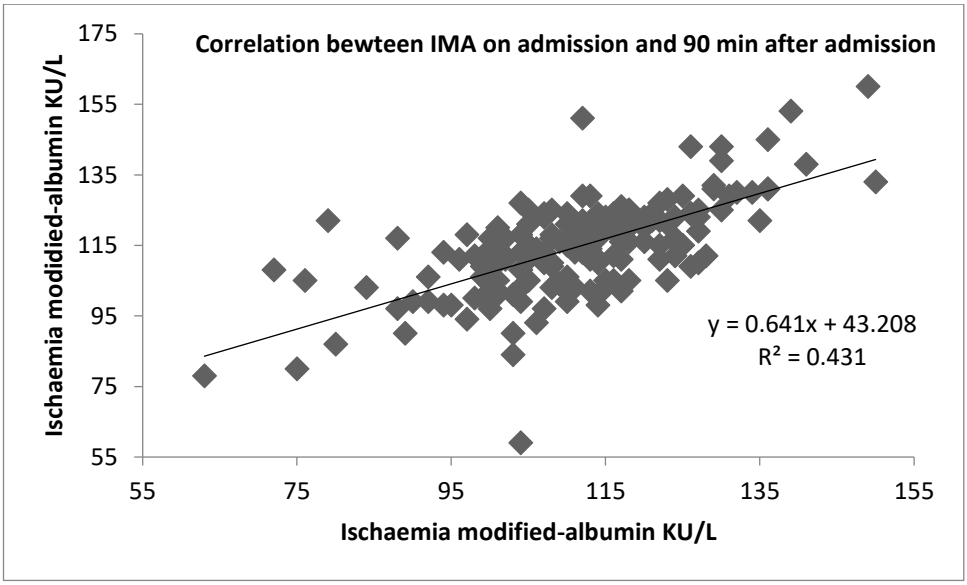


Figure 1: Correlation between ischaemia modified-albumin (n = 174) on admission and 90 min after admission

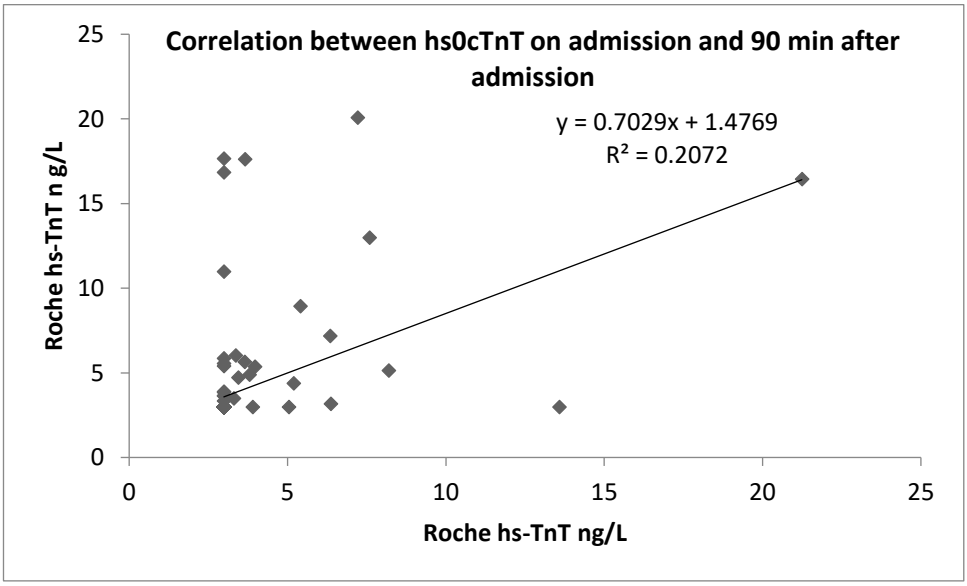


Figure 2: Correlation between Roche Hs-TnT (n = 174) on admission and 90 min after admission

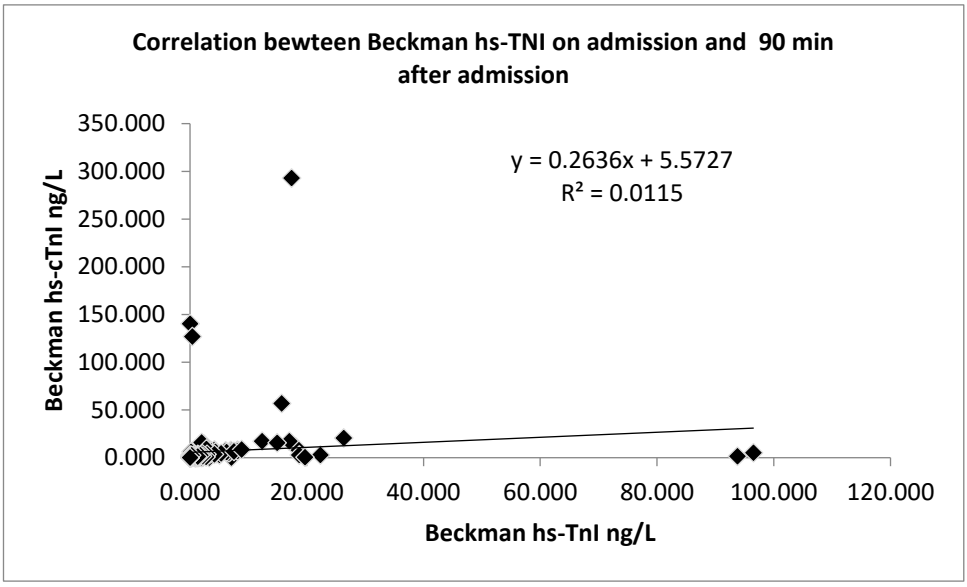


Figure 3: Correlation between Beckman hs-cTnI concentration (n = 174) on admission and 90 min after admission

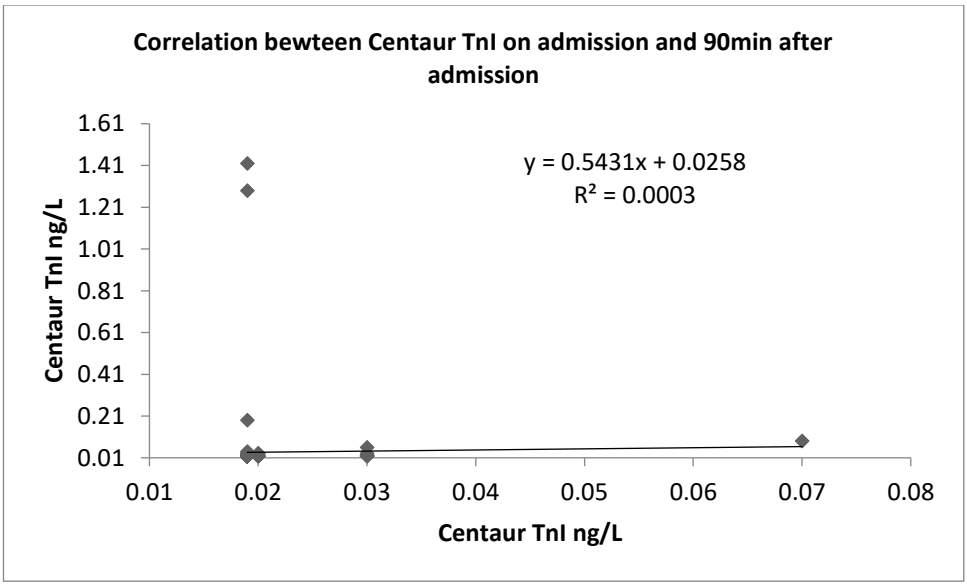


Figure 4: Correlation between Centaur TnI concentration (n = 174) on admission and 90 min after admission

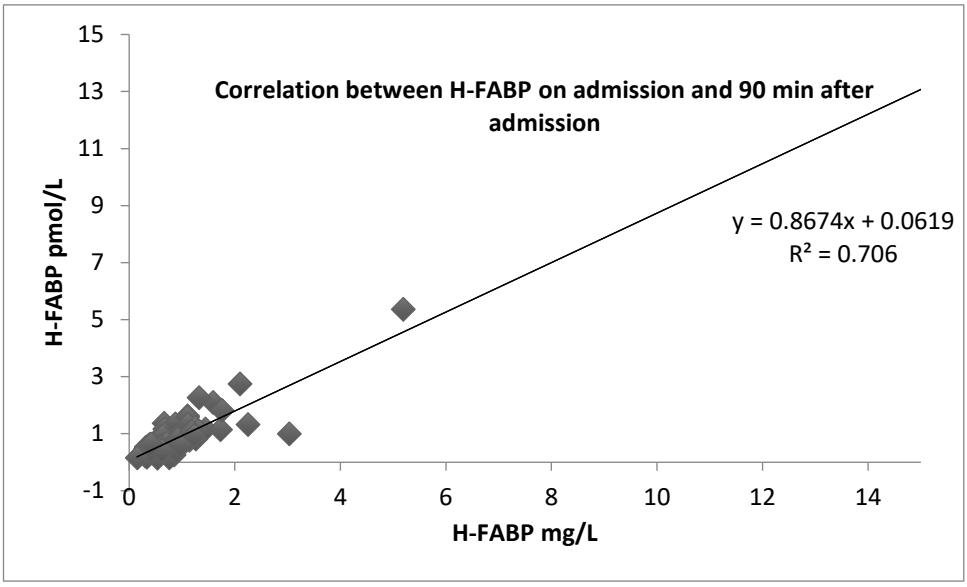


Figure 5: Correlation between H-FABP concentration (n = 174) on admission and 90 min after admission

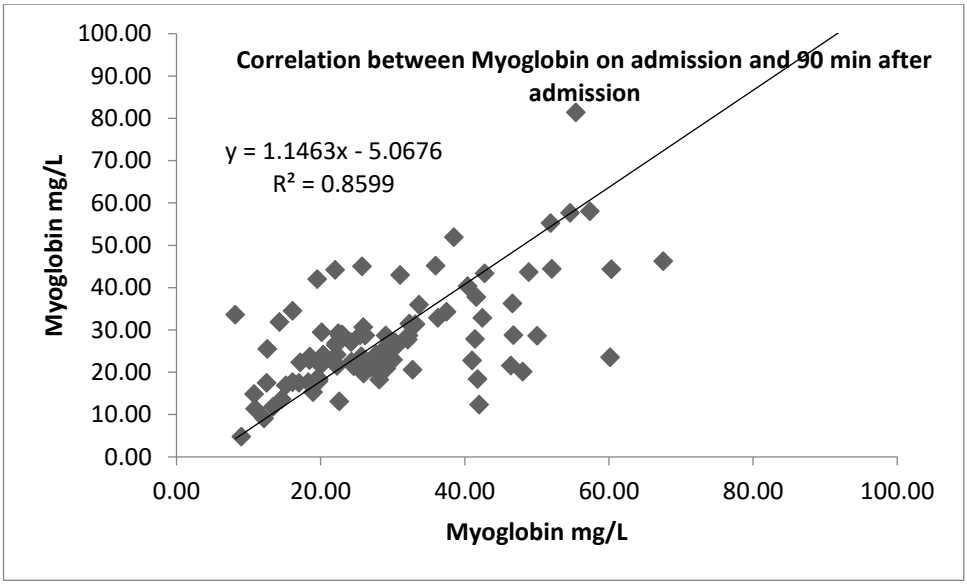


Figure 6: Correlation between myoglobin concentration (n = 174) on admission and 90 min after admission

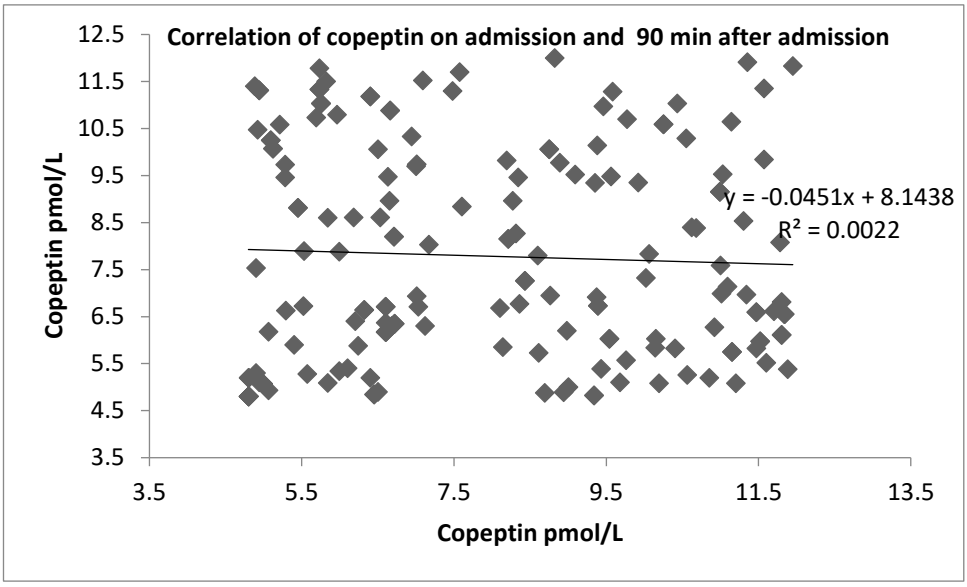


Figure 7: Correlation between copeptin concentration (n = 174) on admission and 90 min after admission

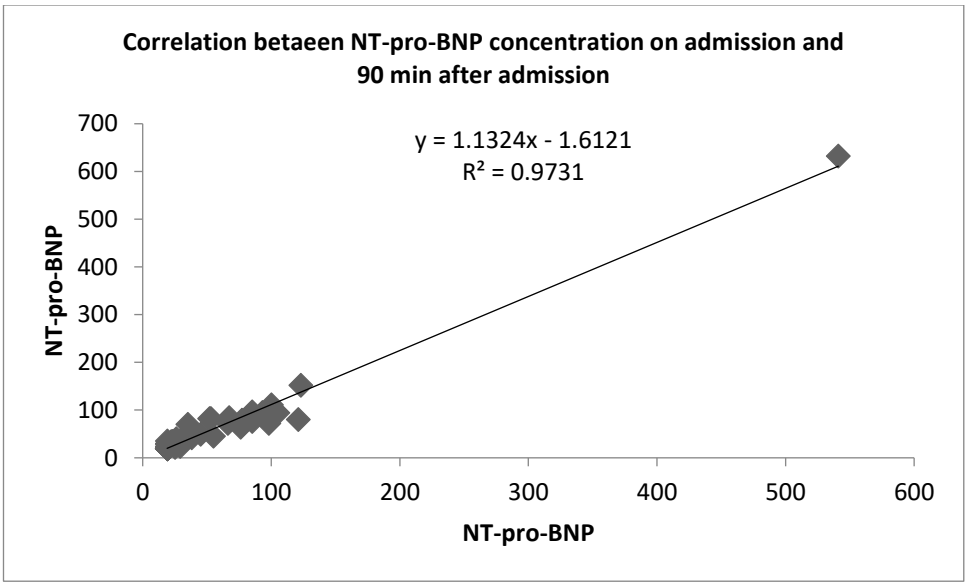


Figure 8: Correlation between NT-pro-BNP concentration (n = 174) on admission and 90 min after admission

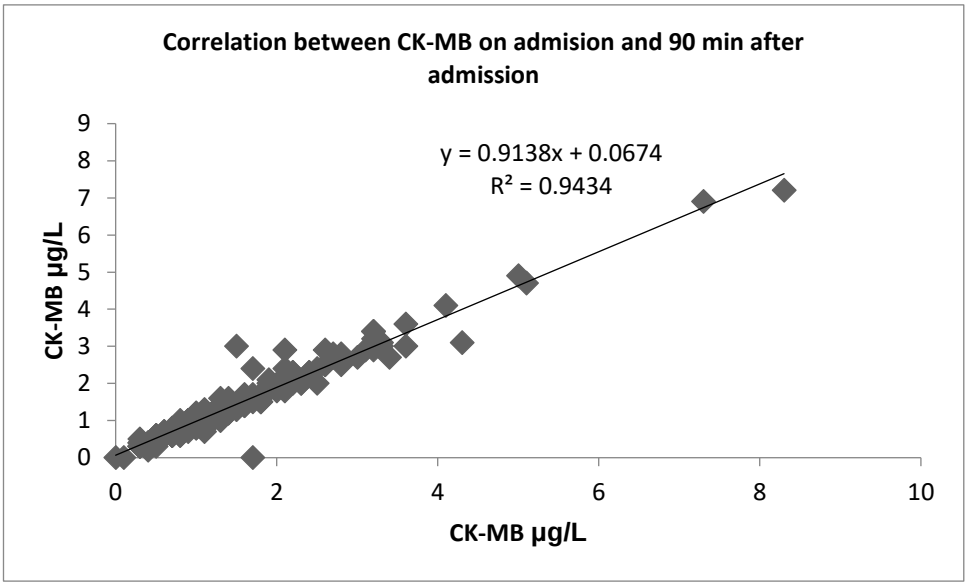


Figure 9: Correlation between CK-MB concentration (n = 174) on admission and 90 min after admission

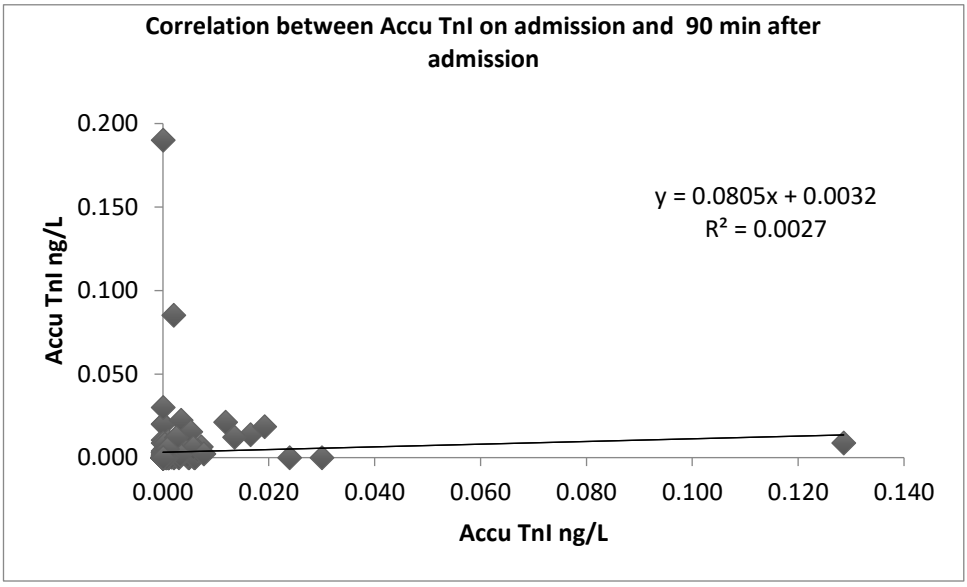


Figure 10: Correlation between Accu TnI concentration (n = 174) on admission and at 90 min after admission

Correlation between participant age and biomarker concentrations

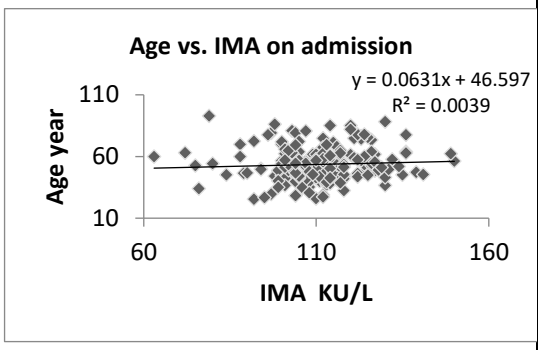


Figure 11: AGE vs. IMA on admission

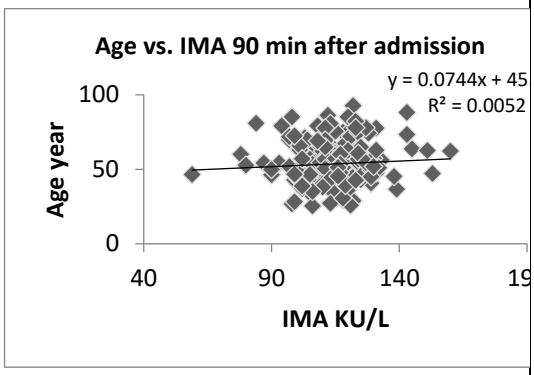


Figure12: AGE vs. IMA at 90 min after admission

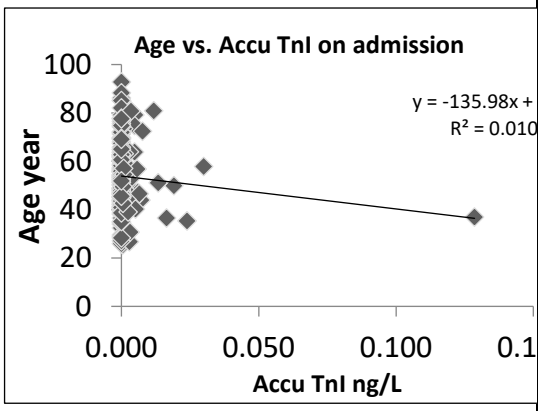


Figure 13: AGE vs. Accu TnI on admission

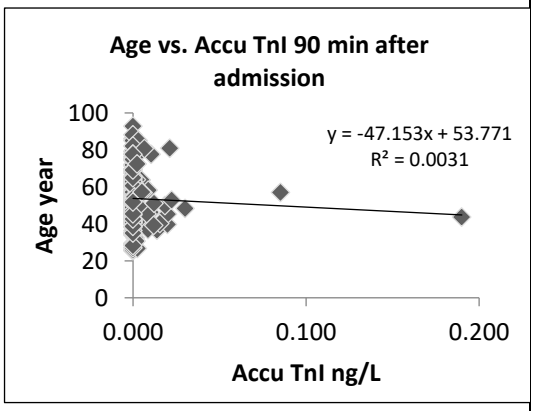


Figure 14: AGE vs. Accu at 90 min after admission

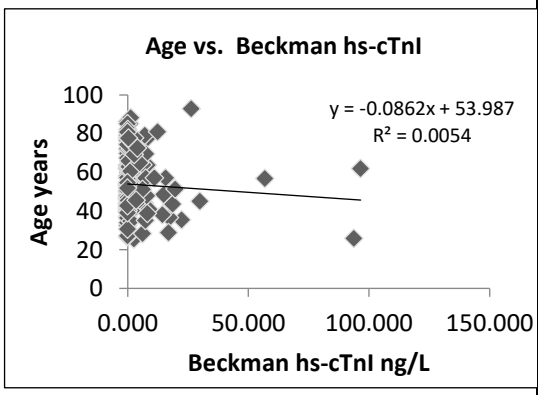


Figure 15: AGE vs. IMA on admission

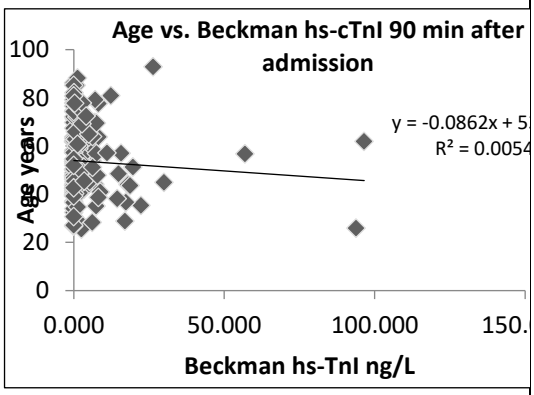


Figure 16: Age vs, hs-TnI at 90 min after admission

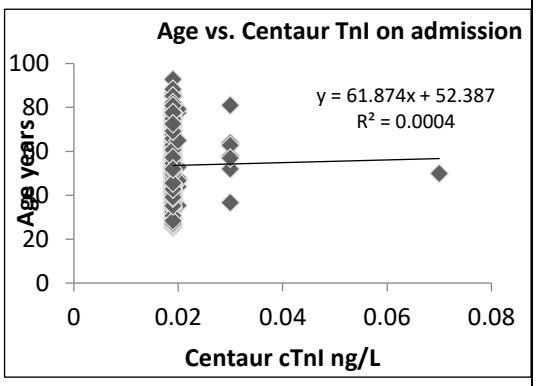


Figure 17: AGE vs. Centaur TnI on admission

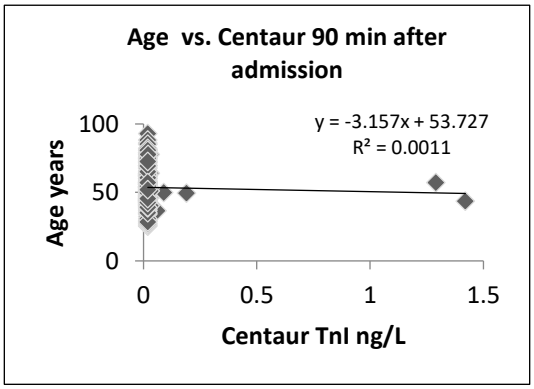


Figure 18: AGE vs. Centaur at 90 min after admission

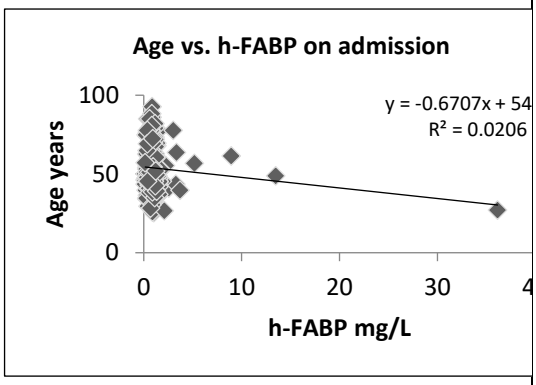


Figure 19: AGE vs. h-FABP on admission

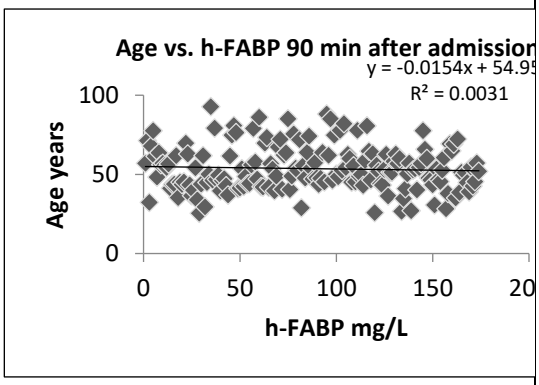


Figure 20: AGE vs. h-FABP at 90 min after admission

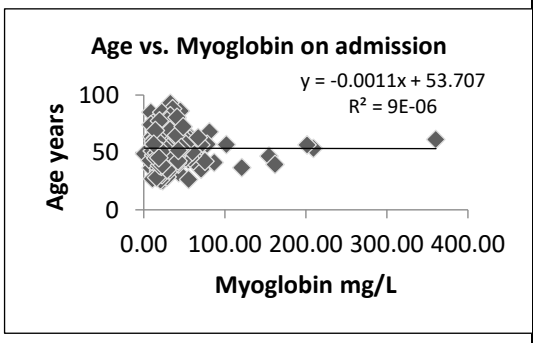


Figure 21: AGE vs. Myoglobin on admission

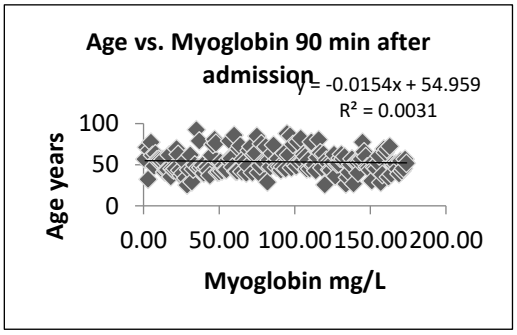


Figure 22: AGE vs. Myoglobin at 90 min after admission

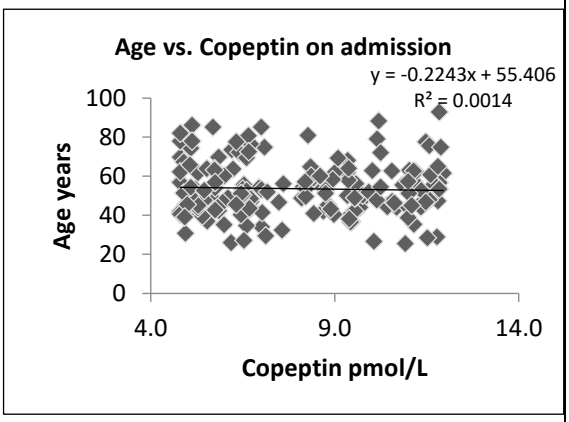


Figure 23: AGE vs. Copeptin on admission

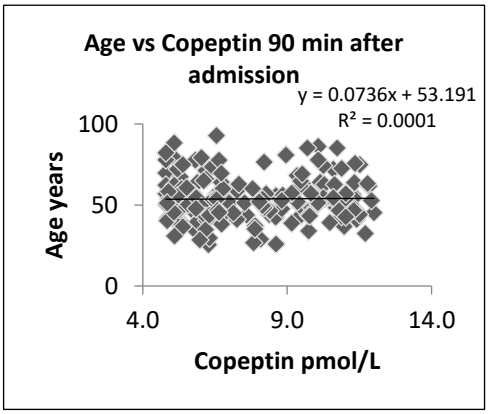


Figure 24: AGE vs. Copeptin at 90 min after admission

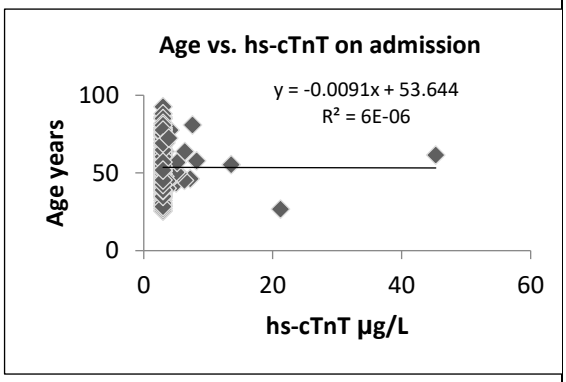


Figure 25: AGE vs. hs-cTnT on admission

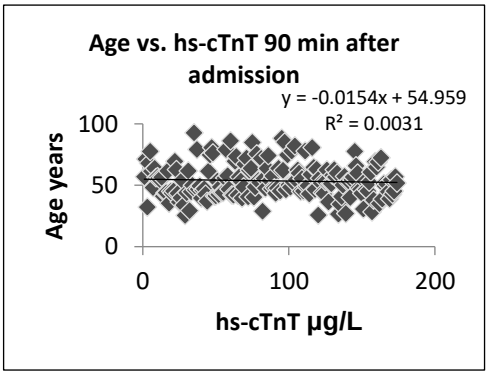


Figure 26: AGE vs. hs-cTnT at 90 min after admission

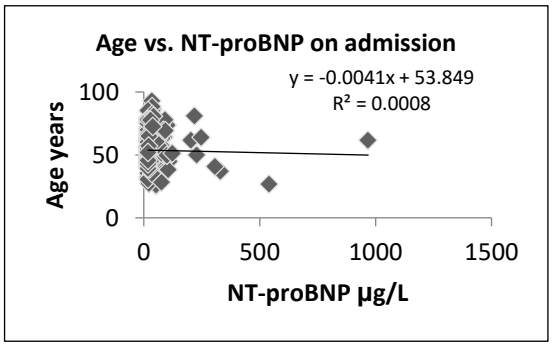


Figure 27: AGE vs. NT-pro-BNP on admission

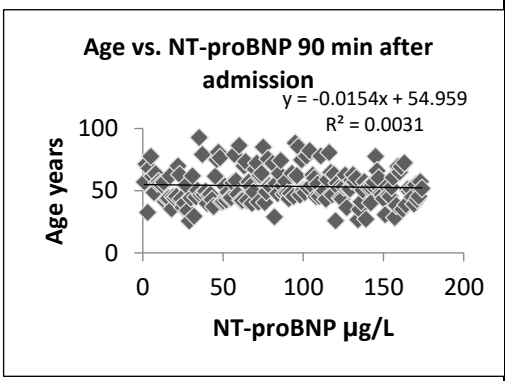


Figure 28: AGE vs. NT-pro-BNP at 90 min after admission

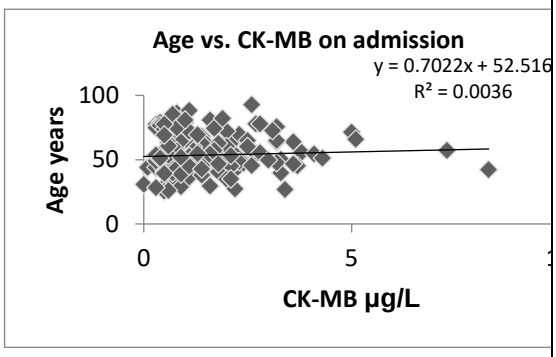


Figure 29: AGE vs. ICK-MB on admission

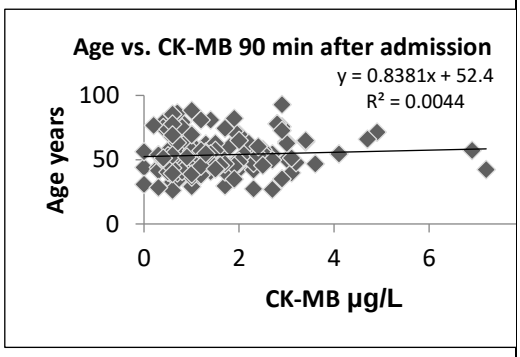


Figure 30: AGE vs. CK-MB at 90 min after admission

Control group (n = 66) age characteristics

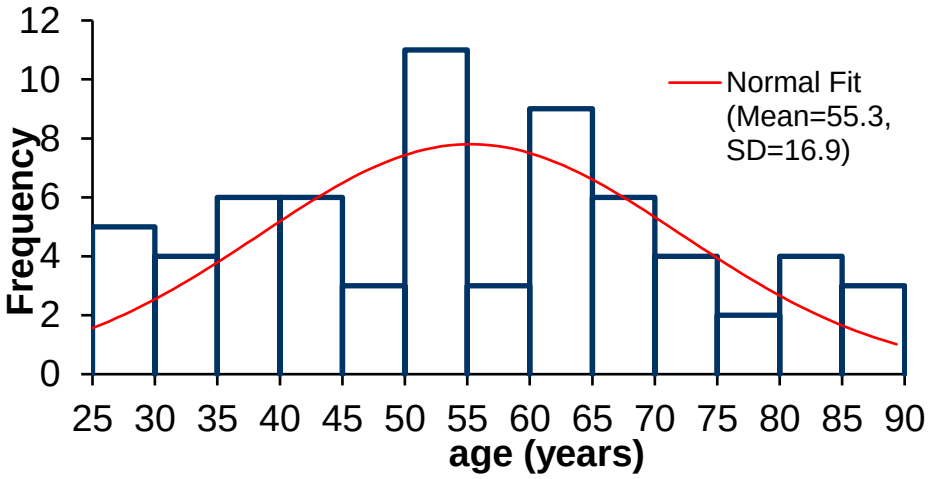


Figure 31: reference range participant age distribution

Table 1: Sample Demographics

Sample	IMA	DOB	age	Sex	Sample	IMA	DOB	age	Sex
948110R	124	21/09/1989	25	M	949294P	117	02/08/1960	54	M
948343D	123	01/08/1987	27	F	948609M	125	11/04/1960	55	F
947020C	115	26/05/1987	27	F	948504M	120	01/01/1960	55	F
948556C	127	14/05/1986	28	F	948133G	126	18/06/1959	55	F
947462R	125	16/04/1986	28	F	950078X	118	26/04/1958	56	F
950036N	110	12/11/1983	31	F	948687Z	118	03/01/1957	58	F
947977X	120	01/07/1983	31	F	949707S	115	24/03/1954	61	F
948010G	125	15/03/1981	34	F	949613L	122	26/01/1954	61	M
948040S	127	01/01/1981	34	F	950502Y	107	02/09/1953	61	M
947283N	119	22/09/1979	35	M	947360D	117	02/11/1952	62	M
947286H	123	29/11/1978	36	F	948336N	124	27/09/1952	62	F
947093Z	124	05/06/1976	38	F	947392B	126	18/05/1952	62	M
949300C	119	01/07/1975	39	M	949596R	118	02/12/1951	63	F
948515K	125	18/06/1975	39	M	949467W	110	09/08/1950	64	F
948937X	120	22/01/1975	40	M	947972M	118	14/06/1950	64	F
949718L	122	10/07/1974	40	M	950169B	119	21/03/1949	66	F
950496N	120	26/07/1972	42	M	947563M	121	13/03/1949	66	F
949000B	122	24/11/1970	44	M	948465K	123	11/10/1946	68	M
948062L	126	13/08/1970	44	F	948934V	120	22/02/1946	69	M
949854A	121	01/05/1970	44	F	947700W	120	29/12/1945	69	F
948056E	127	01/03/1970	45	M	949790Z	123	15/11/1945	69	F
947691G	125	27/10/1966	48	F	947553V	124	01/05/1944	70	F
948472P	124	28/12/1964	50	F	950076B	122	16/06/1942	72	F
947787E	126	15/12/1964	50	F	946959F	126	10/12/1941	73	M
947656M	124	10/07/1964	50	F	946765M	124	31/07/1940	74	M
947703C	124	02/05/1964	50	F	949852W	127	14/02/1939	76	F

Sample	IMA	DOB	age	Sex	Sample	IMA	DOB	age	Sex
948462F	126	14/04/1964	51	F	948853V	120	24/08/1938	76	M
949245X	122	06/01/1964	51	F	949519A	122	16/04/1934	81	F
948445B	123	01/01/1964	51	M	946812C	125	19/07/1933	81	F
949296F	122	13/01/1963	52	M	947081G	122	30/04/1932	82	F
947348F	119	10/10/1960	54	M	949437K	121	29/08/1930	84	F
948168S	126	13/08/1960	54	F	947240Y	121	26/08/1928	86	F
948444V	127	14/08/1925	89	F	948949X	123	02/02/1926	89	M

Over all study descriptive data

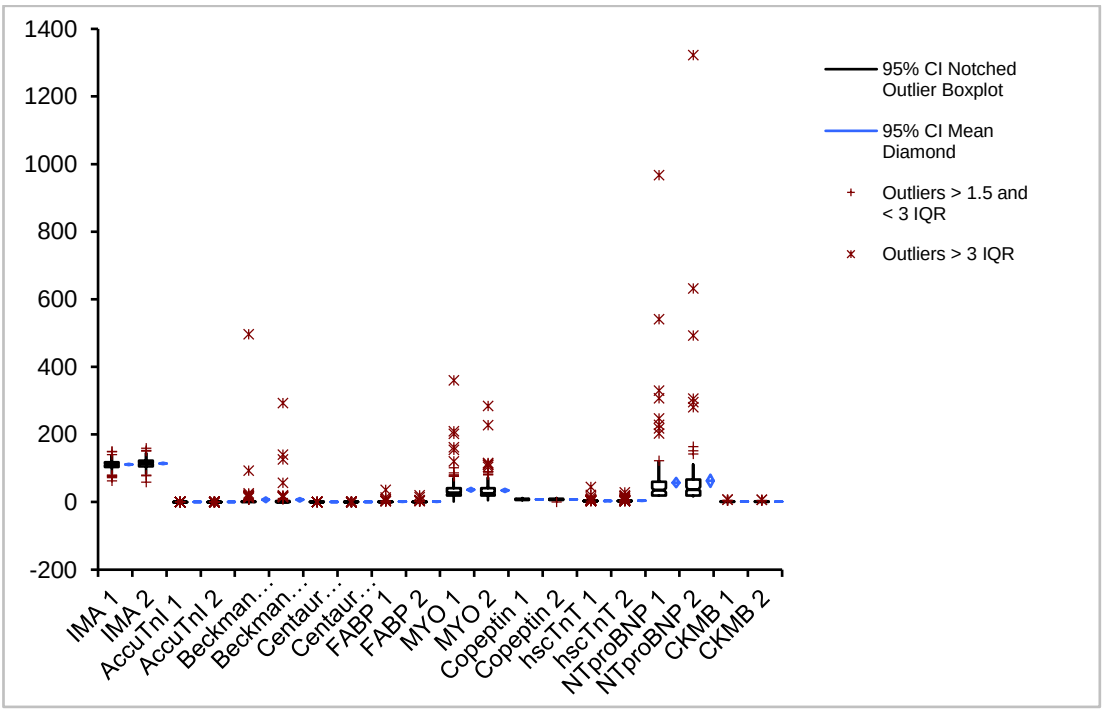


Figure 32: Overall data distribution of all biomarkers on admission and at 90 min after admission

Table 2: Study descriptive data

	n	Mean	95% CI		SE	SD
IMA 1	174	111.2	109.1	to 113.2	1.04	13.71
IMA 2	174	114.5	112.5	to 116.5	1.01	13.39
AccuTnI 1	174	0.002047	0.0	to 0.0	0.0007888	0.0104054
AccuTnI 2	174	0.003372	0.0	to 0.0	0.0012339	0.0162768
Beckman hsTnI 1	143	6.79989	-0.2	to 13.8	3.530085	42.213671
Beckman hs TnI 2	143	6.62771	1.8	to 11.5	2.445310	29.241659
Centaur TnI 1	174	0.0198	0.0	to 0.0	0.00033	0.00440
Centaur TnI 2	174	0.0365	0.0	to 0.1	0.01088	0.14349
FABP 1	170	1.293	0.8	to 1.7	0.2293	2.9896
FABP 2	166	1.195	0.9	to 1.5	0.1540	1.9845
MYO 1	173	36.4469	30.7	to 42.2	2.92366	38.45476
MYO 2	171	34.373	29.5	to 39.3	2.4714	32.3172
Copeptin 1	166	8.155	7.8	to 8.5	0.1768	2.2784
Copeptin 2	168	7.843	7.5	to 8.2	0.1762	2.2840
Hs-cTnT 1	173	3.608	3.1	to 4.2	0.2768	3.6413
Hs-cTnT 2	173	3.915	3.4	to 4.4	0.2536	3.3360
NT-pro-BNP 1	174	57.307	43.6	to 71.0	6.9617	91.8315
NT-pro-BNP 2	171	63.094	45.0	to 81.2	9.1773	120.0090
CKMB 1	174	1.56	1.4	to 1.7	0.089	1.176
CKMB 2	165	1.49	1.3	to 1.7	0.086	1.105

Table 3: Study descriptive data on admission (1) and at 90 min after admission (2)

	n	Min	1st Quartile	Median		95% CI	3rd Quartile	Max	IQR
IMA 1	174	63	103.9	112.0	109.0	to 114.0	118.2	150	14.3
IMA 2	174	59	105.9	115.0	113.0	to 117.0	123.0	160	17.1
AccuTnI 1	174	0.00000	0.000000	0.000000	0.0	to 0.0	0.000600	0.12860	0.000600
AccuTnI 2	174	0.00000	0.000000	0.000000	0.0	to 0.0	0.000808	0.19000	0.000808
Beckman hs-TnI 1	143	0.0000	0.00000	1.03100	0.3	to 1.5	2.79017	496.4950	2.79017
Beckman hs TnI 2	143	0.0000	0.00000	0.84900	0.4	to 1.5	3.29767	292.9420	3.29767
Centaur TnI 1	174	0.019	0.0190	0.0190	0.0	to 0.0	0.0190	0.070	0.0000
Centaur TnI 2	174	0.019	0.0190	0.0190	0.0	to 0.0	0.0190	1.420	0.0000
FABP 1	170	0.16	0.588	0.865	0.8	to 0.9	1.180	36.13	0.592
FABP 2	166	0.14	0.520	0.810	0.7	to 0.9	1.164	20.00	0.644
MYO 1	173	1.020	19.1000	26.7300	23.4	to 29.2	41.5800	360.590	22.4800
MYO 2	171	4.82	19.488	25.760	23.3	to 28.5	40.735	285.04	21.247
Copeptin 1	166	4.88	6.198	8.200	6.9	to 8.8	10.195	11.95	3.997
Copeptin 2	168	0.00	5.995	7.260	6.7	to 8.2	9.968	12.00	3.973
Hs-cTnT 1	173	2.99	2.990	2.990	3.0	to 3.0	2.990	45.28	0.000
h--scTnT 2	173	2.99	2.990	2.990	3.0	to 3.0	2.990	28.93	0.000
NT-pro--BNP 1	174	19.00	19.000	35.350	29.0	to 41.0	60.292	967.70	41.292
NT-pro-BNP 2	171	17.00	19.000	36.000	34.0	to 45.0	67.000	1323.00	48.000
CK-MB 1	174	0.0	0.80	1.30	1.1	to 1.4	2.01	8.3	1.21
CK-MB 2	165	0.0	0.70	1.20	1.0	to 1.4	2.00	7.2	1.30

Table 4: Study Assays characteristics on admission

Assay	Mean	95% CI		SE	SD
IMA	111.17	109.12	113.22	1.04	13.71
AccuTnI	0.00	0.00	0.00	0.00	0.01
Beckman hs-TnI	6.80	-0.18	13.78	3.53	42.21
Centaur TnI	0.02	0.02	0.02	0.00	0.00
H-FABP	1.29	0.84	1.75	0.23	2.99
MYO	36.45	30.68	42.22	2.92	38.45
Copeptin	8.15	7.81	8.50	0.18	2.28
Hs-cTnT	3.61	3.06	4.15	0.28	3.64
NT-pro-BNP	57.31	43.57	71.05	6.96	91.83
CK-MB	1.56	1.38	1.74	0.09	1.18

Table 5: Study assay characteristics at 90 min after admission

Assay	Mean	95% CI		SE	SD
IMA	114.47	112.47	116.47	1.01	13.39
AccuTnI	0.00	0.00	0.01	0.00	0.02
Beckman hs-TnI	6.63	1.79	11.46	2.45	29.24
Centaur TnI	0.04	0.02	0.06	0.01	0.14
H-FABP	1.20	0.89	1.50	0.15	1.98
MYO	34.37	29.49	39.25	2.47	32.32
Copeptin	7.84	7.50	8.19	0.18	2.28
Hs-cTnT	3.91	3.41	4.42	0.25	3.34
NT-pro-BNP	63.09	44.98	81.21	9.18	120.01
CK-MB	1.49	1.32	1.66	0.09	1.11

Table 4: and 5 shows IMA comparative predictive value on admission and at 90 min after admission in the diagnosis of acute myocardial infarction using hs-cTnT as a predicate

Table 6: IMA diagnostic efficiency characteristics on admission (1-way ANOVA) using the combined AMI and hs-cTnT.

IMA by Biochem Dx AMI hs-cTnT	n	Mean	SE	Pooled SE	SD
AMI	7	111.9	4.40	5.11	11.7
Non-AMI	166	111.4	1.05	1.05	13.6

Source of variation	Sum squares	DF	Mean square	F statistic	p
Biochem Dx AMI hs- cTnT	1.7	1	1.7	0.01	0.9235
Residual	31278.9	171	182.9		
Total	31280.6	172			

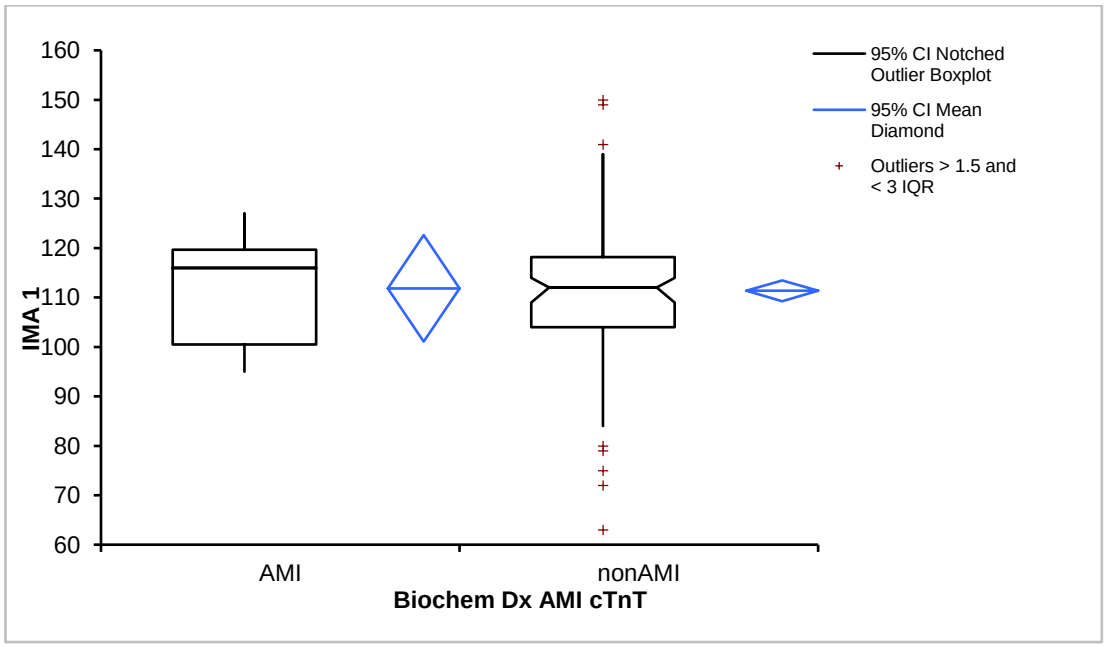


Figure 33: IMA assay diagnostic predictive value for AMI on admission. Hs-cTnT was used as a predicate test

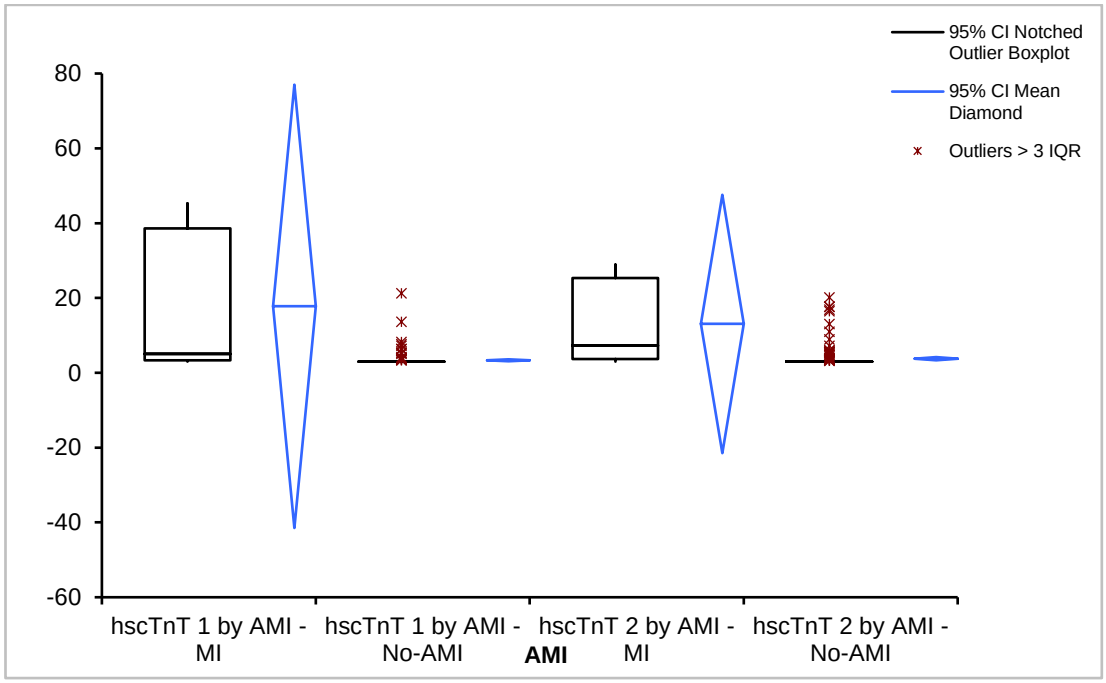


Figure 34: IMA assay diagnostic predictive value for AMI on admission and 90 min after admission. Hs-cTnT was used as a predicate test

Table 7: Summary of Hs-cTnT predictive value for the final diagnosis of AMI.

	n	Mean	95% CI		SE	SD
Hs-cTnT by AMI - MI	3	17.767	-41.478	to 77.011	13.7693	23.8491
Hs-cTnT by AMI - No-AMI	170	3.358	3.090	to 3.626	0.1359	1.7725
Hs-cTnT by AMI - MI	3	13.073	-21.457	to 47.604	8.0254	13.9003
Hs-cTnT by AMI - No-AMI	170	3.753	3.338	to 4.169	0.2104	2.7431

	n	Min	1st Quartile	Median	95% CI		3rd Quartile	Max	IQR
Hs-cTnT by AMI - MI	3	2.99	3.330	5.030	-	to -	38.572	45.28	35.242
Hs-cTnT by AMI - No-AMI	170	2.99	2.990	2.990	2.990	to 2.990	2.990	21.24	0.000
Hs-cTnT by AMI - MI	3	2.99	3.708	7.300	-	to -	25.325	28.93	21.617
Hs-cTnT by AMI - No-AMI	170	2.99	2.990	2.990	2.990	to 2.990	2.990	20.08	0.000

Table 8: Area under the ROC curve of biomarkers of necrosis

Test	Area	95% CI		SE
AccuTnI	0.88	0.71	to 1.00	0.089
Beckman hs-TnI	0.78	0.54	to 1.00	0.120
Centaur TnI	0.84	0.65	to 1.00	0.096
CK-MB	0.62	0.32	to 0.92	0.153
Myoglobin	0.72	0.57	to 0.87	0.076
Hs-cTnT	1.00	-	to -	0.000

Table 9: Comparison of the magnitude of putative biomarkers on admission.

Contrast	Difference	95% CI		SE	p
IMA v H-FABP 1	-0.14	-0.53	to 0.25	0.201	0.4882
IMA v MYO 1	-0.24	-0.51	to 0.03	0.137	0.0771
IMA v Copeptin 1	-0.08	-0.35	to 0.19	0.137	0.5430
IMA v NT-pro-BNP 1	-0.06	-0.42	to 0.29	0.181	0.7303
IMA v CK-MB 1	-0.03	-0.37	to 0.31	0.175	0.8747
H-FABP v MYO 1	-0.10	-0.25	to 0.04	0.075	0.1654
H-FABP v Copeptin 1	0.06	-0.22	to 0.33	0.142	0.6930
H-FABP v NT-pro-BNP 1	0.08	-0.40	to 0.55	0.243	0.7511
H-FABP v CK-MB 1	0.11	-0.06	to 0.29	0.088	0.2062
MYO v Copeptin 1	0.16	-0.05	to 0.37	0.105	0.1290
MYO v NT-pro-BNP 1	0.18	-0.22	to 0.58	0.204	0.3762
MYO v CK-MB 1	0.22	0.05	to 0.39	0.087	0.0128
Copeptin v NT-pro-BNP 1	0.02	-0.42	to 0.46	0.224	0.9255
Copeptin v CK-MB 1	0.06	-0.15	to 0.26	0.103	0.5895
NT-pro-BNP v CK-MB 1	0.03	-0.47	to 0.54	0.260	0.8933

Table 10: Hs-cTnT predictive value of the final diagnosis of AMI on admission and 90 min after admission. Hs-cTnT was used as a predicate thus was statistically significant (p = < 0.0001).

Hs-cTnT by Biochem Dx AMI hs-cTnT	n	Mean	SE	Pooled SE	SD
AMI	7	12.629	5.9634	1.1871	15.778
Non-AMI	166	3.227	0.0840	0.2438	1.082

Source of variation	Sum squares	DF	Mean square	F statistic	p
Biochem Dx AMI hs-cTnT	593.632	1	593.632	60.18	<0.0001
Residual	1686.885	171	9.865		
Total	2280.518	172			

Hs-cTnT 2 by Biochem Dx AMI hs-cTnT	n	Mean	SE	Pooled SE	SD
AMI	7	17.843	2.4008	0.6457	6.352
Non-AMI	166	3.328	0.0968	0.1326	1.248

Source of variation	Sum squares	DF	Mean square	F statistic	p
Biochem Dx AMI cTnT	1415.163	1	1415.163	484.96	<0.0001
Residual	498.992	171	2.918		
Total	1914.155	172			

Table 11: Over all data of biomarkers on admission (1) and at 90 min after admission (2)

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
2048	117	124	0.002	0.0852	15.69	56.856	0.019	1.29	0.9	1.85
2059	115	103	0	0	0.2	0.308	0.019	0.019	0.87	1.05
2105	118	120	0	0.0013	0	0.485	0.019	0.019	1.03	0.66
2108	116	112	0	0	0.536	0.849	0.019	0.019	1.35	1.55
2118	100	108	0	0.0001	0.159	0	0.019	0.019	0.91	1.03
2131	104	113	0	0	0	0	0.019	0.019	0.86	0.37
2140	150	133	0	0	1.961	2.427	0.019	0.019	1.39	1.64
2150	117	126	0	0	0	0.028	0.019	0.019	1.76	1.6
2154	109	104	0	0	3.29	0.172	0.019	0.019	0.71	0.56
2157	106	93	0	0	1.582	1.521	0.019	0.019	0.71	0.89

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
2158	100	107	0	0	0.841	1.261	0.019	0.019	0.88	1.29
2161	105	115	0	0	0.167	6.141	0.019	0.019	2.56	3.6
2172	112	114	0	0	6.946	7.543	0.019	0.019	0.84	0.87
2173	127	119	0	0	496.495	4.964	0.019	0.019	0.79	1.06
2176	108	117	0.0239	0	22.329	2.711	0.02	0.019	1.4	1.23
2181	84	103	0	0.0017	3.541	7.382	0.019	0.019	1.55	0.9
2271	105	105	0	0	4.54	4.078	0.019	0.019	0.92	1.27
2286	104	103	0.0002	0	4.173	8.154	0.019	0.019	1.91	2.01
2293	88	97	0	0	7.011	3.291	0.019	0.019	1.36	1.22
2298	72	108	0	0	5.051	2.613	0.019	0.019	1.2	1.02
2303	108	125	0.0059	0	4.339	6.198	0.019	0.019	1.51	2.29
2326	130	139	0.0165	0.0138	17.348	292.942	0.03	0.06	1.08	1.13

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
6002	111	103	0	0	0	0	0.019	0.019	0.81	0.47
6007	80	87	0	0	0	0	0.019	0.019	0.32	0.28
6020	92	106	0.0005	0	2.544	3.489	0.019	0.019	1.03	0.91
6023	118	121	0.0072	0.0065	18.068	11.067	0.02	0.02		1.02
6038	109	104	0	0	0	0	0.019	0.019	0.41	0.49
6041	97	118	0	0	0.89	0	0.019	0.019	0.43	0.65
6044	114	101	0.0006	0	2.82	1.695	0.019	0.019	0.59	0.84
6049	94	113	0	0	0	0	0.019	0.019		0.23
6060	79	122	0	0	26.299	20.614	0.019	0.019	0.88	0.68
6061	89	90	0	0	0	0	0.019	0.019	0.28	0.24
6063	97	94	0.0049	0	7.042	0	0.019	0.019	1.15	1
6067	124	116	0.0001	0	0.212	0	0.019	0.019	0.85	0.79

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
6071	94	98	0.0028	0.0019	2.206	0.554	0.019	0.19	1.18	0.71
6076	108	103	0.0006	0.0008	0	0	0.019	0.019	0.25	0.41
6077	122	111	0	0	0	0.186	0.019	0.019		
6080	99	109	0	0	0	0.386	0.019	0.019	0.22	0.25
6086	110	99	0.0027	0.0041	0.863	0	0.019	0.019	0.6	0.62
6087	113	102	0.1286	0.0087	0	0	0.019	0.019	1.56	1.71
6092	110	102	0.0004	0.0051	0	2.807	0.019	0.019	0.48	0.67
6100	112	118	0.0048	0	0.112	1.598	0.019	0.019	0.94	0.96
6172	123	121	0	0	0	0.186	0.019	0.019	0.37	0.44
6174	116	121	0	0	0.129	0.329	0.019	0.019	1.12	2.14
6177	107	110	0	0	0	0	0.019	0.019	0.65	0.98
6179	124	117	0	0	0	0	0.019	0.019	0.71	0.55

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
6189	105	115	0	0	0	0.193	0.019	0.019	0.94	0.47
6200	113	129	0	0	1.479	0	0.019	0.019	0.48	
6204	98	100	0	0	0	0	0.019	0.019	0.43	0.47
6211	107	123	0	0.0007	1.468	1.159	0.019	0.019	0.71	0.77
6222	104	108	0	0	0	0	0.02	0.019	0.56	20
6239	117	111	0	0	0	0	0.019	0.02	1.54	1.26
6247	98	112	0	0	0	0	0.019	0.019	0.85	1.24
6249	101	105	0	0	0	0	0.019	0.019	0.74	0.9
6252	118	105	0.001	0	0.3969	0.489	0.019	0.019	1.25	0.65
6288	100	104	0	0	0	0	0.019	0.019	1.03	0.84
6295	126	143	0.0009	0.001	1.2394	1.541	0.019	0.019	1.15	1.08
6296	114	117	0	0	3.2965	3.115	0.019	0.019	1.55	1.3

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
6299	123	105	0	0	1.548	1.594	0.019	0.019	1.93	1.21
6306	110	106	0.001	0	0.694	0.811	0.019	0.019	1.6	14.2
6310	104	118	0	0.02	0	0.329	0.019	0.02	3.7	2.64
6315	99	100	0	0	0	0	0.019	0.019	13.48	0.86
6316	112	119	0	0	0	0	0.019	0.019	1.24	0.98
6318	105	121	0	0	0	0	0.019	0.019	0.7	
6329	112	129	0	0	0	0	0.019	0.019	1.17	1.49
6330	101	110	0	0	1.1894	1.293	0.019	0.019	0.93	0.52
5012	114	98	0	0	0.859	0	0.019	0.019	0.41	1
5020	113	114	0	0	1.885	2.264	0.019	0.019	0.52	0.67
5023	102	112	0	0	1.862	1.245	0.019	0.019	1.2	2.02
5031	125	115	0	0	1.176	0.553	0.019	0.019	0.51	

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
5037	100	114	0.0026	0.00093	6.082	7.052	0.019	0.019	0.45	0.52
5075	108	109	0	0	2.625	3.48	0.019	0.019	0.56	0.57
5117	100	97	0	0	1.031	0.16	0.019	0.019	1.2	0.99
3005	112	122	0	0			0.019	0.019	1.12	0.34
3006	127	110	0	0			0.019	0.019	0.38	0.16
3068	112	120	0	0			0.019	0.019	0.31	0.21
3157	123	128	0	0			0.019	0.019	0.3	0.27
3159	115	123	0	0			0.019	0.019	0.54	0.43
3167	139	153	0.006	0			0.02	0.019	1.28	1.08
3168	132	130	0	0			0.019	0.019	0.97	0.66
3169	117	116	0	0			0.02	0.019	0.81	1.32
3174	122	122	0.001	0			0.019	0.019	0.16	0.44

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
3237	129	131	0.002	0			0.019	0.019	0.8	0.47
3241	105	112	0	0			0.019	0.019	0.43	0.41
3298	130	143	0	0			0.019	0.019	0.82	1.52
3300	149	160	0	0			0.019	0.019	0.51	0.72
3301	120	120	0	0.003			0.019	0.019	0.59	0.94
3312	121	124	0	0			0.019	0.019	0.23	
3317	110	116	0	0			0.019	0.019	0.48	0.33
3343	127	125	0	0.03			0.019	0.04	0.85	0.25
3346	122	127	0	0.003			0.019	0.019	0.78	0.62
3358	126	124	0	0			0.019	0.02	0.66	1.36
3360	120	123	0	0			0.019	0.019	1.16	1.14
3370	126	109	0	0			0.019	0.02	0.67	1.16

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
3404	112	151	0	0			0.019	0.019	0.31	0.47
3406	124	112	0	0			0.019	0.019	0.68	0.72
3410	135	122	0	0			0.019	0.019	0.42	0.4
3417	124	123	0	0			0.019	0.019	3.03	0.99
3418	128	112	0	0			0.019	0.019	1.44	1.16
4062	105	115	0	0	1.965	1.064	0.019	0.019	0.57	0.87
4064	121	122	0	0	0	0	0.019	0.019	0.68	0.77
4066	107	113	0.0035	0.0065	6.266	5.899	0.019	0.019	0.84	0.8
4075	117	125	0	0	0	0	0.019	0.019	0.64	0.61
4077	115	105	0.00656	0.0042	3.602	4.934	0.019	0.019	1.1	1.62
4081	103	101	0.0011	0	1.94	0.217	0.019	0.019	0.71	0.7
4082	110	121	0	0	0	0	0.019	0.019	0.89	0.7

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
4090	112	121	0	0	0	0.33	0.019	0.019	0.87	1.34
4092	107	97	0	0	18.693	2.686	0.03	0.019	1.14	0.77
4096	105	125	0.0021	0	1.592	0.759	0.019	0.019	1.02	0.69
4109	130	125	0.0002	0	0.974	0.251	0.019	0.019	1.06	0.91
4111	116	124	0	0	5.327	4.596	0.019	0.019	0.32	0.53
4112	117	120	0	0	1.222	0	0.03	0.019	0.66	0.98
4117	101	120	0	0	0	0	0.019	0.019	0.36	0.44
4119	117	118	0	0	0	0	0.019	0.019	0.6	0.56
4123	101	113	0	0	0.398	126.729	0.019	0.019	0.63	0.49
4125	112	113	0	0.0088	2.356	14.342	0.019	0.019	0.71	0.79
4130	136	131	0	0	0	0	0.019	0.019	0.88	0.83
4135	105	111	0.0016	0.0004	14.879	15.671	0.03	0.02	0.67	0.41

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
1020	63	78	0	0	0	1.234	0.019	0.019	1.26	0.81
1028	106	114	0	0	0.097	0.805	0.019	0.019	0.3	0.22
1030	114	119	0	0	2.443	3.57	0.019	0.019	0.56	0.49
1037	122	115	0.003	0	1.509	1.319	0.019	0.019	0.98	0.76
1040	112	113	0	0	0.206	0	0.019	0.019	36.13	
1041	118	125	0.0034	0.0224	1.945	15.963	0.019	0.03	0.81	0.3
1044	118	124	0	0	0.133	0	0.019	0.019	1.1	1.58
1055	107	124	0.0001	0	1.298	1.739	0.019	0.019	0.76	
1060	96	111	0	0	1.265	1.528	0.019	0.02	0.38	0.6
1245	101	101	0	0	1.703	2.232	0.019	0.019	0.7	0.57
1247	88	117	0	0	0	1.315	0.019	0.019	0.53	0.14
1252	104	59	0	0	0.866	0.464	0.019	0.019	0.72	0.78

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
1256	111	113	0	0	0	0	0.019	0.019	0.63	0.91
1257	108	118	0.0032	0.0017	93.754	1.498	0.019	0.019	0.87	0.51
1261	102	117	0	0	2.711	0.835	0.019	0.019	1.73	1.14
1262	90	99	0	0	1.303	3.299	0.02	0.02	1.03	0.71
1264	118	125	0.0003	0.008	2.051	3.951	0.019	0.019	1.11	1.32
1268	75	80	0	0	0.102	2.228	0.02	0.019	1.76	1.81
1271	114	124	0	0	1.303	1.981	0.019	0.019	1.25	1.22
1274	104	99	0	0	2.129	1.483	0.019	0.019	0.7	0.67
1281	114	110	0	0	0	0	0.019	0.019	0.6	0.51
1287	102	111	0	0	19.686	0.364	0.02	0.019	0.33	0.17
1289	104	108	0	0	0	0	0.019	0.019	0.36	0.38
1290	99	106	0	0	0.517	2.024	0.019	0.019	1.14	0.82

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
1295	117	102	0	0	1.765	2.289	0.019	0.019	0.89	0.41
1303	118	116	0	0.0006	0	0.385	0.019	0.019	0.39	0.43
1304	131	129	0.0006	0.0032	1.282	1.041	0.019	0.019	1.31	1.11
1308	129	132	0.0135	0.0122	8.808	8.35	0.019	0.03	0.62	0.44
1312	113	119	0.0025	0.012	0	0	0.019	0.019	1.32	2.26
1315	104	127	0.001	0.0009	0	0	0.019	0.019	0.97	0.61
1319	114	123	0.0006	0	0	0	0.019	0.019	1.18	1.07
1320	109	116	0	0	0	0	0.019	0.019	0.41	0.63
1326	101	102	0.0008	0.0051	0.257	2.382	0.019	0.019		
1328	134	130	0	0	0	0	0.019	0.019	1.23	0.94
1250	108	117	0	0.0039	2.92	3.508	0.019	0.019	0.38	0.61
3073	126	124	0.003	0.002			0.03	0.03	1	0.79

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
1284	113	111	0	0	1.053	1.397	0.019	0.019	0.94	0.84
1311	125	129	0.0001	0	0	0.155	0.019	0.019	0.62	0.41
1046	99	110	0.0052	0.0154	2.806	9.02	0.019	0.03	0.93	0.81
6208	100	117	0.0046	0.0011	6.844	6.137	0.019	0.019	0.95	1.03
1292	92	99	0.0077	0.0023	7.501	6.357	0.019	0.019	0.92	0.81
3296	116	119	0	0.02			0.019	0.019	1.57	1.34
2111	136	131	0	0.0104	8.103	7.846	0.02	0.03	1.51	0.84
2166	108	110	0	0.19	0	140.443	0.019	1.42	3.27	6.45
1033	114	111	0	0.003	1.171	3.269	0.019	0.019	1.01	1.1
1057	110	124	0.0057	0.0058	4.155	3.405	0.019	0.019	5.19	5.36
4021	103	90	0.0192	0.0185	16.966	17.3325	0.07	0.09	0.39	0.5
3416	141	138	0	0.001			0.019	0.02	1.59	2.09

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2
5004	136	145	0.0048	0.0047	8.105	6.82	0.019	0.019	3.36	3.43
3311	116	105	0.001	0.01			0.02	0.03	0.5	0.58
6106	103	84	0.0118	0.0212	12.339	17.133	0.03	0.02	0.78	0.67
6236	110	115	0.03	0	6.598	5.187	0.03	0.019	1.05	1.48
3412	127	123	0	0.001			0.019	0.019	2.25	1.32
1024	95	98	0.0029	0.0026	1.637	1.054	0.019	0.019	2.1	2.75
2114	120	116	0	0	1.624	0.829	0.019	0.019	8.94	5.33
6014	76	105	0	0	0	0	0.019	0.019	0.34	0.42
26.73	27.62	<4.8	<4.8	2.99	2.99	non-AMI	19	19	1.7	2.4	MI	ACS
41.62	30.83	6.61	6.18	2.99	2.99	non-AMI	19	19	5	4.9	No-AMI	Musculoskeletal
43.29	23.59	7.57	11.7	2.99	2.99	non-AMI	19	19	0.6	0.6	No-AMI	Probable arterial
81	104.75	9.35	9.34	2.99	2.99	non-AMI	19	19	1.4		No-AMI	Probable arterial
30.32	43.54	9.77	10.7	2.99	2.99	non-AMI	67	22	0.3	0.5	No-AMI	Unknown

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
57.28	72.29	6.24	5.88	2.99	2.99	non-AMI	52	36	1.9	2.1	No-AMI	Non specific
38.32	52.26	9.58	11.28	2.99	2.99	non-AMI	40	22	0.7	0.6	No-AMI	Non specific
78.58	36.22	11.15	5.75	2.99	2.99	non-AMI	19	19	3.6	3	No-AMI	Non specific
26.22	26.71	6.53	8.61	2.99	2.99	non-AMI	19	19	1.4	1.3	No-AMI	Non specific
46.05	53.1	4.94	11.31	2.99	3.4	non-AMI	34	53	1.3	1.4	No-AMI	Non specific
70.46	64.03	11.84	6.55	2.99	2.99	non-AMI	19	19	3.8		No-AMI	Non specific
86.93	115.8	4.99	5.07	2.99	2.99	non-AMI	64	19	2.7	2.7	No-AMI	Non specific
17.86	10.79	10.68	8.38	2.99	2.99	non-AMI	19	19	1.2	1.2	No-AMI	Other-CVT
23.12	26.97	9.34	4.82	2.99	2.99	non-AMI	19	19	0.8	0.9	No-AMI	Anxiety
71	43.35	11.15	5.75	2.99	2.99	non-AMI	19	19	1.6	1.6	No-AMI	Non specific
18.97	28.45	4.94	11.31	2.99	2.99	non-AMI	34	47	0.1	0	No-AMI	Other-LRT
12.49	11.26	11	7.59	2.99	2.99	non-AMI	19	19	0.9	1	No-AMI	Non specific
28.14	31.97	11.3	8.53	2.99	2.99	non-AMI	19	19	1	0.9	No-AMI	Non specific
44.41	43.91	5.84	5.09	2.99	2.99	non-AMI	19	19	2.1	1.8	No-AMI	Gastro-oesophageal

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
20.45	21.17	11.03	9.53	2.99	2.99	non-AMI	19	19	1.4	1.2	No-AMI	Non specific
19.18	23.9	6.63	9.47	2.99	2.99	non-AMI	19	19	1.3	1.6	No-AMI	Non specific
9.43	8.78	9.46	10.97	2.99	2.99	non-AMI	41	38	1	1	No-AMI	ACS
42.77	54.25	5.57	5.28	2.99	2.99	non-AMI	34.7	29.7	2.1	2.2	No-AMI	Skeletal
18.69	13.45	11.8	6.81	2.99	2.99	non-AMI	29.7	25.3	0.7	0.8	No-AMI	Non specific
23.58	15.23	10.92	6.27	2.99	2.99	non-AMI	53.2	51.2	1.1	0.8	No-AMI	Gastro-oesophageal
	21.09	9.68	5.1	2.99	2.99	non-AMI	60	54	0.8	0.9	No-AMI	ACS
22.25	17.08	9	5	2.99	2.99	non-AMI	44.39	48.25	1	1	No-AMI	Gastro-oesophageal
27.18	25.38	7.12	6.3	2.99	2.99	non-AMI	42.46	47.67	0.8	0.7	No-AMI	ACS
7.95	9.37	10.4	5.82	2.99	2.99	non-AMI	110	95	0.5		No-AMI	Other-known WPW stable
10.76	6.84	9.34	4.82	2.99	2.99	non-AMI	58.2	98.05	0.3	0.3	No-AMI	Chest infection
32.88	25.52	11.84	6.55	2.99	2.99	non-AMI	35	47	1.1	1	No-AMI	Non specific
58.08	42	11.47	6.59	2.99	2.99	non-AMI	74	65	1.6	1.7	No-AMI	Gastro-oesophageal
28.97	11.46	4.99	5.07	2.99	3.65	non-AMI	55	95	1.3	1.3	No-AMI	Non specific

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
154.58	110.74	6.72	6.35	2.99	2.99	non-AMI	56	67	3.3	3	No-AMI	Non specific
62.4	90.47	11.52	5.97	2.99	2.99	non-AMI	59	280.4	2.6	2.9	No-AMI	Non specific
21.78	26.33	9.39	6.73	2.99	2.99	non-AMI	69	82	0.5	0.5	No-AMI	Pneumonia
10.12	7.13	6.6	6.71	2.99	2.99	non-AMI	52	66.27	0.4	0.4	No-AMI	Non specific
33.52	37.06	11.8	6.11	2.99	2.99	non-AMI	83.98	93	2.1	1.9	No-AMI	Non specific
61.37	70.74	6.66	10.88	2.99	2.99	non-AMI	29	36	0.8	0.7	No-AMI	Non specific
121.16	86.48	5.53	7.89	2.99	3.65	non-AMI	330.4		3.3	3.1	No-AMI	
56.89	72.01	5.28	9.46	2.99	2.99	non-AMI	967.7	143	1.6	1.5	No-AMI	Muscularskeletal
30.29	50.25	7.09	11.52	2.99	2.99	non-AMI	37	17	1	1	No-AMI	other-mild pancreatitis
12.54	17.94	6.71	8.2	2.99	5.87	non-AMI	19	19	1.2	1.2	No-AMI	Non specific
33.66	40.81	4.88	11.4	2.99	2.99	non-AMI	26.3	26.9	1.1	1.1	No-AMI	Non specific
22.12	22.83	7.03	6.71	2.99	2.99	non-AMI	26.7	32.1	0.9	0.7	No-AMI	Non specific
18.06	19.81	5.21	10.58	2.99	2.99	non-AMI	27	21.6	0.3	0.4	No-AMI	Angina-No ACS
75.82	38.96	5.84	8.6	2.99	2.99	non-AMI	35.5	26.9	1.6	1.4	No-AMI	other-ACS ruled out

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
19.94	7.98	8.1	6.68	2.99	2.99	non-AMI	42.1	32.7	0.4	0.2	No-AMI	Anxity
17.16	22.14	11.34	6.97	2.99	2.99	non-AMI	44.8	34.2	0.7	0.6	No-AMI	Non specific
12.11	15.94	7.6	8.84	2.99	2.99	non-AMI	40.3	44.1	0.5	0.3	No-AMI	gastro-oesophageal
41.06	42.08	10.15	6.03	2.99	2.99	non-AMI	19	34	0.3	0.3	No-AMI	Non specific
19	26.34	6.4	11.18	2.99	2.99	non-AMI	19	19	1.9	2	No-AMI	Anxity
45.2	19.45	5.12	10.07	2.99	2.99	non-AMI	39.1	42.6	0.3	0.4	No-AMI	Non specific
18.31	18.87	9.54	6.03	2.99	2.99	non-AMI	44.8	42.5	1.3	1.3	No-AMI	Self dischard before diagnosis
19.96	18.33	8.69	4.88	2.99	2.99	non-AMI	51.1	47.7	1.2	1.1	No-AMI	Non specific
24.68	20.8	<4.8	<4.8	2.99	2.99	non-AMI	80.3	32.5	1.7	0	No-AMI	Other?cariac arrythmia
17.15	19.25	6.2	6.4	2.99	2.99	non-AMI	101.5	93.75	1	0.8	No-AMI	
18.25	20.54	<4.8	5.2	2.99	2.99	non-AMI	66.72	45.19	2.6	2.5	No-AMI	Gastro-oesophageal
102.12	81.08	<4.8	<4.8	2.99	2.99	non-AMI	63.5	65.09	2.1	2	No-AMI	Muscularskeletal
20.65	8.56	6.5	4.9	2.99	2.99	non-AMI	41	63	0.8	0.7	No-AMI	Muscularskeletal
162.22	114.25	5.4	5.9	2.99	10.98	non-AMI	25	1323	8.3	7.2	No-AMI	Muscularskeletal

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
1.02	8.41	6.1	5.4	2.99	2.99	non-AMI	72	94	1.1	1.1	No-AMI	Gastro-oesophageal
17.09	20.24	4.9	5.3	2.99	2.99	non-AMI	48	59	2.3	2	No-AMI	Musculoskeletal
21.76		6.4	5.2	2.99	2.99	non-AMI	68	59	0.5	0.5	No-AMI	Non specific
19.15	29.86	<4.8	5.2	2.99	2.99	non-AMI	307	70	2.2	2.3	No-AMI	Other-hyperventilation episode
13.6	5.52	<4.8	<4.8	2.99	2.99	non-AMI	45	101	0.5	0.5	No-AMI	Angina-No ACS
8.77	17.56	5.69	10.73	2.99	2.99	non-AMI	36	22	1	1	No-AMI	Non specific
34.26	43.77	8.98	6.2	2.99	2.99	non-AMI	29	46	0.7	0.7	No-AMI	Non specific
36.44	45.7	9.56	9.48	2.99	2.99	non-AMI	59	83	2.4	2.3	No-AMI	Other-possible cardiac but MI rule
11.46		11.57	11.35	2.99	2.99	non-AMI	43	62	0.8	0.6	No-AMI	Non specific
25.621	23.82	6.94	10.33	2.99	2.99	non-AMI	59	84	2	1.9	No-AMI	Other-possible cardiac but MI rule
19.7	18.44	6.6	6.37	2.99	2.99	non-AMI	35.2	34.1	1.5	1.4	No-AMI	Non specific
32.14	28.73	10.25	10.59	2.99	2.99	non-AMI	47.3	34.9	1.5	1.3	No-AMI	Angina-No ACS
28.91	25.76	11.78	8.08	2.99	2.99	non-AMI	26	32	0.6	0.7	No-AMI	Musculoskeletal
22.27	21.53	8.61	5.73	2.99	2.99	non-AMI	19	19	0.7	0.6	No-AMI	Gastro-oesophageal

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
41.96	12.4	5.73	11.33	2.99	2.99	non-AMI	73	19	1.8	1.7	No-AMI	Gastro-oesophageal
8.99	4.82	5.09	10.25	2.99	2.99	non-AMI	76.04	37	0.7	0.6	No-AMI	Other- arrythmia
30.01	22.94	5.75	11.03	2.99	2.99	non-AMI	23	52.45	3.2	2.9	No-AMI	Muscularskeletal
60.14	23.55	10.56	5.26	2.99	2.99	non-AMI	19	19	2.2	2.1	No-AMI	Other-stress related
22.2	24.18	8.76	6.95	2.99	2.99	non-AMI	32	19	1.4	1.4	No-AMI	Othre-infection gram negative spt
54.59	57.68	11.57	9.84	2.99	2.99	non-AMI	19	28	1.1	0.7	No-AMI	Non specific
22.09	26.43	5.28	9.73	2.99	2.99	non-AMI	19	19	0.9	1	No-AMI	Non specific
26.16	28.71	5.45	8.81	2.99	2.99	non-AMI	82	54	1.8	1.6	No-AMI	Gastro-oesophageal
48.03	20.19	5.52	6.72	2.99	2.99	non-AMI	19	19	2.2	2	No-AMI	Non specific
35.94	45.22	10.19	5.08	2.99	2.99	non-AMI	36	54	3.2	3.4	No-AMI	Other- LRTI
31.02	43.04	11.2	5.08	2.99	2.99	non-AMI	19	28	1.7	1.7	No-AMI	Muscularskeletal
22.44	29.26	7	9.7	2.99	3.35	non-AMI	19	19	0.9	0.9	No-AMI	Unknown
25.73	45.05	11.88	5.38	2.99	2.99	non-AMI	19	19	2.4	2.3	No-AMI	Gastro-oesophageal
19.98	21.66	9.76	5.57	2.99	2.99	non-AMI	19	19	0.9	0.9	No-AMI	Gastro-oesophageal

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
50.01	28.66	6.71	8.2	2.99	16.85	AMI	26	43	1.1	1	No-AMI	Non specific
37.42	34.28	<4.8	<4.8	2.99	2.99	non-AMI	92	103	1.2	1	No-AMI	Non specific
20.11	29.44	11.35	11.91	2.99	5.55	non-AMI	81	67	3.2	3.2	No-AMI	Gastro-oesophageal
33.14	31.33	<4.8	<4.8	2.99	2.99	non-AMI	37	61	0.9	0.8	No-AMI	Muscularskeletal
33.64	35.96	8.14	5.85	2.99	2.99	non-AMI	91	74	1.1	1.1	No-AMI	Non specific
41.56	37.8	10.55	10.29	2.99	2.99	non-AMI	49	75	1.1	1	No-AMI	Gastro-oesophageal
38.48	51.97	5.99	5.34	2.99	2.99	non-AMI	29	34	1.8	1.5	No-AMI	Non specific
46.37	21.62	5.29	6.63	2.99	2.99	non-AMI	33	48	0.7	0.6	No-AMI	Angina-No ACS
28.99	28.73	5.12	10.07	2.99	2.99	non-AMI	19	19	1.4	1.4	No-AMI	Gastro-oesophageal
29.18	22.15	9.54	6.03	2.99	2.99	non-AMI	19	19	0.8	0.8	No-AMI	Non-specific
8.15	33.64	6.6	6.17	2.99	2.99	non-AMI	36	35	0.4	0.4	No-AMI	Non-specific
20.35	24.2	8.43	7.26	2.99	2.99	non-AMI	32	43.84	2.3	2	No-AMI	Angina-No ACS
41.38	27.91	6.65	8.96	2.99	2.99	non-AMI	42	53	2.7	2.8	No-AMI	Unknown
24.56	21.48	8.34	9.46	2.99	2.99	non-AMI	46	78	0.9	0.8	No-AMI	Gastro-oesophageal

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
24.2	27.16	5.96	10.79	2.99	2.99	non-AMI	88		1.9	1.9	No-AMI	Angina-No ACS
67.52	46.27	5.73	11.78	2.99	2.99	Non-AMI	79	73	4.1	4.1	No-AMI	Angina-No ACS
20.62	23.29	6.18	8.61	2.99	2.99	Non-AMI	53	86	1.5	3	No-AMI	Angina-No ACS
18.41	22.91	11.7	6.61	2.99	2.99	Non-AMI	33		2	1.8	No-AMI	Unknown
22.64	28.64	9.92	9.35	2.99	2.99	Non-AMI	98	72	2.1	1.9	No-AMI	Muscularskeletal
27.69	24.18	5.82	11.5	2.99	2.99	Non-AMI	52	82	2.8	2.5	No-AMI	Gastro-oesophageal
25.9	30.67	8.89	9.77	2.99	2.99	Non-AMI	29	24	3.7		No-AMI	Gastro-oesophageal
13.37	11.85	7.01	6.93	2.99	2.99	Non-AMI	53	82	2.8	2.8	No-AMI	Angina-No ACS
14.25	31.89	11.14	10.64	2.99	2.99	Non-AMI	38	42	1.8		No-AMI	Muscularskeletal
18.48	23.73	9.43	5.39	2.99	2.99	Non-AMI	85	76	0.6		No-AMI	Unknown
10.76	14.91	11.01	6.99	2.99	2.99	Non-AMI	76	64	1.1	1	No-AMI	Non specific
18.26	17.69	4.9	7.53	2.99	2.99	Non-AMI	26	40	1.4	1.4	No-AMI	Anxity
25.94	19.68	8.94	4.89	2.99	2.99	Non-AMI	24	29	1	1.2	No-AMI	Angina-No ACS
21.91	22.1	10.02	7.32	2.99	2.99	Non-AMI	66	72	2.5	2	No-AMI	Gastro-oesophageal

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
25.45	28.77	8.31	8.27	2.99	2.99	Non-AMI	78	72	0.6	0.7	No-AMI	Unknown
30.65	26.5	8.36	6.77	2.99	2.99	Non-AMI	19	19	0.6	0.7	No-AMI	Other-chest infection
32.3	31.53	6.6	6.17	2.99	2.99	Non-AMI	34	39	0.6	0.6	No-AMI	Non specific
41.76	18.42	8.43	7.26	2.99	2.99	Non-AMI	19	29	1.4	1.3	No-AMI	Other-URTI
52.06	44.45	10.99	9.15	2.99	2.99	Non-AMI	45	49	1.2	1	No-AMI	Other-chest infection
10.71		6.53	0	2.99	2.99	Non-AMI	23	34	1	0.8	No-AMI	Non specific
32.08	27.74	8.75	10.06	2.99	17.66	AMI	19	29	1	1.2	No-AMI	Angina-No ACS
209.47	285.04	8.21	8.15	2.99	2.99	Non-AMI	19	19	2.1	2.4	No-AMI	Other-left sided pneumonia
30.32	27.03	5.09	10.25	2.99	2.99	Non-AMI	19	19	1.8	1.5	No-AMI	Angina-No ACS
19.66	17.87	6.32	6.64	2.99	2.99	Non-AMI	29	34	1.1	1	No-AMI	Non specific
19.6	18.65	5.06	6.18	2.99	2.99	Non-AMI	19	35	1.5	1.3	No-AMI	Non specific
12.11	9.12	8.6	7.8	2.99	2.99	Non-AMI	19	19	1.3	1	No-AMI	Non specific
17.16	22.37	7.48	11.3	2.99	2.99	Non-AMI	19	19	1.3	1.1	No-AMI	Non specific
24.25	22.49	6.68	6.3	2.99	2.99	Non-AMI	19	19	1.4		No-AMI	Muscularskeletal

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
22.58	13.17	4.94	5.1	2.99	2.99	Non-AMI	19	19	0.8	1	No-AMI	Other-?ACS ?PE
27.21	21.37	11.84	6.55	2.99	2.99	Non-AMI	19	19	1.2		No-AMI	Muscularskeletal
41	22.85	11.47	6.59	2.99	2.99	Non-AMI	19	19	3.4	2.7	No-AMI	Angina-No ACS
36.25	32.91	4.99	5.07	2.99	2.99	Non-AMI	19	19	2	1.9	No-AMI	Angina-No ACS
46.7	28.82	5.45	8.81	2.99	2.99	Non-AMI	19	19	0.9	0.8	No-AMI	Muscularskeletal
16.98	17.55	11.6	5.52	2.99	2.99	Non-AMI	55	45	1.2	1.2	No-AMI	Muscularskeletal
16.1	34.58	11.52	5.97	2.99	2.99	Non-AMI	77	79	1.7	1.5	No-AMI	Anxity
22	44.2	9.39	6.73	2.99	2.99	Non-AMI	105	94	2.2	2.3	No-AMI	Anxity
40.39	40.36	11.8	6.11	2.99	2.99	Non-AMI	23	34	0.5	0.5	No-AMI	Non specific
14.55	13.69	9.09	9.52	2.99	3.9	Non-AMI	93	95	0.3	0.3	No-AMI	Muscularskeletal
29.09	20.89	5.99	7.88	2.99	3.85	Non-AMI	35	70	0.5	0.5	No-AMI	Non specific
19.48	42.05	10.99	9.15	2.99	2.99	Non-AMI	25	22	1.6	1.4	No-AMI	Anxity
10.86	11.42	8.75	10.06	2.99	2.99	Non-AMI	121	80	1.2	1.2	No-AMI	Non specific
16.1	17.69	8.21	8.15	2.99	2.99	Non-AMI	100	111	0.5	0.6	No-AMI	Non specific

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
22.14	26.91	6.45	4.84	2.99	5.41	Non-AMI	123	152	5.1	4.7	No-AMI	ACS
22.96	28.95	4.92	10.47	2.99	2.99	Non-AMI	19	19	2.5	2.4	No-AMI	Gastro-oesophageal
46.59	36.26	10.25	10.59	2.99	2.99	Non-AMI	19	19	1.5	1.4	No-AMI	Non specific
42.43	32.86	5.75	11.03	2.99	2.99	Non-AMI	19	19	1.8	1.7	No-AMI	Non specific
18.93	15.31	6.32	6.64	2.99	2.99	Non-AMI	19	19	0.8	0.6	No-AMI	Gastro-oesophageal
12.47	17.53	8.19	9.82	2.99	2.99	Non-AMI	22	34	0	0	No-AMI	ACS
42.74	43.38	7.17	8.03	2.99	2.99	Non-AMI	19	19	0.6	0.6	No-AMI	Non specific
12.56	25.53	10.14	5.84	3.3	3.5	Non-AMI	19	19	3.6	3.6	No-AMI	Non specific
28.1	18.27	9.38	10.14	3.36	6.03	Non-AMI	88	69	1.4	1.6	No-AMI	Anxity
32.75	20.62	6.6	6.71	3.44	4.72	Non-AMI	85	96	1.1	1.3	No-AMI	Muscularskeletal
15.16	16.91	11.09	7.14	3.65	5.65	Non-AMI	29	36	0.6		No-AMI	Non specific
29.36	22.85	6.45	4.84	3.66	17.63	AMI	19	19	0.3	0.3	No-AMI	Other-SUT
23.4	24.56	10.43	11.03	3.8	4.9	Non-AMI	37.3	43.4	1.4	1.2	No-AMI	Non specific
48.86	43.7	6.66	10.88	3.9	2.99	Non-AMI	39	54	1.3	1	No-AMI	Angina-No ACS

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
57.32	58.08	8.82	12	3.97	5.36	Non-AMI	19	19	3.2	3.4	No-AMI	Non specific
27.94	11.37	11.47	5.82	4.11	3.23	Non-AMI	24	28	0.5	0.6	No-AMI	Unknown
55.16	71.85	6.72	6.35	5.03	28.93	AMI	19	19	2.1	2.9	MI	ACS
60.32	44.4	6.5	10.06	5.04	2.99	Non-AMI	19	24	3.1	2.9	No-AMI	Non specific
201.81	227.96	5.75	11.03	5.19	4.39	Non-AMI	67	84	0.9	1	No-AMI	Other-porforated D/V-che
20.48	23.25	5.06	4.93	5.41	8.94	Non-AMI	229	164	4.3	3.1	No-AMI	Non specific
51.88	55.3	6.4	11.18	6.35	7.19	Non-AMI	69	86	3	2.7	No-AMI	Non specific
10.46	15.65	5.73	11.33	6.37	3.17	Non-AMI	248	295.9	0.4	0.4	No-AMI	Unknown
26.9	21.12	10.62	8.4	7.21	20.08	AMI	19	19	1.1	1.2	No-AMI	Non specific
39.43	20.05	8.27	8.96	7.59	12.99	Non-AMI	218.2	492.9	0.5	0.6	No-AMI	Non specific
35.11	44.54	9.37	6.91	8.2	5.14	Non-AMI	45.3	48.7	1.3	1.3	No-AMI	Angina-No ACS
26.92	22.14	10.85	5.2	13.58	2.99	Non-AMI	26	34	1.4	1.5	No-AMI	Unknown

ID	IMA 1	IMA 2	AccuTnI 1	AccuTnI 2	Beckman hs-TnI 1	Beckman hs TnI 2	Centaur TnI 1	Centaur TnI 2	FABP 1	FABP 2	
55.37	81.47	10.06	7.83	21.24	16.45	AMI	541	632	2.6	2.5	No-AMI	Muscularskeletal
360.59	109.9	11.95	11.83	45.28	7.3	AMI	203	306	7.3	6.9	MI	Angina-No ACS

Appendix 4: Meta-analysis of Ischemia modified-albumin supplementary data

Table 12: Example of 2x2 table used to extract sensitivity and specificity.

Authors					
Title					
Year of publication					
Abstract in folder					
Abstract					
Number of subjects					
ACS group studied					
Length of follow up					
Mean age at enrolment					
% male					
Sample storage					
IMA			Assay generation		
IMA cut off used for diagnosis			Assay generation		
Odds ratio quoted		Lower 95% CI		Upper 95% CI	
2 x 2 data contingency table or Odds ratio check		**Reference standard is positive**	**Reference standard is negative**		
		Test is positive			
		Test is negative			
Odds ratio calculated		Lower 95% CI		Upper 95% CI	
Sensitivity		Lower 95% CI		Upper 95% CI	
Specificity		Lower 95% CI		Upper 95% CI	
Data input in excel	06/07/2017	Double entry		Data check	

Authors	Study reference	N	Sensitivity	Lower 95%	Upper 95%	Sen SE	Specificity	Lower 95%	Upper 95%	Spec SE	NPV	Lower 95%	Upper 95%	NPV SE	PPV	Lower 95%	Upper 95%	PPV SE	Comments
(Aparci,Kardesoglu,Ozmen *et al.*, 2007)	Prognostic significance of ischemia-modified albumin in patients with acute coronary syndrome	95	70.0	69.0	71.0	0.5102	82.0	81.0	83.0	0.5102									
(Lee,Kim,Cho *et al.*, 2007)	Application of albumin-adjusted ischemia modified albumin index as an early screening marker for acute coronary syndrome	413	98.4	97.9	98.9	0.2551	34.5	34.0	35.0	0.2551	98.0	97.5	98.5	0.2551	40.6	40.1	41.1	0.2551	
Bali,Cuisset,Giorgi *et al.*, 2008)	Prognostic value of ischaemia-modified albumin in patients with non-ST-segment elevation acute coronary syndromes	79	75.0	73.9	76.1	0.5612	74.6	90.6	92.8	9.2859	91.7	90.6	92.8	0.5612	44.4	43.3	45.5	0.5612	
(Consuegra-Sanchez,Bouzas-Mosquera,Sinha *et al.*, 2008)	Ischemia-modified albumin predicts short-term outcome and 1-year mortality in patients attending the emergency department for acute ischemic chest pain	207	75.7	66.1	83.8	4.8980	74.1	64.8	82.0	4.0307	76.9	69.8	82.8	3.6225	72.8	65.7	79.0	3.6225	
(Liyan,Jie,Yonghua *et al.*, 2008)	Assay of ischemia-modified albumin and C-reactive protein for early diagnosis of acute coronary syndromes	113	94.4	93.5	95.3	0.4592	82.6	81.7	83.5	0.4592	79.2	78.3	80.1	0.4592					PPV not reported. Cannot abstract data for 2 x 2
(Talwalkar,Bon Homme,Miller *et al.*, 2008)	Ischemia modified albumin, a marker of acute ischemic events: a pilot study	89	53.9	25.1	80.8	14.2094	75.0	63.7	84.2	4.6940	90.5	83.9	94.6	3.3674	26.9	16.3	41.0	5.4083	
(Manini,Ilgen,Noble *et al.*, 2009)	Derivation and validation of a sensitive IMA cut-point to predict cardiac events in patients with chest pain	106	80.9	58.0	94.6	11.6839	36.5	26.3	47.6	5.4338	88.6	75.4	95.1	6.7348	23.9	19.5	29.1	2.2449	
(Charpentier,Ducasse,Cournot *et al.*, 2010)	Clinical assessment of ischemia-modified albumin and heart fatty acid-binding protein in the early diagnosis of non-ST-elevation acute coronary syndrome in the emergency department	677	69.2	65.7	72.7	1.7857	36.0	32.4	39.6	1.8368	75.6	72.4	78.9	1.6582	28.9	25.5	32.3	1.7347	
(Pan,Tong,Lin *et al.*, 2010)	Ischemia-modified albumin measured with ultra-filtration assay in early diagnosis of acute coronary syndrome	169	79.8	79.0	80.6	0.4082	65.2	64.5	65.9	0.3571	69.4	68.9	70.5	0.2551	77.7	76.9	78.4	0.4082	
MA takhshid, J Kojuri, SMB Tabei	Early Diagnosis of acute coronary syndrome with sensitive Troponin I and Ischemia Modified Albumin	123	84.0	81.0	93.0	1.5306	85.0	75.0	92.0	3.5715	88.0	77.0	94.0	5.6123	81.0	68.0	90.0	6.6328	
(Wudkowska,Goch & Goch, 2010)	Ischemia-modified albumin in differential diagnosis of acute coronary syndrome without ST elevation and unstable angina pectoris	121	77.6	64.7	87.5	6.5818	23.8	14.6	36.2	6.3266	53.6	37.6	68.9	7.9848	48.4	43.5	53.3	2.5000	
(Hjortshoj,Kristensen & Ravkilde, 2010)	Diagnostic value of ischemia-modified albumin in patients with suspected acute coronary syndrome	107	86.0	69.0	95.0	8.6736	49.0	36.0	60.0	5.6123	88.0	72.0	96.0	8.1634					PPV not reported. Cannot abstract data for 2 x 2
(Dawie,Chawla,Worku *et al.*, 2011)	Diagnosis of ischemic heart disease using CK-MB, troponin-I and ischemia modified albumin	55	100.0	98.7	101.3	0.6633	71.1	69.8	72.4	0.6633	100.0	98.7	101.3	0.6633	80.6	79.3	81.9	0.6633	
(Maneewong,Mekrungruangwong,Luangaram *et al.*, 2011)	Combinatorial Determination of Ischemia Modified Albumin and Protein Carbonyl in the Diagnosis of Non ST-Elevation Myocardial Infarction	33	51.5	33.5	69.2	9.1073	87.9	71.8	96.0	4.1327	64.4	62.7	66.1	0.8674	80.9	79.2	82.7	0.8929	
(Shen,Lin,Han *et al.*, 2011)	Assessment of ischemia-modified albumin levels for emergency room diagnosis of acute coronary syndrome	113	89.0	88.1	89.9	0.4592	94.4	83.5	85.3	5.5613	96.2	95.3	97.1	0.4592	83.4	82.5	84.3	0.4592	
(Zhong,Wang,Xu *et al.*, 2012)	Ischemia-modified albumin in stable coronary atherosclerotic heart disease: clinical diagnosis and risk stratification	129	49.0	48.1	49.8	0.4592	90.0	89.1	90.8	0.4082	45.0	44.1	45.9	0.4592	91.0	90.1	91.9	0.4592	
(Sokhanvar,Mellati,Mousavinasab *et al.*, 2012)	The clinical assessment of ischaemia modified albumin and troponin I in the early diagnosis of the acute coronary syndrome	226	54.0	51.0	56.0	1.5306	87.0	83.0	92.0	2.5511	62.0	59.0	66.0	1.5306	82.0	78.0	88.0	2.0409	
(Ertekin,Kocak,Defne Dundar *et al.*, 2013)	Diagnostic value of ischemia-modified albumin in acute coronary syndrome and acute ischemic stroke	90	83.0	71.0	90.0	6.1226	90.0	77.0	97.0	3.5715	84.0	73.0	91.0	5.6123	89.0	76.0	97.0	6.6328	
(Kountana,Tziomalos,Semertzidis et al., 2013)	Comparison of the diagnostic accuracy of ischemia-modified albumin and echocardiography in patients with acute chest pain	33	60.0	58.3	61.7	0.8674	89.3	87.6	91.0	0.8674	92.6	90.9	94.3	0.8674	50.0	483.0	51.7		
(Patil,Banker,Padalkar *et al.*, 2013)	The clinical assessment of ischaemia modified albumin and troponin I in the early diagnosis of the acute coronary syndrome	102	88.0	87.0	88.0	0.5102	93.0	92.0	93.0	0.5102	89.6	88.6	91.0	0.5102	92.8	91.8	93.8	0.5102	
(Sygitowicz,Janas,Bialek et al., 2013)	Prognostic value of ischaemia-modified albumin in patients with non-ST-segment elevation acute coronary syndromes and negative cTnI	88	72.1	56.3	84.7	8.0614	75.6	60.5	87.1	5.8675	73.9	58.9	85.7	7.6532	73.8	58.0	86.1	8.0614	
(Gurumurthy,Borra,Yeruva *et al.*, 2014)	Estimation of Ischemia Modified Albumin (IMA) Levels in Patients with Acute Coronary Syndrome	675	88.0	87.6	88.4	0.2041	89.0	88.6	89.3	0.1531									
(Bhakthavatsala Reddy,Cyriac & Desle, 2014)	Role of "Ischemia Modified Albumin" (IMA) in acute coronary syndromes	89	92.0	91.0	93.0	0.5102	87.0	86.0	88.0	0.5102	94.0	93.0	95.0	0.5102	88.0	87.0	89.0	0.5102	
Arun Kumar. K, Sheila Uthappa, Sudarshan Surrendran	Correlation of albumin concentration and ischemia modified albumin in the diagnosis if acute myocardial infarction	120	69.2	68.3	70.1	0.4592	67.7	66.8	68.6	0.4592	68.7	67.8	69.6	0.4592	68.2	67.3	69.1	0.4592	
(Mehta,Marwah,Ghosh *et al.*, 2015)	A synergistic role of ischemia modified albumin and high-sensitivity troponin T in the early diagnosis of acute coronary syndrome	120	91.3	90.4	92.2	0.4592	81.1	80.2	82.0	0.4592	93.9	93.0	94.8	0.4592	74.4	73.5	75.2	0.4592	
(Bakula,Milicevic,Bakula *et al.*, 2016)	Kinetics of Ischemia-Modified Albumin Following Exercise-Induced Myocardial Ischemia	87	70.0	60.0	80.0	5.1021	64.0	45.0	80.0	8.1634	52.0	36.0	65.0	8.1634	79.0	68.0	88.0	5.6123	

Appendix 5: Current study sample size calculation

Current study sample size calculations

RESULTS

Dichotomous Endpoint, One-Sample Study

Sample Size	
Group 1	1168
Total	**1168**

Study Parameters	
Incidence, population	7%
Incidence, study group	5%
Alpha	0.05
Beta	0.2
Power	0.8

http://clincalc.com/stats/samplesize.aspx used to calculate the sample size for the current study.

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ISCHEMIA MODIFIED ALBUMIN ASSAY: BIOCHEMISTRY AND CLINICAL UTILITY

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CONTENTS

Keywords: Acute Coronary Syndrome; Albumin Cobalt Binding Assay; Cardiac Ischemia; Ischemia Modified Albumin.

I. INTRODUCTION

Ischemia (from the Greek ισχαιμία, *ischaimía*; *isch-* root denoting a restriction or thinning or to make or grow thin, *haema* blood) is the restriction of blood supply and thus the inadequate delivery of oxygen and removal of carbon dioxide from cellular tissue. This imbalance may lead to dysfunctional or permanent damage to the affected tissue and organ.

Cardiac ischemia occurs when there is a supply versus demand mismatch in coronary blood flow. In patients who present with unstable angina, ischemia occurs due to partial or total occlusion of a coronary artery due to plaque rupture. In stable angina however, there is progressive vascular occlusion resulting ultimately in a luminal stenosis of greater than 70%, impeding blood flow to the distal tissue. If the ischemia is reversible, no permanent myocardial damage occurs. If however the ischemic episode is prolonged; there will be cellular necrosis which will lead to acute myocardial infarction (AMI) or a heart attack. The immediate clinical challenge is to be able to identify acutely impaired myocardial perfusion before the necrotic process starts. Currently, the only strategy for this is to detect ST-segment changes on the electrocardiogram (ECG), however the ECG is non-diagnostic in many cases. The sensitivity of the admission ECG is typically around 50%. Reperfusion, be it pharmacological or surgical, is the essential life-saving intervention with the aim of salvaging myocardial tissue localised at the affected site. Many patients however who present with chest pain to the emergency department (ED) do not have a final diagnosis of AMI. There is therefore a need for a strategy which could detect cardiac ischemia before necrosis occurs and result in prompt revascularisation. Blood borne biomarkers for ischemia may be of diagnostic and prognostic value.

To date, a number of candidate biomarkers of ischemia are being researched. However, one, Ischemia modified albumin (IMA®), has been developed into a commercially available cardiac biomarker assay and licensed for routine clinical application both by CE marking in Europe and Food and Drug Administration (FDA) approval in the United States. This chapter will explore the rational for the necessity of cardiac ischemia biomarker testing and detail the development of the IMA assay with emphasis on its clinical and prognostic utility.

The cardiovascular disease epidemic

Cardiovascular disease (CVD) accounts for the majority of global deaths. CVD was responsible for 29% of all global deaths in 2004. According to the World Heart Federation, CVD is responsible for 17.1 million deaths globally each year. Surprisingly, 82% of these deaths occur in the developing world. Such numbers are often difficult to comprehend. CVD is responsible for one in every five deaths disease kills one person every 34 seconds in the USA alone. 35 people under the age of 65 die prematurely in the United Kingdom every day due to CVD. It is predicted that by 2030 23 million people will die from a cardiovascular related disease. Data from the USA suggests that CVD was responsible for 34% of all deaths in 2006 and over 151,000 Americans who died were under 65 years of age.

Acute chest pain

Patients with chest pain constitute the largest single category of patients admitted to hospitals in the UK [1]). In the USA, registry data recoded 11.2 million chest pain presentations to the ED in 2008 alone. The presentations are also diagnostically challenging. The majority of admissions have either stable ischemic heart disease (IHD) or no ischemic heart disease [2]). Such admission episodes are often short and clinically inappropriate. Conversely, it has been estimated that between 2 and 7% of patients with acute myocardial infarction (AMI) are inappropriately discharged from the ED [3;4]) and suffer disproportionate morbidity and mortality. Attempts to improve diagnosis have included risk scoring systems [5]), computerised decision support [6;7]) and automated ECG interpretation [8]). Although clinical assessment remains integral to assessment of the patents with chest pain, cardiac biomarker measurement has become an essential component in the diagnostic armamentarium.

Pathophysiology of cardiac ischemia

The mechanisms involved in the development of cardiovascular disease are multifactorial and include abnormalities in cholesterol and lipid metabolism, inflammation and oxidative stress processes within the vascular wall, cellular disruption to the endothelium and intra-lumenal platelet activation/aggregation. The ischemia cascade from initiation of local ischemia to the development of symptomatic chest pain is depicted in figure 1. The pathological processes responsible for the development of atherosclerotic lesions and endothelial dysfunction are advanced far earlier than when patients typically become symptomatic and present with chest pain. The disease process does not occur in distinct episodes but rather is a continuum from asymptomatic vascular dysfunction thorough to angina in those with myocardial ischemia, which,

without intervention can progress to non-ST segment elevation myocardial infarction (NSTEMI) or cumulate into ST segment elevation myocardial infarction (STEMI). Patients presenting at any stage in the process may be diagnosed with acute coronary syndrome (ACS). The earlier in the disease continuum the presentation is; the greater the opportunity for successful myocardial tissue preservation. As there is no definitive biomarker for ischemia, current treatment focuses on the need for urgent therapeutic revascularisation in patients with established cardiac necrosis, identified by the cardiac troponins.

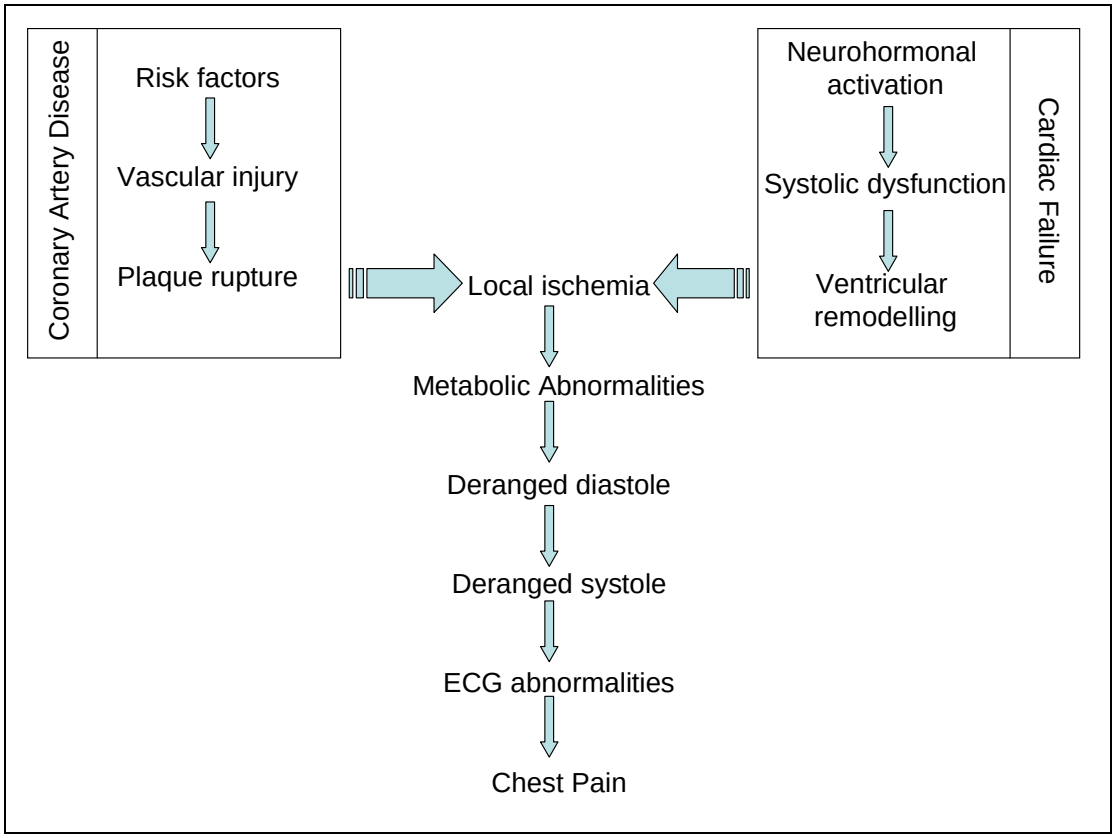

Fig 1: Development of Ischemia in the ischemia cascade.

The pathological process unless disrupted by therapeutic intervention results in the death of cardiac myocytes. Predisposing this terminal event, a vulnerable atherosclerotic plaque becomes disrupted exposing the thrombogenic lipid core

and sub endothelium to the luminal milieu. Exposure results in platelet activation and aggregation and along with the coagulation cascade, an intracoronary thrombus forms. The thrombus may not obstruct the lumen and the patient is asymptomatic, however if the lumen is totally occluded AMI will ensue. A partially occluded lumen and reduced oxygen supply both contribute to the development of ischemic myocardium.

II. CLINICAL DETECTION OF CARDIAC ISCHEMIA

The clinical presentation of cardiac ischemia is difficult to definitively diagnose. Currently there is no gold standard test to detect ischemia however a number of reliable tests exist. Historically patients were admitted for monitoring or discharged on the basis of clinical interpretation by the ED physician. It is accepted that this is no longer acceptable clinical practice.

The typical presentation is exertional or stress induced central chest pain. These episodes usually last from a few minutes to hours and can resolve upon rest. Common descriptions by the patient include tightness, crushing stabbing or burning pain. Patients may also have nausea and vomiting, dyspnoea, palpitations. Typical symptoms increase the likelihood of an AMI however atypical presentations can not be used to exclude AMI. Women, the elderly and those with diabetes mellitus often present with atypical chest pain.

The clinical history and physical examination will assess the presence of risk factors for AMI, however alone; the initial clinical examination is insensitive and unspecific for diagnosis. It may however give insight to differential or alternative diagnoses in those patients who, upon further investigation do not have an AMI. The 12 lead ECG is additive to the physical examination. The majority of ECG traces performed at admission are non-diagnostic with approximately 5% of suspected AMI patients having a diagnostic trace indicative of AMI. Although relatively insensitive, ST segment elevation however is 100% diagnostic for AMI and serves as the criterion for induction of fibrinolytic therapy.

A. Cardiac imaging

Recently cardiac imaging has played an important role in the detection of ischemia. Perfusion abnormalities can be detected by single-photon emission computer tomography (SPECT) myocardial perfusion imaging (MPI) and mechanical dysfunction can be detected by echocardiography or gated MPI. Gated SPECT MPI can identify regional and global dysfunction of the left ventricle as ischemia impairs myocellular contractility. SPECT requires uptake of an isotope by active membrane transport mechanisms and caution should be advised in those patients with impaired renal clearance. Both echocardiography and SPECT are sensitive and specific and have a high negative predictive vale for the diagnosis and prognosis of patients with suspected ACS. These diagnostic modalities however are grossly expensive, time consuming, technically more challenging and are not as widely available as compared to the

simple ECG or a blood borne biomarker; therefore, their use in the ED on a 24 hour basis is therefore compromised.

B. Candidate biomarkers

There have been progressive developments within basic and clinical research to identify candidate biomarkers of ischemia and to develop simple to use assays. Any such assay needs to have similar analytical (limit of detection, precision, reference intervals) and clinical performance (sensitivity, specificity, risk stratification and predictive value) compared to that of markers of necrosis, such as high sensitivity cardiac troponin assays. A number of candidate biomarkers have been identified. Malondialdehyde low density lipoprotein (MDA-LDL) is a sensitive biomarker for ACS patients with unstable angina and AMI. MDA is a candidate compound which causes oxidative modification of LDL. MDA (propanedial, $C_3H_4O_2$) is a reactive aldehyde produced by degradation of polyunsaturated lipids or released during prostanoid metabolism. This reactive oxygen species causes oxidative modification to LDL. MDA-LDL reacts with the charged amino group of B-100 protein lysyl residues. Plasma concentrations of MDA-LDL identify patients with coronary artery disease. Modified LDL may also instigate an immune response leading to autoantibody and LDL immune complex production. MDA-LDL not only serves as an oxidative stress marker but as a marker of plaque destabilisation.

Myeloperoxidase (MPO, 150 kDa, EC 1.11.1.7) is a white blood cell enzyme, stored in azurophilic granules of polymorphonucleocytes and macrophages. MPO catalyses the conversion of chloride and hydrogen peroxide into hypochlorite. MPO may contribute to the pathophysiology of ACS, as the hypochlorite end product is an oxidizing agent of low density lipoprotein (LDL) and play a key role in the degradation of collagen and contributing to the destabilisation of the plaque. Patients with ACS who have elevated MPO are at risk of short and long-term adverse outcomes. A study of the ED population demonstrated that MPO was associated with a 4 fold risk of major adverse cardiac events at 30 and 180 days in patients who were cardiac troponin negative.

Choline is a product of phosphodiesteric cleavage of membrane phospholipids such as phosphatidylcholine and sphingomyelin; catalysed by phospholipase D (EC 3.1.4.4). Physiologically choline provides cell structural integrity, is the precursor for acetylcholine production and a source of methyl groups that participate in the S-adenosylmethionine synthesis pathway.

Whole blood (WBCHO) and plasma choline concentrations increase after stimulation of phospholipase D and the activation of coronary plaque cell surface receptors or ischemia. Phospholipase D activation in coronary plaques causes stimulation of macrophage by oxidised LDL, secretion of matrix metalloproteinase enzymes and activation of platelets. WBCHO can be measured by high performance liquid chromatography coupled to mass spectrometry (HPLC-MS). In a study of over 300 patients with suspected ACS, WBCHO measured at admission was a significant predictor of cardiac death, cardiac arrest, arrhythmia, heart failure or the need for percutaneous coronary intervention at 30 day follow up [9]). The predictive power was enhanced by the addition of either cTnT or cTnI and served not as a marker of myocardial cell necrosis but identified patients at high risk with unstable angina. WBCHO is therefore a better predictive tool than plasma choline for early risk stratification in patients who are cardiac troponin negative on admission. The current detection methodology using HPLC-MS is not suitable for urgent clinical use.

Free fatty acids are produced from the breakdown of triglyceride. The majority of free fatty acids (FFA) circulate bound to albumin with a very small percentage appearing as the unbound free fatty acid (FFAu) form [10]). The circulating level of FFA is limited to the availability of the albumin binding sites. The mechanism of FFAu release is not fully understood however increased catecholamines following cardiac ischemia may activate FFA release following lipolysis in adipocytes. FFAu are 14-fold higher post percutaneous coronary intervention (PCI), compared to pre procedural concentrations and were higher in those with associated ischemic ST segment changes on the ECG [11]). Using a recombinant fatty acid binding protein bound to a fluorescent tag (ADIFAB) [12;13]) has been developed and a second generation assay using a fluorescent molecular probe (ADIFAB2) and a portable reader makes this a potential early marker for the point of care setting. Whilst this marker shows promise in the early phase of ischemia induced ACS, further trials are required to evaluate the diagnosis and prognostic value of FFAu in the chest pain population.

A reduction in oxygen supply versus causes localized acidosis and the generation of free radicals. Copper and zinc ions, normally bound to proteins in the plasma are released from protein binding sites to circulate in the free form [14-16]). The N-terminus of albumin binds transition metals. The N-terminus however, is susceptible to biochemical alteration [17]). The altered form is referred to as ischemia modified albumin (IMA). Following a period of ischemia, a reduction in the ability of albumin to bind cobalt is apparent. This is the basis of the albumin cobalt-binding test (ACB® test) for IMA. IMA has been extensively studied in the basic science and clinical research settings and is an FDA cleared CE marked clinical assay for the detection of cardiac ischemia.

III. BIOCHEMISTRY OF ISCHEMIA MODIFIED ALBUMIN

A. Mechanism of IMA generation

The NH_2-terminal of human serum albumin (HSA, 66.5 kDa, 585 amino acids) is known to be a binding site for transition metal ions such as cobalt, copper and nickel [18]). Using one and two dimensional 1H-NMR studies, Sadler and colleagues demonstrated binding of Ni^{++}, Cu^{++}, Co^{++}, Cd^{++} and Al^{+++} to bovine and human serum albumin. Strong binding was associated with three N-terminal amino acid residues (Asp-Thr-His in bovine albumin and Asp-Ala-His in human albumin). A Lysine residue designated Lys4 is also involved in the binding site. The authors demonstrated for the fist time selective reduction in the intensities of resonances to the $\square CH_2$ resonance of Lys4 on the addition of Co^{++} to HSA. There are in fact, four metal-binding sites with different specificities in HSA. In addition to the NH_2-terminal, three other sites occur at (i) reduced cysteine at residue Cys34, (ii) site A, including histidine at His67 as a ligand and (iii) the non-localized site B. Cu^{++} and Ni^{++} preferentially bind the NH_2-terminus site. Cd^{++} bind sites A and B, Zn^{++} binds site A and Au^{+} and Pt^{++} bind at residue Cys34.

It is currently not known if there are any significant changes in total human serum albumin between ischemic and non ischemic patients in the general chest pain population. Many divalent metals bind HSA in the circulation but in concentrations far lower than that required to impact albumin directly. The N-terminal portion of HSA is susceptible to biochemical degradation and is less stable than the albumin of other species [17]) including bovine, dog, goat, horse, pig rabbit, rat and sheep but not Chicken. Using electrospray-mass spectrometry and N-terminal sequencing, Chan and colleagues have demonstrated degradation corresponding to the first two resides (Asp-Ala) which is dependent both on temperature and the N-terminal alpha-amino group.

IMA however is a form of HSA where the N-terminal amino acids are unable to bind transition metal ions. Myocardial ischemia is known to generate free radicals [16;19]), induce localised acidosis [15]) and the release of free iron and copper ions bound to enzymes and proteins. [20;21]). Direct evidence of Cu/Fe mobilization in the coronary flow following prolonged (25-60 minute) ischemia but not short (15-21 minute) ischemia has been demonstrated [21]). Both copper and iron concentrations in the first coronary flow fraction were 50-fold and 15-fold higher respectively following prolonged ischemia, compared to pre-ischemic concentrations. This suggests that both copper and iron play a causative role in ischemic cardiac injury by their ability to catalyse the production of free radicals

and could be the target of therapeutic intervention to salvage tissue damage [20]). It was therefore postulated that following a period of cardiac ischemia, these processes would result in a change in the ability of the N-terminus of HSA to bind transition metal ions. The release of these ions likely initiates one potential pathway for IMA generation, rather than be considered an interference that may negatively affect IMA. In support of this suggestion, decreased albumin cobalt binding was reported in 99 acute chest pain patients with myocardial ischemia [22]) compared to 44 chest pain patients with no evidence of myocardial ischemia. Albumin cobalt binding was also assessed in 41 patients undergoing elective coronary artery angioplasty. Samples were tested using the Albumin Cobalt Binding (ACB) assay before, immediately after, 6 and 24 hours post procedure and compared to results from 13 patients undergoing cardiac catheterization without balloon angioplasty, thus serving as the control group. ACB concentrations were significantly elevated immediately post procedure, compared to the control population and ACB concentrations returned to baseline after six hours [23]); suggesting that HSA undergoes a significant reduction in the capacity to bind exogenous Co^{++} immediately after coronary artery occlusion induced during elective angioplasty. Modification of the Asp-Ala-His-Lys site by N-terminal acetylation or deletion of one or more residues abolishes this cobalt binding [24]).

The postulated mechanism (figure 2) of IMA generation is that localised ischemia results in acidosis. The localised acidotic environment stimulates the release of Cu^{++} ions from weak binding sites on circulating proteins such as caeruloplasmin. Caeruloplasmin (EC 1.16.3.1, 151kDa) is a ferroxidase enzyme encoded by the *CP* gene located on Chromosome 3. The enzyme is synthesised in the liver and carries approximately 70% of the total copper in human plasma (a further 15% carried by HSA and the remainder by macroglobulins). Each enzyme molecule contains 6 atoms of copper within

In the presence of a reducing agent such as ascorbic acid, free copper II is converted to copper I which can react with oxygen to form copper II and generate superoxide free radicals ($O_2^{\bullet -}$). Superoxide dismutase (EC 1.15.1.1) converts the superoxide free radical to hydrogen peroxide which is then degraded by catalase. The copper II ions released are immediately scavenged by human serum albumin but they are tightly bound to the N-terminus. Copper bound albumin is then damaged by hydroxyl free radicals ($OH^{\bullet}$), causing removal of the three N terminal amino acids and release of the copper II ion to repeat the process in a chain reaction [25]). Marx and Chevion demonstrated by SDS/polyacrylaminde gel electrophoresis the site specific alteration of HSA in the presence of 50 □M Cu^{++} and increasing portions of 0.2 mM ascorbate; where after the addition of 5 portions, bands at 3, 18, 22, 47 and 50 kDa were observed and also demonstrated that degradation does not occur in the

absence of Cu++ or in the addition of 1 mM ethylenediaminetetraacetic acid (EDTA) or citrate chelating agents [25]).

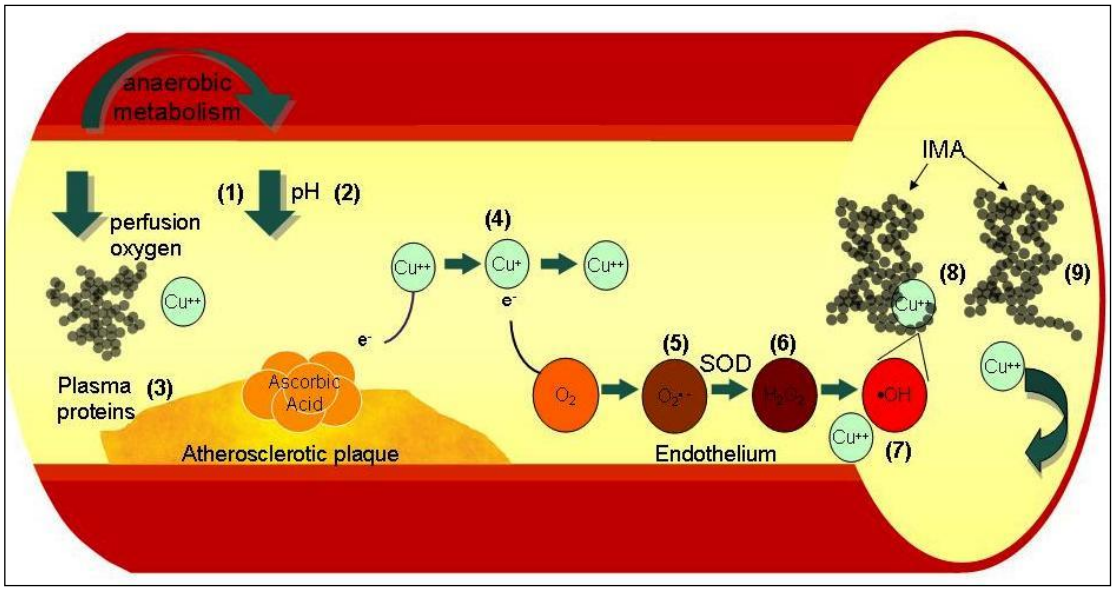

Fig 2: Mechanism of Ischemia Modified Albumin generation. (1)Tissue hypoxia from anaerobic metabolism reduces ATP and causes a (2)lower localized pH inducing acidosis. (3)Cu^{++} ions are released from plasma proteins such as caeruloplasmin. In the presence of ascorbic acid, (4)Cu^{++} is converted to Cu^{+}. Cu^{+} reacts with O_2 to form (5) $O_2^{\bullet-}$. Superoxide dismutase dismutates the $O_2^{\bullet-}$ to (6)H_2O_2, which in presence of Cu^{++} or Fe^{+}, undergoes the Fenton reaction forming (7)$OH^{\bullet}$ hydroxyl radicals. Free Cu^{++} is scavenged by (8)HSA, where it binds tightly to the N-terminus. $OH^{\bullet}$ radicals alter the amino acid N-terminus of (9)HSA rendering it incapable of binding Cu^{++}. These two altered forms are known as IMA.

This postulated mechanism, although theoretically attractive has not been borne out in practice. In a study of patients with increased IMA, the N-terminal portion of albumin was sequenced in 8 cases [26]) by cleavage of the 11 amino acid resides at the NH_2 terminus, by rapid liquid-phase Edman degradation. The N-terminal amino acid sequence showed normal residues for 6 of 7 patient samples with elevated IMA and one non-ischemic sample. The remaining patient sample with high IMA demonstrated two missing amino acids at the N-terminus. Clinically the patient did not have an ischemic cardiac event (table 1).

Table 1

NH_2-terminal HSA sequence analysis from 6 ischemic patients, a control (wild type) and one non ischemic patient with a high serum IMA (Source: Adapted from Bhagavan et al, Clin Chem 2003;49:581-585)

Subject	NH_2-terminal HSA Sequence

Control (wild type)	DAHKSEVAHRF
Non-ischemic patient, high serum IMA	--HKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF
Ischemic patient, high serum IMA	DAHKSEVAHRF

Furthermore, the *in vivo* half life of HSA is 19-20 days. HSA with a truncated NH_2-terminus would presumably have similar *in vivo* half life properties and yet IMA returns to baseline rapidly after an ischaemic cardiac event. This indicates that the alteration to albumin to create IMA is transient and reversible, rather than a finite chemical alteration. Recent physicochemical studies using electronic absorption EPR and NMR spectroscopy of Co-binding to HSA under anaerobic conditions to prevent Co^{++} oxidation have suggested a different explanation. Using competition experiments with cadmium (Cd^{++}) which binds sites A and B and Cu^{++} which binds the NH_2-terminus, three binding sites for Co^{++} were identified on HSA. Sites A and B showed greater avidity for Co^{++} binding than the NH_2-terminal binding site [27]). Fatty acid binding to albumin occurs at one of the additional cobalt binding sites with a negative allosteric interaction. It is hypothesised, that in myocardial ischemia the release of fatty acids results in binding of fatty acids to albumin. This would then reduce the ability of albumin to take up cobalt hence account for the presence of IMA [27]). If this also produced a conformational change in the albumin affecting the N terminal site, this would also reduce cobalt binding.

B. Kinetic release of Ischemia Modified Albumin

Studies in patients receiving angioplasty where ischemia is induced in a controlled manner, have defined the kinetics of IMA production. There is a rapid rise in IMA values after balloon inflation with a subsequent fall at 6 hours and return to normal by 24 hours [28;29]). The rise in IMA occurs earlier than the rise in cardiac troponin and natriuretic peptides (figure 3) and occurs early after the onset of plaque rupture.

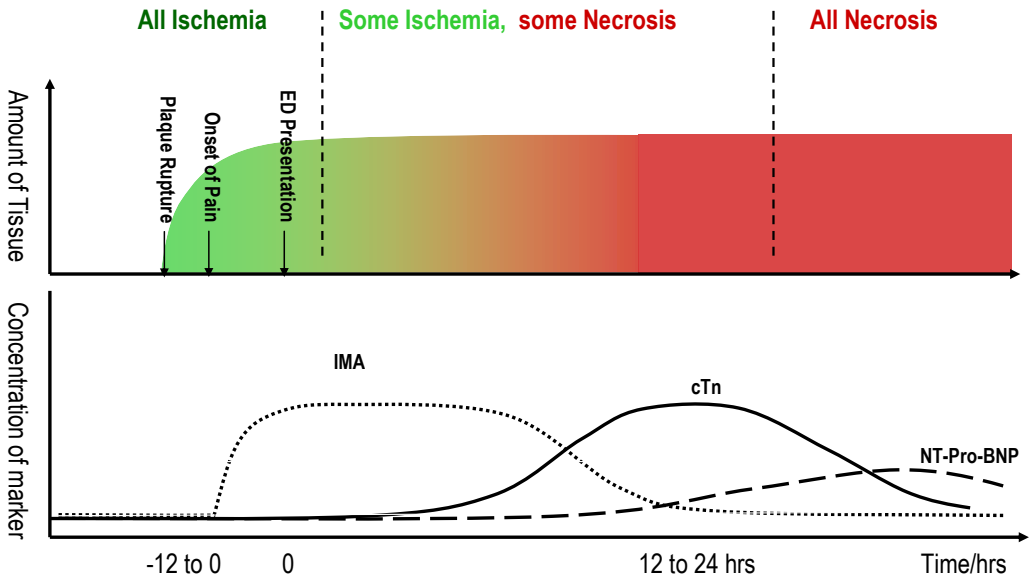

Fig 3. Kinetic release of Ischemia modified albumin (IMA, dotted line) and other cardiac markers, cardiac troponin (cTn, solid line) and natriuretic peptide (NTproBNP, dashed line) [bottom panel], in relation to extent and timing of tissue damage [top panel]

The magnitude of IMA elevation has been found to correlate with the number and frequency of transluminal balloon inflations during the PCI procedure [30]). 34 patients received standard routine care for elective single vessel PCI for the management of stable angina pectoris. 44% of patients received 1-4 balloon inflations whilst, 56% received ≥5 inflations. IMA concentrations were higher in those with more balloon inflations, higher pressure load of the balloon and the longer the duration of the inflation, thus IMA is not only a marker of the occurrence of ischemia but is also an indicator of the severity of the ischemic episode.

Furthermore, IMA concentrations are lower in patients who demonstrate angiographic evidence of collateral vessels present in the coronary circulation, according to Rentrop's classification [31]). IMA levels post PCI are higher than baseline, however post-PCI values are lower compared to post-PCI values in those patients without a collateral circulation; irrespective of the extent of coronary artery disease or those who underwent a large number of balloon inflations for longer duration. [32]) The lower IMA concentrations in patients with a collateral circulation likely represent a cardioprotective effect against PCI-induced ischemia. IMA elevation is also correlated to the need for subsequent

revascularization [33]). Elevated IMA greater than 130 kU/L was associated with a higher frequency of target lesion revascularization at 4-years follow-up in 60 patients who underwent a successful elective single vessel PCI for stable angina pectoris at baseline. The accepted gold standard blood marker for myocardial ischemia is myocardial lactate extraction. Simultaneous IMA and lactate was measured in 10 patients undergoing PCI for chronic stable angina. Post-PCI IMA concentrations paralleled that of transmyocardial lactate[29]).

Elevation in serum IMA has been recorded following coronary vasospasm [34]). Twenty six patients with variant angina underwent intracoronary ergonovine spasm provocation testing. Arterial IMA concentrations were measured pre and post procedure and compared to 18 patients undergoing elective PCI and 10 patients with normal coronary angiography. IMA was significantly elevated following drug induced coronary vasospasm compared to baseline and elevated values detected coronary vasospasm with an area under the ROC curve of 0.98 (95%CI 0.92-1.00). Other studies involving invasive cardiac procedures have shown rises in IMA where ischemia might occur, occurring concurrently with ECG changes in cardioversion [35]), but show a variable picture when there is non-ischaemic myocardial damage as in cardiac ablation [36;37]).

C. Measurement of Ischemia Modified Albumin

The original biochemical test for IMA was known as the albumin cobalt binding (ACB®) assay. This was developed by Ischemia Technologies Inc, Colorado, USA). The assay measures the cobalt binding capacity of albumin in a sample of serum. A known amount of cobalt is added to the patient serum sample. Dithiothreitol (DTT) is added which binds any remaining unbound cobalt and the colorimetric change is measured spectrophotometrically. In serum from non-ischaemic patients, cobalt binds to the N-terminus of HSA, leaving little free cobalt to react with DTT and form a coloured product. Conversely, in serum of patients with ischemia, cobalt does not bind to the N-terminus of modified HSA, leaving more free cobalt to react with DTT and form a darker colour. As normal albumin will bind cobalt, the amount of free cobalt, hence the absorbance will be proportional to the amount of IMA present (figure 4).

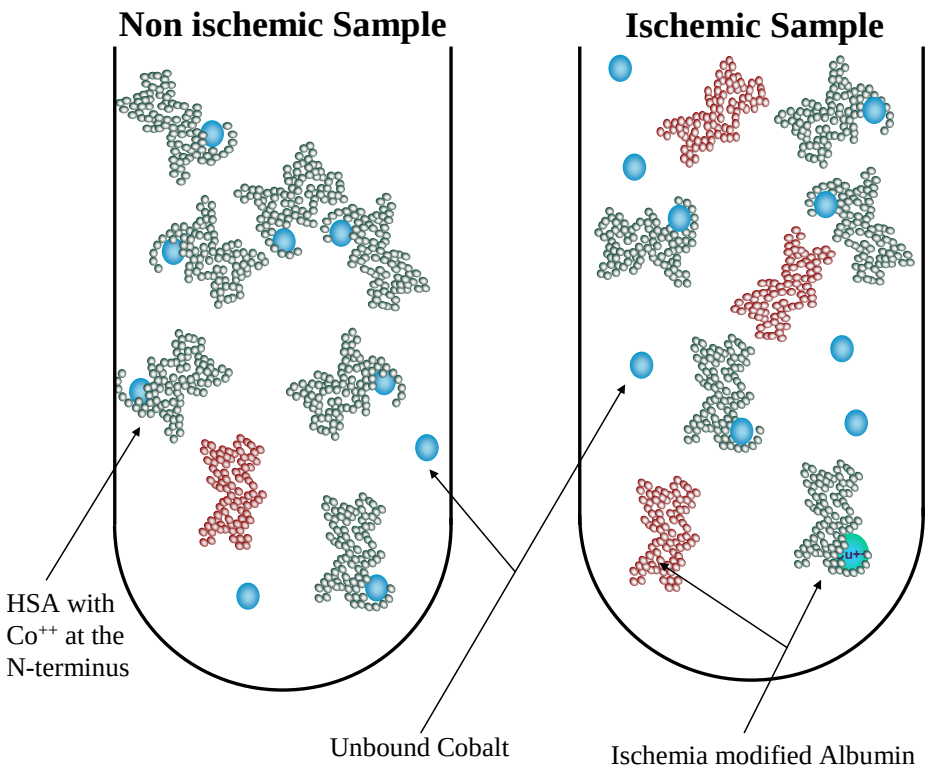

Fig 4. Measurement of Ischemia Modified Albumin by the Albumin Cobalt Binding (ACB) assay. A known amount of $CoCl_2$ is added to a serum sample. DTT is added which binds unbound Co^{++} causing a colorimetric change read spectrophotometrically.

The first generation assay was semi-automated and required a sample pre-treatment step where 500□L of serum sample was added to an Eppendorf tube containing 0.45g $CaCl_2$. The sample was inverted twice and centrifuged at 1200g for 10 minutes. 300□L of supernatant was removed for assay of IMA. For the assay, powdered DTT was provided which required reconstitution in the ratio 15mg DTT: 10 mL diluent. The reconstituted reagent and power required storage at 2-8ºC and the working solution had a shelf life of 3-5 days. The second generation of the assay used 7.5mg DTT to 10mL diluent. The third generation assay which became commercially available did not contain the sample pre treatment step and the assay kit contained a concentrated liquid form of DTT which was reconstituted in buffer with a fixed volume 200 □L pipette. This assay has been approved by the Food and Drug Administration (FDA) in the USA as an adjunct test to rule out myocardial ischemia. The assay is also CE marked in Europe. The assay can be performed manually with a spectrophotometer, however it was also initially automated on the Cobas MIRA Plus (Roche Diagnostics) automated spectrophotometer [38]). The assay has since been adapted for other automated clinical chemistry platforms including the LX-20 (Beckman Coulter, Brea, CO, USA) [39]), Hitachi 911 (Hitachi, Japan) [40])Hitachi 7600 (Hitachi, Japan) [41]) and the Konelab 20 (Thermo Scientific,

United Kingdom) [42]). To date a commercialised point of care device for IMA remains to be developed however a pre-commercial portable spectrophotometer and IMA assay has been developed by (Microwells Biotechnology Co Ltd, Shanghai, China). In the Microwells assay, the DTT has been replaced with a more stable azo dye chromogen in the Microwells assay. An automated method to measure ischemia induced alterations of the binding capacity of HSA for nickel [43]) has been described [44]). The nickel binding assay correlated well to the ACB assay ($r=0.5387$, $p<0.001$) however the area under the curve (AUC) of the Receiver Operating Characteristic (ROC) curve was higher for the nickel binding assay (0.7582) compared to the ACB assay (0.7289) suggesting the nickel binding has a superior ability to discriminate between ACS and non-ACS compared to the ACB assay. There are rumours of an ELISA assay for IMA. This assay is not validated for clinical diagnostic or therapeutic use and an independent performance validation and comparison to the ACB assay does not appear in the literature. The development of an ELISA however is probably not valid given the rapid alteration and return to baseline of IMA following ischemia, suggesting the alteration is transient and not a permanent change to a specific epitope which could be detected by an antibody.

The *in vitro* stability of the IMA has been shown to be two hours at either 4°C or 20°C, but values increase significantly after four hours irrespective of the storage temperature [30]). It is likely that the changes are due to *in vitro* pH changes altering the metal binding capacity of human serum albumin. Samples frozen at -20° C are stable although values have been reported to be slightly higher once thawed, compared to freshly analysed samples [45]).

A study of 109 subjects (55 men and 54 women; age range, 20 to 85 years) to determine the 95th percentile reference range for IMA has been performed [46]). The concentrations ranged from 25.7 to 84.5 kU/L with an upper 95th percentile of 80.2 kU/L. This study used the first generation of the assay utilising the pre-handling sample preparation step. Further studies of healthy subjects have reported higher IMA ranges. Abadie and colleagues demonstrated a mean IMA value of 89 kU/L from 69 subjects with a mean age of 49 years [47]) whilst Maguire and colleagues demonstrated a 97.5th percentile of 110 kU/L [39]) from a population of 81 healthy volunteers (28 men and 53 women aged 22-86 years). Values ranged from 82.0 to 110 kU/L and values were similar between males and females (99.1 vs 100.7 kU/L, $p=0.12$). The biological variation of IMA has been studied [48]). In a population of 17 apparently healthy individuals (7 male, 10 female, aged 26-61 years), the within subject coefficient of variation was 2.89% and the between subject coefficient of variation was 6.76%, calculated from weekly blood draws performed at the same time by the same phlebotomist for 5 consecutive weeks. Again there was no specific gender difference in IMA concentrations however the authors reported statistically different IMA

concentrations between Caucasian and Black populations, with higher IMA concentrations in Black males and females compared to Caucasian counterparts.

Total serum albumin concentrations might be expected to affect the performance of IMA measurement. There is a relationship between IMA values and serum albumin concentration, although this is much less marked across the reference interval for albumin [49]). The use of an albumin adjusted correction has been proposed [50]) although a reference interval study found albumin correction to have little impact compared to other analytical factors [39]). It has been reported that the changes in IMA observed in patients with chest pain was attributable only to changes in the serum albumin concentration [51]).

IV. UTILITY OF ISCHEMIA MODIFIED ALBUMIN IN CHEST PAIN PATIENTS

Clinical validation of any test for ischemia is difficult as there is currently no accepted diagnostic gold standard, although blood lactate has been used previously. In addition, there is no predicate test which can be used against which to perform an initial validation. The initial studies using IMA were based on the ability of an early measurement to predict the final diagnosis of AMI as defined by the elevation of cardiac troponin at 6-12 hours post chest pain. Two studies utilised the first generation pre-release ACB test and a third study manufactured an in-house method. The first study examined acute coronary syndrome (ACS) patients and utilised serial sampling on admission and two subsequent samples [52]). Diagnostic sensitivity of the admission sample for a final diagnosis of AMI was 23.9% for cardiac troponin I (cTnI) alone, 39.1% for IMA alone and 55.9% for the two combined. The second study examined enrolled 256 ACS patients [53]). AUC of the ROC curve for the ACB test was 0.78 with a sensitivity and specificity of 83% and 69% respectively at the optimised decision threshold for AMI. The third study enrolled 75 patients with ischemia and 92 non-ischaemic patients [26]). IMA had poor predictive power in discriminating between AMI and non-AMI in patients with underlying ischaemic heart disease (AUC of 0.66). However, the test gave good discrimination between patients with or without ischemia. The AUC for the ROC curve for diagnosis of ischemia was 0.95 with sensitivity of 94% and specificity of 88%. In these initial studies there were significant problems with sample stability and the assay involved in addition of calcium chloride and centrifugation as part of the routine method. This made the method unsuitable from routine analysis and the assay was reformulated.

The majority of patients who present to hospital with chest pain and suspected ACS are eventually ruled out for acute myocardial infarction and active unstable coronary disease. The ideal role of an ischemia marker would therefore be as rule out test. The most logical place to use such a test is therefore in the ED. A study of ED presentations examined 208 patients the diagnostic sensitivity of IMA measurement alone was 82% at 46% specificity in samples taken within the first 3 hours. The combination of ECG, cardiac troponin T (cTnT) and IMA showed 95% sensitivity for diagnosis of ACS at presentation [54]). One year follow up performed on this population demonstrated a survival disadvantage in patients with IMA greater than the median concentration of the study group [55]). A subsequent study of 538 patients admitted to a chest pain evaluation unit found admission measurement of IMA plus cTnT had 100% sensitivity for prediction of a final diagnosis of AMI [1]). The presence of an elevated IMA and an elevated cTnT on admission predicted 21% risk of major adverse cardiac events (MACE) compared to patients where both were not elevated, even in patients where the final diagnosis excluded AMI by troponin based criteria. IMA measurement appears to work best as part of a panel of other tests or a test sequence [47]). Admission measurement of IMA has been found to be superior to biomarkers of necrosis and to show 97% sensitivity when combined with them. Not all investigators have considered the diagnostic performance of IMA either alone or in combination with cardiac troponin, or other biomarkers of necrosis, to be adequate. A prospective ED study enrolling 277 patients and using a positive IMA or troponin as the index test and an 8 hour troponin as the definitive test found only a 97.6% sensitivity with 97% negative predictive value. The investigators did not consider this to be adequate when compared with troponin but did not provide any follow-up data [56]). A second large study prospectively enrolling 189 patients presenting to the ED with chest pain and found an elevated IMA was a poor predictor of cardiac events within the next 72 hours [57]). Conversely, another study found elevated IMA predicted long-term cardiac events [58]). The most consistent finding across all studies of IMA is of a high negative predictive value. This has been highlighted in a meta-analysis specifically examining the role of IMA as a rule out test [59]). The summarised data of over 1800 patients demonstrated a triple negative prediction test (non-diagnostic ECG, negative cTn and negative IMA) with a sensitivity and negative predictive value for ACS of 94.4% and 97.1% respectively.

The prognostic value of IMA in the ACS setting has been investigated [55;58;60;61]). Using a ROC derived cut off of 477 KU/L, Aparci and colleagues found significantly higher mortality at one year in those who had serum IMA >477 KU/L, compared to those with IMA <477 KU/L [58]). Furthermore, using cox regression modelling, IMA was related to mortality, independently of the presence of hypertension, diabetes or advanced age. In a larger cohort of 245 consecutive attendances to the ED, in which there were 31 composite endpoint (cardiac death, AMI or recurrent angina) at 30-days from presentation and 16 deaths at one year; the short and long term ability of IMA to predict outcome

was assessed. Short term survival was significantly compromised in those with IMA > 93.3 KU/L compared to those with lower IMA concentrations at both 30 days and 1 year [55]). Using the cohort of the French Nationwide OPERA study IMA, cTn, CRP and BNP were measured within 24hours from admission in 471 patients hospitalized with AMI. Using a primary end point of death, resuscitated cardiac arrest, recurrent AMI or ischemia, heart failure or stroke, 75 in-hospital events and 144 events at 1 year were recorded. Using quartile analysis, 40% of patients reached the end point with IMA concentrations in the highest quartile (>104 KU/L), compared to only 20% of patients in the lowest quartile of 83 KU/L [61]). In those STEMI patients who are treated with primary PCI, IMA is a powerful predictor of 30-day mortality however it does not add to the validated Thrombolysis In Myocardial Infarction (TIMI) risk score [62]).

V. UTILITY OF ISCHEMIA MODIFIED ALBUMIN IN NON-CHEST PAIN PATIENTS

Any marker associated with pathological processes upstream of cardiac necrosis will invariably suffer from a lack of specificity; unlike the cardiac troponins for cardiac necrosis. The further upstream in the ischemic continuum the more likely is the lack of cardiac specificity of the biomarker. Elevations in circulating IMA concentrations are not specific for myocardial ischemia. Mechanistically, IMA can be generated during any ischemic process within the body. A comprehensive review of IMA elevations in non-cardiac conditions is beyond the scope of this chapter but an in-depth summary is given in table 2. Those conditions that have been studied most are explained in more detail below.

Table 2

Alteration to serum IMA concentrations in conditions other than acute coronary syndrome.

Skeletal muscle ischemia

Studies of subjects with skeletal muscle ischemia have produced contradictory results. In healthy subjects undergoing arduous physical exertion, IMA has been reported to fall immediately post exercise and then subsequently rise [63-65]) or return to normal [66]). Subjects undergoing a forearm ischemia test when the forearm muscles are exercised for 1 minute with the external compression of the arm blood supply showed a fall in IMA, maximal at 3 minutes from the test, returning to baseline by 30 minutes [67]). A similar rise in serum lactate occurred. Conversely during standardized exercise in a plantar flexion pedal combined with inflation of a femoral blood pressure cuff (at 0, 60, 90, 120 and 150 mmHg) to induce calf muscle ischemia an increase in IMA was observed after release

of the cuff and returned to baseline within 30 minutes [68]). Peri-operative skeletal muscle ischemia induced by femoral blood pressure cuff being inflated to 300 mmHg in 23 patients undergoing arthroscopic knee surgery. Increased IMA and myoglobin and decreased albumin were observed following release of the cuff [69]). In patients with peripheral vascular disease (PVD) undergoing a treadmill walk test, a decrease in serum IMA immediately post-test has been documented [70;71]). In 40 consecutive patients undergoing exercise electrocardiography, a significant decrease in IMA at peak exercise then a subsequent rise in IMA has been observed, however there was no difference in IMA concentrations between those patients with positive and negative stress test results [72]). Revascularisation for PVD is accompanied by a post procedural rise in IMA [70;71;73]). In skeletal muscle ischemia, an initial fall with subsequent rise appears to be a consistent finding without adequate explanation. Smooth muscle ischemia does not appear to be associated with a rise in IMA [74]). The effect of skeletal muscle on ischemia will limit the application of IMA measurement after cardiac stress testing for detection of myocardial ischemia and may explain the inconsistent findings[51;72;75;76]).

Ischemic Stroke

Patients with acute ischemic stroke demonstrate abnormalities in a number of biomarkers of nitrosative and oxidative stress. In 41 patients with ischemic stroke, Senes and colleagues demonstrate that nitrate, IMA and thiobarbituric acid-reactive substances (TBARS) concentrations are significantly increased compared to 37 age and gender matched controls [77]). In a larger cohort of 118 patients presenting within 3 hours of neurological deficit, IMA was elevated in those with cerebral infarction and intracranial haemorrhage (ICH) but normal reference values were observed in those with transient ischemic attacks (TIA) lasting less than 1 hour or those with epileptic seizures [78]). Within 24 hours of injury IMA increased during cerebral infarction but not in intracranial haemorrhage and may offer diagnostic utility in the differential diagnosis of neurological deficit. IMA also correlated with National Institutes of Health Stroke Scale (NIHSS) Score in both cerebral infarction and ICH. Conversely, Herisson and colleagues did not demonstrate a causal relationship between IMA or heart type fatty acid binding protein and NIHSS score or stroke volume. Ahn and colleagues have utilised an albumin-adjusted IMA index for the early detection of ischemic stroke [79]). In 52 patients, 28 (54%) with Ischemic stroke, 24 (46%) non-stroke, the AUC of ROC curve analysis was 0.928 for IMA but 0.99 for albumin-adjusted IMA index. The sensitivity and specificity of the IMA index was superior to IMA concentration alone.

Pulmonary embolus

Pulmonary embolus (PE) is an acute medical emergency estimated to occur in 3.5/1000 hospitalized patients. Patients experience sudden onset dyspnoea, tachypnoea, pleuritic-type chest pain, cyanosis and haemoptysis. PE has an

associated mortality of 26%. Diagnosis is primarily based on typical clinical presentation using the Wells and Geneva clinical probability scores. D-dimer measurement and pulmonary angiography are often clinically useful. The ECG can demonstrate acute *cor pulmonale* in large PE's but lacks specificity. IMA has been measured in a number of studies of PE patients. Turedi and colleagues [80]) have demonstrated that IMA was significantly elevated in 30 PE patients compared to 30 healthy controls and adequately discriminated between the presence and absence of PE. The positive predictive value of IMA for PE is higher than that for D-dimer (79.4% compared to 69.4%) and in combination with the Wells and Geneva criteria, IMA offers an alternative to D-dimer testing [81]).

Chronic kidney disease

Patients with chronic kidney disease (CKD) have a reduced life span compared to those without renal disease. Mortality rates are highest in those receiving haemodialysis as renal replacement therapy (RRT). Cardiovascular mortality accounts for the majority of renal deaths. Between 2001 and 2006, 24% of deaths in UK RRT patients were due to ischemic heart disease [82]). This rate is consistent with data from other countries. Cardiovascular morbidity is also increased. 55% of patients receiving haemodialysis RRT also have concomitant congestive cardiac failure. [83]). IMA levels have been determined in patients with CKD [84-86]) and in patients receiving haemodialysis (HD) [87-91]).

In 2006, Sharma and colleagues demonstrated that patients with elevated IMA have a significantly large left ventricle, decreased systolic function and greater estimated left ventricular filling pressure [85]). Further, in multivariate analysis, a positive dobutamine stress echocardiogram (DSE) combined with elevated IMA and cTnT and E/Ea ratio were independent prognostic factors for death. IMA values increase significantly in those patients with a positive DSE compared to those with no ischemic response [84]). In a modestly small study of 17 anaemic CKD patients and 19 controls, Cichota and colleagues demonstrated that IMA increased in patients compared to the control group. IMA correlated to lactate, haemoglobin and creatinine [86]).

Pre and post-HD IMA concentrations are significantly correlated [87]), however in this study IMA concentrations were not significantly different between those CKD patients with or without ischemic heart disease, diabetes mellitus or peripheral vascular disease. Fast intravenous iron administration during HD is associated with oxidative stress and inflammation. In a study of 20 HD patients receiving slow intravenous iron administration, IMA concentrations were significantly increased across three HD sessions independently of slow i.v. iron administration [88]). Following adjustment of albumin by two methods, post

dialysis IMA levels remain significantly increased following HD [89]). Paroxonase-1 (PON-1) is a calcium dependent esterase (arylesterase, aromatic esterase 1, serum aryldialkylpohosphatase 1, EC 3.1.8.1) is a major anti-atherosclerotic component of HDL cholesterol. PON-1 concentrations are lower in CKD patients with and without haemodialysis RRT compared to controls suggesting chronic oxidative stress and accelerated atherosclerosis are a feature of CKD. In a pilot study of CKD patients receiving HD, PON-1 concentrations were significantly and inversely correlated to IMA suggesting an oxidative stress and ischemic process occurs during HD [90]). Recently Albarello and colleagues have evaluated the effect of IMA and protein carbonyl groups as markers of protein oxidation in 23 CKD patients receiving HD. The authors confirm previous reports of higher IMA post-HD than pre-HD and observed a significant correlation between IMA and protein carbonyl groups, attributed to oxidative stress associated with HD [91]).

Hyperlipidaemia and Obesity

IMA measurement may be of benefit in hypercholesterolaemic patients. IMA is correlated to cholesterol, low density lipoprotein (LDL) and antibodies to oxidised LDL (ox-LDL) [21]). In a study of 37 subjects with hypercholesterolaemia compared to 37 controls, Duarte and colleagues [92]) confirm these findings observing IMA correlations to cholesterol, LDL ox-LDL antibodies and to high sensitivity C-reactive protein, suggesting that hypercholesterolaemia is associated with inflammatory and oxidative stress processes, contributing to the advancement of atherosclerosis. IMA is related to the presence of metabolic syndrome independently of age, gender, presence of diabetes or hypercholesterolaemia [93]). Furthermore, the use of 10 mg/day ezetimibe immunotherapy for a duration of 12 weeks in 31 hypercholesterolaemic patients reduced both LDL cholesterol and IMA [94]). The reduction of IMA was independent of the reduction in LDL suggesting that ezetimibe may reduce the burden of oxidative stress in hypercholesterolaemia.

IMA concentrations are higher in obese subjects, with a positive correlation between IMA and body mass index (BMI). In a large study of 148 volunteers in Brazil; subjects were classified as normal, overweight or obese, defined as BMI of 18.5-24.9, 25.0-29.9 and $\geq$30 kg/m^2 respectively. IMA concentrations increased exponentially between the three groups, the highest being in those subjects with BMI $\geq$30 kg/m^2. Similar findings have been demonstrated in obese postmenopausal women where IMA and IMA:Albumin ratio are higher in those subjects with BMI 26-32 kg/m^2 compared to those with BMI 21-25 kg/m^2. The obese concentrations were similar to those with documented coronary artery disease but normal BMI. In the obese women IMA was positively correlated to BMI, hs-CRP, insulin concentrations and homeostasis assessment model score [95]).

Diabetes Mellitus

Patients with type 2 diabetes mellitus who demonstrate poor glycaemic control have higher IMA concentrations than those with good glycaemic control. IMA was significantly higher in 76 diabetic patients compared to 25 control subjects and IMA concentrations are correlated to HbA1c [96]), glucose and hs-CRP [97]). Conversely, Dahiya and colleagues suggest no significant changes in IMA occur in 60 newly diagnosed type 2 diabetics, compared to 30 control subjects [98]). Diabetic patients who undertake chronic exercise for three months demonstrate lower post exercise IMA concentrations suggesting that exercise alleviates some of the oxidative stress associated with diabetes mellitus [99]).

Bowel Ischemia

Bowel (mesenteric) ischemia occurs infrequently however if not recognised early, carries a devastatingly high mortality. The presentation is often characterised by generalised abdominal pain, fever, diarrhoea or constipation, tachycardia, hematochezia (blood per rectum), nausea and vomiting. Diagnosis is difficult due to non-specific signs and symptoms, plain x-ray or laboratory tests (increased white blood cell count and serum lactic acid). Mesenteric angiography is considered to be the gold standard test which can differentiate between embolic, thrombotic or nonocclusive ischemia. In a preliminary study of 26 patients presenting with symptoms of internal ischemia, Polk and colleagues [100]) identified 12 with a positive clinical diagnosis. Positive patients had higher IMA concentrations than those without intestinal ischemia. IMA detected bowel ischemia with a sensitivity of 100% and a specificity of 86%. In a case-controlled study from Turkey, Gunduz and colleagues [101]) demonstrated that pre-operative IMA concentrations were significantly higher in patients with thromboembolic occlusion of the superior mesenteric artery (SMA) compared to an age-matched control group of healthy volunteers. A number of animal studies of mesenteric ischemia have provided conflicting results. In a Wistar rat model [102]) a time dependent response in IMA in mesenteric ischemia has been demonstrated. 36 mature female rats underwent either simple laparotomy in the control groups or laparotomy followed by clamping of the SMA in the subject group. IMA concentrations were highest 6 hours from ischemic onset, however IMA at 30 minutes and 2 hours were also significantly higher in the clamped group compared to the control group. Elevations of IMA tracked changes in both lactate and malondialdehyde. A similar time dependent change in IMA was demonstrated in New Zealand rabbits undergoing ligation of the SMA compared to either a control group or those undergoing a sham procedure [103])with elevation of IMA at 2 and 6 hours significantly higher than baseline and higher than IMA concentrations in the control rabbits. IMA concentrations mimicked elevations in serum IL-6 with elevated IL-6 in the ischemia group at 1, 3 and 6 hours, but no elevations in the sham operated or control group. In a further study of mesenteric ischemia in a Wistar rat model, Uygun and colleagues demonstrated similar IMA concentrations in control, sham, 2-hour and 6-hour post-SMA ischemia refuting the previous animal studies. It seems likely that IMA may offer additional diagnostic value in the early presentation of

mesenteric ischemia. Further prospective studies are required to assess both the diagnostic and prognostic ability of IMA in conjunction with mesenteric angiography to detect bowel ischemia. *Obstetric and gynaecological use of IMA*

The care of women and their unborn child during pregnancy is greatly challenging for obstetricians. The adult can interact and provide a history of signs and symptoms whereas the unborn child can only be examined indirectly by means of imaging, foetal heart monitors and a limited number of direct interventions. Women achieving spontaneous preterm (<37 weeks) labour account for 10% of all births and are attributable to 75% of neonatal deaths. The foetus relies entirely on the maternal placenta for O_2/CO_2 exchange. This delicate dependence, between the placenta and the foetus is crucial to normal healthy growth. Any malfunction or disruption to the adequate supply of oxygen can cause hypoxia and potentially fatal acidosis. A limited degree of acidosis is well tolerated by the foetus; however chronic acidosis or hypoxia may lead to a significant mortality and morbidity with potential log-term sequelae. Currently the mechanism of foetal hypoxia and acidosis is unclear, and physiological consequence of foetal acidosis is believed to target the cell energy availability and /or cell poisoning.

During pregnancy plasma proteins change markedly due to increased plasma volume, increased renal blood flow and altered protein synthesis in response to hormonal changes. Plasma volume expansion of up to 45% (1300mL) compared to the non-pregnant state causes an overall net decrease in plasma protein concentration by 10-12 g/L which is reached around week 28 of gestation. The predominant cause of lowered albumin is dilutional, oestrogen is known to affect albumin. The alteration to plasma albumin concentrations throughout the pregnancy period is shown in table 3. The lower concentration of albumin also results in an apparent decrease in substances normally bound to this protein.

Table 3

Alteration to plasma HSA concentrations during the gestational period.

Time point	Mean albumin concentration (g/L)	Reference interval (g/L)
Non-pregnant control	41	36-46
12 weeks	38	33-43
18 weeks	35	30-39
24 weeks	33	29-37
28 weeks	32	28-37
32 weeks	32	38-36
36 weeks	32	38-36
Full term	32	26-38
1 day post partum	29	23-38
6 weeks post partum	42	37-47

The HSA reference interval in the full term healthy neonate between term and day 4 is 28-44 g/L. Albumin concentrations increase a little from birth to puberty where the adolescent reference interval (day 4 to 14 years) is 38-54 g/L.

Current experimental studies suggest that foetal development occurs in a hypoxic intrauterine environment and the presence of reperfusion and oxidative stress is believed to be crucial for trophoblast development [104;105]). Trophoblast invasion of the maternal spiral arteries allows the increase of uterine blood supply necessary to maintain the pregnant state. Serum IMA during normal pregnancy is elevated compared to non-pregnant controls [106-109]). Prefumo and colleagues [106]) demonstrate supra-physiological IMA concentrations in early normal pregnancy (11-13 weeks of gestation) suggesting that trophoblast development occurs in a hypoxic uterus. In a large population of 117 pregnant women compared to non-pregnant healthy women, Guven and colleagues demonstrated a cross-sectional elevation in IMA in pregnant women. IMA increased significantly through each trimester. Further, there the authors demonstrated a significant negative correlation between IMA and HSA,

suggesting that elevated IMA in pregnancy represents a physiologic state of oxidative stress.

Increased intrauterine hypoxia predisposes to defective endovascular trophoblast invasion of the maternal spiral arteries which may possibly lead to the development of pre-eclampsia; a hypertensive state (>140/90 mmHg) associated with significant proteinuria (≥300mg/dL). Pre-eclampsia affects 6-8% of pregnancies worldwide. Papageorghiou and colleagues have demonstrated that first trimester serum IMA are significantly higher in women who develop pre-eclampsia compared to those with a normal pregnancy [110]). Both IMA and normalised IMA (IMA:Albumin, ratio) were higher in 20 pre-eclamptic women compared to 22 normal pregnancies [109]). These data suggest IMA could be a biological marker of pre-eclampsia however larger studies are required to fully characterise the supra-normal IMA and normalised IMA reference interval in normal pregnancy.

Maternal IMA and normalised IMA concentrations are also increased in women with recurrent pregnancy loss (two or more unexplained miscarriages in the first trimester) compared to healthy pregnancy [111]), suggesting that an increase of intrauterine oxidative stress and hypoxia contribute to placental deficiency and subsequently recurrent early miscarriage.

The use of umbilical cord blood for IMA has also been examined. Neonatal cord blood IMA concentrations are higher than IMA concentrations in healthy adults [112]) but is not attributable to changes in HSA concentration. Elevated fetal IMA may reflect transient localised ischemia from external forces exerted on the foetus during labour. In a case-control study of 26 newborns, 12 delivered at normal term and 14 with complicated labour or delivered pre-term; cord blood IMA concentrations were significantly higher (50%) than those with uneventful deliveries, suggesting IMA is a marker of fetal distress. Doubly-clamped cord blood IMA concentrations are similar in intrauterine growth restriction, compared to those delivered with appropriate for gestational age full-term pregnancies [113]). The similar IMA concentrations may be due to the ‘brain sparing effect’ accompanied by oligohydraminos (deficiency in amniotic fluid), which is characterised by rerouting the blood supply and the nutrient to vital organs such as the heart, brain and adrenal glands. IMA concentrations in cord blood are higher following caesarean section compared to vaginal delivery and in multigravida compared to primigravida [113]) and may be attributable to higher oxidative stress on both accounts. Cigarette smoking during the gestational period alters the oxidant/antioxidant balance in favour of oxidative stress. In response, IMA and MDA concentrations in pregnant smokers are significantly higher and vitamins A and E, SOD and total antioxidant capacity are significantly lower, compared to non-smoking pregnant women [114]).

Although there are a number of postulates, the mechanisms by which IMA is generated remain unexplained. Indeed the evidence that intact HSA exists in patients with ischemia and the remarkably rapid return of IMA concentrations to pre-ischemic levels questions the N-terminal deletion theory. It has been suggested that IMA is in fact a marker of oxidative stress as conditions associated with raised IMA can be associated with other markers of oxidative stress such as carbonyl residues, nitrate and TBARS. Unfortunately such markers may also be associated with elevated markers of cardiac damage and dysfunction, such as cardiac troponin and natriuretic peptides.

Despite this, ischemia modified albumin currently remains the only ischemia assay to have reached the clinical validation stage and obtained CE marking and FDA approval for the diagnosis of cardiac ischemia. The most suitable clinical role for IMA in the acute chest pain population is in conjunction with the ECG and cTn for a triple negative rule out test. IMA alone due to its lack of cardiospecificty cannot be used as a single rule in or rule out cardiac biomarker. For non acute populations it seems that IMA is associated with oxidative stress and may identify affected patients. Furthermore, as some studies have shown, patients who receive anti-oxidative agents demonstrate lower IMA concentrations post therapy. IMA therefore may be useful in the monitoring and guidance of anti-oxidant therapy.

IMA has the potential to be enormously valuable in assessing both chest pain patients and those with conditions associated with oxidative stress. A better understanding of this marker is required before it is acceptable for prime time use.

REFERENCES

1. Collinson PO, Gaze DC, Bainbridge K et al.: Utility of admission cardiac troponin and "Ischemia Modified Albumin" measurements for rapid evaluation and rule out of suspected acute myocardial infarction in the emergency department. *Emerg.Med.J.* 23:256-61 (2006)

2. Collinson PO, Gaze DC.: Ischaemia-modified albumin: clinical utility and pitfalls in measurement. *J.Clin.Pathol.* 61:1025-8 (2008)

3. Pope JH, Aufderheide TP, Ruthazer R et al.: Missed diagnoses of acute cardiac ischemia in the emergency department. *N.Engl.J.Med.* 342:1163-70 (2000)

4. Collinson PO, Premachandram S, Hashemi K.: Prospective audit of incidence of prognostically important myocardial damage in patients discharged from emergency department. *BMJ* 320:1702-5 (2000)

5. Pozen MW, D'Agostino RB, Selker HP, Sytkowski PA, Hood WB, Jr.: A predictive instrument to improve coronary-care-unit admission practices in acute ischemic heart disease. A prospective multicenter clinical trial. *N.Engl.J.Med.* 310:1273-8 (1984)

6. de Dombal FT, Clamp SE, Softley A, Unwin BJ, Staniland JR.: Prediction of individual patient prognosis: value of computer-aided systems. *Med.Decis.Making* 6:18-22 (1986)

7. Goldman L, Cook EF, Brand DA et al.: A computer protocol to predict myocardial infarction in emergency department patients with chest pain. *N.Engl.J.Med.* 318:797-803 (1988)

8. Willems JL, Willems RJ, Bijnens I, Doerr R, Verstraete M.: Value of electrocardiographic scoring systems for the assessment of thrombolytic therapy in acute myocardial infarction. The European Cooperative Study Group for Recombinant Tissue Type Plasminogen Activator. *Eur.Heart J.* 12:378-88 (1991)

9. Danne O, Mockel M, Lueders C et al.: Prognostic implications of elevated whole blood choline levels in acute coronary syndromes. *Am.J.Cardiol.* 91:1060-7 (2003)

10. Richieri GV, Kleinfeld AM.: Unbound free fatty acid levels in human serum. *J.Lipid Res.* 36:229-40 (1995)

11. Kleinfeld AM, Prothro D, Brown DL, Davis RC, Richieri GV, DeMaria A.: Increases in serum unbound free fatty acid levels following coronary angioplasty. *Am.J.Cardiol.* 78:1350-4 (1996)

12. Richieri GV, Ogata RT, Kleinfeld AM.: The measurement of free fatty acid concentration with the fluorescent probe ADIFAB: a practical guide for the use of the ADIFAB probe. *Mol.Cell Biochem.* 192:87-94 (1999)

13. Richieri GV, Ogata RT, Kleinfeld AM.: A fluorescently labeled intestinal fatty acid binding protein. Interactions with fatty acids and its use in monitoring free fatty acids. *J.Biol.Chem.* 267:23495-501 (1992)

14. Chevion M, Jiang Y, Har-El R, Berenshtein E, Uretzky G, Kitrossky N.: Copper and iron are mobilized following myocardial ischemia: possible predictive criteria for tissue injury. *Proc.Natl.Acad.Sci.U.S.A* 90:1102-6 (1993)

15. Cobbe SM, Poole-Wilson PA.: The time of onset and severity of acidosis in myocardial ischaemia. *J.Mol.Cell Cardiol.* 12:745-60 (1980)

16. McCord JM.: Oxygen-derived free radicals in postischemic tissue injury. *N.Engl.J.Med.* 312:159-63 (1985)

17. Chan B, Dodsworth N, Woodrow J, Tucker A, Harris R.: Site-specific N-terminal auto-degradation of human serum albumin. *Eur.J.Biochem.* 227:524-8 (1995)

18. Sadler PJ, Tucker A, Viles JH.: Involvement of a lysine residue in the N-terminal Ni2+ and Cu2+ binding site of serum albumins. Comparison with Co2+, Cd2+ and Al3+. *Eur.J.Biochem.* 220:193-200 (1994)

19. Levine RL.: Ischemia: from acidosis to oxidation. *FASEB J.* 7:1242-6 (1993)

20. Chevion M, Jiang Y, Har-El R, Berenshtein E, Uretzky G, Kitrossky N.: Copper and iron are mobilized following myocardial ischemia: possible predictive criteria for tissue injury. *Proc.Natl.Acad.Sci.U.S.A* 90:1102-6 (1993)

21. Berenshtein E, Mayer B, Goldberg C, Kitrossky N, Chevion M.: Patterns of mobilization of copper and iron following myocardial ischemia: possible predictive criteria for tissue injury. *J.Mol.Cell Cardiol.* 29:3025-34 (1997)

22. Bar-Or D, Lau E, Winkler JV.: A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. *J.Emerg.Med.* 19:311-5 (2000)

23. Bar-Or D, Winkler JV, Vanbenthuysen K, Harris L, Lau E, Hetzel FW.: Reduced albumin-cobalt binding with transient myocardial ischemia after elective percutaneous transluminal coronary angioplasty: a preliminary

comparison to creatine kinase-MB, myoglobin, and troponin I. *Am.Heart J.* 141:985-91 (2001)

24. Bar-Or D, Curtis G, Rao N, Bampos N, Lau E.: Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia. *Eur.J.Biochem.* 268:42-7 (2001)

25. Marx G, Chevion M.: Site-specific modification of albumin by free radicals. Reaction with copper(II) and ascorbate. *Biochem.J.* 236:397-400 (1986)

26. Bhagavan NV, Lai EM, Rios PA et al.: Evaluation of human serum albumin cobalt binding assay for the assessment of myocardial ischemia and myocardial infarction. *Clin.Chem.* 49:581-5 (2003)

27. Mothes E, Faller P.: Evidence that the principal CoII-binding site in human serum albumin is not at the N-terminus: implication on the albumin cobalt binding test for detecting myocardial ischemia. *Biochemistry* 46:2267-74 (2007)

28. Collinson PO, Stubbs PJ, Kessler AC.: Multicentre evaluation of the diagnostic value of cardiac troponin T, CK-MB mass, and myoglobin for assessing patients with suspected acute coronary syndromes in routine clinical practice. *Heart* 89:280-6 (2003)

29. Sinha MK, Vazquez JM, Calvino R, Gaze DC, Collinson PO, Kaski JC.: Effects of balloon occlusion during percutaneous coronary intervention on circulating Ischemia Modified Albumin and transmyocardial lactate extraction. *Heart* 92:1852-3 (2006)

30. Quiles J, Roy D, Gaze D et al.: Relation of ischemia-modified albumin (IMA) levels following elective angioplasty for stable angina pectoris to duration of balloon-induced myocardial ischemia. *Am.J.Cardiol.* 92:322-4 (2003)

31. Rentrop KP, Cohen M, Blanke H, Phillips RA.: Changes in collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects. *J.Am.Coll.Cardiol.* 5:587-92 (1985)

32. Garrido IP, Roy D, Calvino R et al.: Comparison of ischemia-modified albumin levels in patients undergoing percutaneous coronary intervention for unstable angina pectoris with versus without coronary collaterals. *Am.J.Cardiol.* 93:88-90 (2004)

33. Dusek J, St'asek J, Tichy M et al.: Prognostic significance of ischemia modified albumin after percutaneous coronary intervention. *Clin.Chim.Acta* 367:77-80 (2006)

34. Cho DK, Choi JO, Kim SH et al.: Ischemia-modified albumin is a highly sensitive serum marker of transient myocardial ischemia induced by coronary vasospasm. *Coron.Artery Dis.* 18:83-7 (2007)

35. Conroy S, Kamal I, Cooper J.: Troponin testing: beware pulmonary embolus. *Emerg.Med.J* 21:123-4 (2004)

36. Roy D, Quiles J, Sinha M et al.: Effect of radiofrequency catheter ablation on the biochemical marker ischemia modified albumin. *Am.J.Cardiol.* 94:234-6 (2004)

37. Sbarouni E, Georgiadou P, Panagiotakos D, Livanis EG, Theodorakis GN, Kremastinos DT.: Ischaemia modified albumin in radiofrequency catheter ablation. *Europace.* 9:127-9 (2007)

38. Gidenne S, Ceppa F, Fontan E, Perrier F, Burnat P.: Analytical performance of the Albumin Cobalt Binding (ACB) test on the Cobas MIRA Plus analyzer. *Clin.Chem.Lab Med.* 42:455-61 (2004)

39. Maguire OC, O'Sullivan J, Ryan J, Cunningham SK.: Evaluation of the albumin cobalt binding (ACB) assay for measurement of ischaemia-modified albumin (IMA) on the Beckman Coulter LX-20. *Ann.Clin.Biochem.* 43:494-9 (2006)

40. Anwaruddin S, Januzzi JL, Jr., Baggish AL, Lewandrowski EL, Lewandrowski KB.: Ischemia-modified albumin improves the usefulness of standard cardiac biomarkers for the diagnosis of myocardial ischemia in the emergency department setting. *Am.J.Clin.Pathol.* 123:140-5 (2005)

41. Su JY, Ju SQ, Wang HM.: Measurement of ischemia modified albumin by albumin cobalt binding test. *Chin.Med.J.of Commmunications* 19:211-4 (2005)

42. Lefevre GF.: Analytical performance of the ACB test for ischemia in the Konelab 20. *Clin.Chem.* 49:A33 (2003)

43. Bar-Or D, Curtis G, Rao N, Bampos N, Lau E.: Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia. *Eur.J.Biochem.* 268:42-7 (2001)

44. da Silva SH, Pereira RS, Hausen BS et al.: Assessment of the nickel-albumin binding assay for diagnosis of acute coronary syndrome. *Clin.Chem.Lab Med.* 49:541-6 (2011)

45. Beetham R, Monk C, Keating L, Benger JR, Kendall J.: Effects of storage at -20 degrees C on ischaemia-modified albumin results. *Ann.Clin.Biochem.* 43:500-2 (2006)

46. Christenson RH, Duh SH, Sanhai WR et al.: Characteristics of an Albumin Cobalt Binding Test for assessment of acute coronary syndrome patients: a multicenter study. *Clin.Chem.* 47:464-70 (2001)

47. Abadie JM, Blassingame CL, Bankson DD.: Albumin cobalt binding assay to rule out acute coronary syndrome. *Ann.Clin.Lab Sci.* 35:66-72 (2005)

48. Govender R, De Greef J, Delport R, Becker PJ, Vermaak WJ.: Biological variation of ischaemia-modified albumin in healthy subjects. *Cardiovasc.J.Afr.* 19:141-4 (2008)

49. Gaze DC, Crompton L, Collinson P.: Ischemia-modified albumin concentrations should be interpreted with caution in patients with low serum albumin concentrations. *Med.Princ.Pract.* 15:322-4 (2006)

50. Lee YW, Kim HJ, Cho YH, Shin HB, Choi TY, Lee YK.: Application of albumin-adjusted ischemia modified albumin index as an early screening marker for acute coronary syndrome. *Clin.Chim.Acta* 384:24-7 (2007)

51. van der Zee PM, Verberne HJ, van Straalen JP et al.: Ischemia-modified albumin measurements in symptom-limited exercise myocardial perfusion scintigraphy reflect serum albumin concentrations but not myocardial ischemia. *Clin.Chem.* 51:1744-6 (2005)

52. Apple FS, Wu AH, Mair J et al.: Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. *Clin.Chem.* 51:810-24 (2005)

53. Christenson RH, Duh SH, Sanhai WR et al.: Characteristics of an Albumin Cobalt Binding Test for assessment of acute coronary syndrome patients: a multicenter study. *Clin.Chem.* 47:464-70 (2001)

54. Sinha MK, Roy D, Gaze DC, Collinson PO, Kaski JC.: Role of "Ischemia modified albumin", a new biochemical marker of myocardial ischaemia, in the early diagnosis of acute coronary syndromes. *Emerg.Med.J.* 21:29-34 (2004)

55. Consuegra-Sanchez L, Bouzas-Mosquera A, Sinha MK, Collinson PO, Gaze DC, Kaski JC.: Ischemia-modified albumin predicts short-term outcome and 1-year mortality in patients attending the emergency department for acute ischemic chest pain. *Heart Vessels* 23:174-80 (2008)

56. Keating L, Benger JR, Beetham R et al.: The PRIMA study: presentation ischaemia-modified albumin in the emergency department. *Emerg.Med.J.* 23:764-8 (2006)

57. Worster A, Devereaux PJ, Heels-Ansdell D et al.: Capability of ischemia-modified albumin to predict serious cardiac outcomes in the short term among patients with potential acute coronary syndrome. *CMAJ.* 172:1685-90 (2005)

58. Aparci M, Kardesoglu E, Ozmen N et al.: Prognostic significance of ischemia-modified albumin in patients with acute coronary syndrome. *Coron.Artery Dis.* 18:367-73 (2007)

59. Peacock F, Morris DL, Anwaruddin S et al.: Meta-analysis of ischemia-modified albumin to rule out acute coronary syndromes in the emergency department. *Am.Heart J.* 152:253-62 (2006)

60. Bali L, Cuisset T, Giorgi R et al.: Prognostic value of ischaemia-modified albumin in patients with non-ST-segment elevation acute coronary syndromes. *Arch.Cardiovasc.Dis.* 101:645-51 (2008)

61. Van Belle E, Dallongeville J, Vicaut E, Degrandsart A, Baulac C, Montalescot G.: Ischemia-modified albumin levels predict long-term outcome in patients with acute myocardial infarction. The French Nationwide OPERA study. *Am.Heart J.* 159:570-6 (2010)

62. Dominguez-Rodriguez A, Abreu-Gonzalez P, Jimenez-Sosa A, Samimi-Fard S, Idaira HB.: Does ischemia-modified albumin add prognostic value to the Thrombolysis In Myocardial Infarction risk score in patients with ST-segment elevation myocardial infarction treated with primary angioplasty? *Biomarkers* 14:43-8 (2009)

63. Apple FS, Quist HE, Otto AP, Mathews WE, Murakami MM.: Release characteristics of cardiac biomarkers and ischemia-modified albumin as measured by the albumin cobalt-binding test after a marathon race. *Clin.Chem.* 48:1097-100 (2002)

64. Lippi G, Brocco G, Salvagno GL, Montagnana M, Dima F, Guidi GC.: High-workload endurance training may increase serum ischemia-modified albumin concentrations. *Clin.Chem.Lab Med.* 43:741-4 (2005)

65. Lippi G, Salvagno GL, Montagnana M, Schena F, Ballestrieri F, Guidi GC.: Influence of physical exercise and relationship with biochemical variables of NT-pro-brain natriuretic peptide and ischemia modified albumin. *Clin.Chim.Acta* 367:175-80 (2006)

66. Middleton N, Shave R, George K et al.: Novel application of flow propagation velocity and ischaemia-modified albumin in analysis of postexercise cardiac function in man. *Exp.Physiol* 91:511-9 (2006)

67. Zapico-Muniz E, Santalo-Bel M, Merce-Muntanola J, Montiel JA, Martinez-Rubio A, Ordonez-Llanos J.: Ischemia-modified albumin during skeletal muscle ischemia. *Clin.Chem.* 50:1063-5 (2004)

68. Falkensammer J, Stojakovic T, Huber K et al.: Serum levels of ischemia-modified albumin in healthy volunteers after exercise-induced calf-muscle ischemia. *Clin.Chem.Lab Med.* 45:535-40 (2007)

69. Refaai MA, Wright RW, Parvin CA, Gronowski AM, Scott MG, Eby CS.: Ischemia-modified albumin increases after skeletal muscle ischemia during arthroscopic knee surgery. *Clin.Chim.Acta* 366:264-8 (2006)

70. Roy D, Quiles J, Sharma R et al.: Ischemia-modified albumin concentrations in patients with peripheral vascular disease and exercise-induced skeletal muscle ischemia. *Clin.Chem.* 50:1656-60 (2004)

71. Hacker M, Hoyer HX, la Fougere C et al.: Effects of peripheral vascular intervention on ischemia-modified albumin. *Coron.Artery Dis.* 18:375-9 (2007)

72. Sbarouni E, Georgiadou P, Theodorakis GN, Kremastinos DT.: Ischemia-modified albumin in relation to exercise stress testing. *J.Am.Coll.Cardiol.* 48:2482-4 (2006)

73. Troxler M, Thompson D, Homer-Vanniasinkam S.: Ischaemic skeletal muscle increases serum ischaemia modified albumin. *Eur.J.Vasc.Endovasc.Surg.* 31:164-9 (2006)

74. Banu NS, Gaze DC, Bruce H, Collinson PO, Belli AM, Manyonda IT.: Markers of muscle ischemia, necrosis, and inflammation following uterine artery embolization in the treatment of symptomatic uterine fibroids. *Am.J.Obstet.Gynecol.* 196:213-5 (2007)

75. Kalay N, Cetinkaya Y, Basar E et al.: Use of ischemia-modified albumin in diagnosis of coronary artery disease. *Coron.Artery Dis.* 18:633-7 (2007)

76. Kurz K, Voelker R, Zdunek D et al.: Effect of stress-induced reversible ischemia on serum concentrations of ischemia-modified albumin, natriuretic peptides and placental growth factor. *Clin.Res.Cardiol.* 96:152-9 (2007)

77. Senes M, Kazan N, Coskun O, Zengi O, Inan L, Yucel D.: Oxidative and nitrosative stress in acute ischaemic stroke. *Ann.Clin.Biochem.* 44:43-7 (2007)

78. Abboud H, Labreuche J, Meseguer E et al.: Ischemia-modified albumin in acute stroke. *Cerebrovasc.Dis.* 23:216-20 (2007)

79. Ahn JH, Choi SC, Lee WG, Jung YS.: The usefulness of albumin-adjusted ischemia-modified albumin index as early detecting marker for ischemic stroke. *Neurol.Sci.* 32:133-8 (2011)

80. Turedi S, Gunduz A, Mentese A et al.: Value of ischemia-modified albumin in the diagnosis of pulmonary embolism. *Am.J.Emerg.Med.* 25:770-3 (2007)

81. Turedi S, Gunduz A, Mentese A et al.: The value of ischemia-modified albumin compared with d-dimer in the diagnosis of pulmonary embolism. *Respir.Res.* 9:49 (2008)

82. The Renal Association. UK Renal Registry Report 2007. 2007.

Ref Type: Generic

83. National Institutes of Health, National Institure of Diabetes and Digestive and Kidney Diseases. US Renal Data System, USRDS 2007 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. 138-154. 2007.

Ref Type: Thesis/Dissertation

84. Sharma R, Gaze DC, Pellerin D et al.: Evaluation of ischaemia-modified albumin as a marker of myocardial ischaemia in end-stage renal disease. *Clin.Sci.(Lond)* 113:25-32 (2007)

85. Sharma R, Gaze DC, Pellerin D et al.: Ischemia-modified albumin predicts mortality in ESRD. *Am.J.Kidney Dis.* 47:493-502 (2006)

86. Cichota LC, Moresco RN, Duarte MM, da Silva JE.: Evaluation of ischemia-modified albumin in anemia associated to chronic kidney disease. *J.Clin.Lab Anal.* 22:1-5 (2008)

87. Montagnana M, Lippi G, Tessitore N et al.: Effect of hemodialysis on traditional and innovative cardiac markers. *J.Clin.Lab Anal.* 22:59-65 (2008)

88. Malindretos P, Sarafidis PA, Rudenco I et al.: Slow intravenous iron administration does not aggravate oxidative stress and inflammatory biomarkers during hemodialysis: a comparative study between iron sucrose and iron dextran. *Am.J.Nephrol.* 27:572-9 (2007)

89. Kiyici A, Mehmetoglu I, Karaoglan H, Atalay H, Solak Y, Turk S.: Ischemia-modified albumin levels in patients with end-stage renal disease patients on hemodialysis: does albumin analysis method affect albumin-adjusted ischemia-modified albumin levels? *J.Clin.Lab Anal.* 24:273-7 (2010)

90. Kotani K, Kimura S, Gugliucci A.: Paraoxonase-1 and ischemia-modified albumin in patients with end-stage renal disease. *J.Physiol Biochem.* 67:437-41 (2011)

91. Albarello K, Dos Santos GA, Bochi GV et al.: Ischemia modified albumin and carbonyl protein as potential biomarkers of protein oxidation in hemodialysis. *Clin.Biochem.* 2012)

92. Duarte MM, Rocha JB, Moresco RN et al.: Association between ischemia-modified albumin, lipids and inflammation biomarkers in patients with hypercholesterolemia. *Clin.Biochem.* 2009)

93. Valle Gottlieb MG, Da Cruz IB, Duarte MM et al.: Associations among metabolic syndrome, ischemia, inflammatory, oxidatives, and lipids biomarkers. *J.Clin.Endocrinol.Metab* 95:586-91 (2010)

94. Kotani K, Caccavello R, Sakane N, Miyamoto M, Gugliucci A.: Influence of ezetimibe monotherapy on ischemia-modified albumin levels in hypercholesterolemic patients. *Pharmacol.Rep.* 63:1248-51 (2011)

95. Kazanis K, Dalamaga M, Kassi E et al.: Serum levels of ischemia modified albumin in overweight/obese postmenopausal women: a potential biomarker of atherosclerotic burden associated with oxidative stress. *Maturitas* 70:182-7 (2011)

96. Piwowar A, Knapik-Kordecka M, Warwas M.: Ischemia-modified albumin level in type 2 diabetes mellitus - Preliminary report. *Dis.Markers* 24:311-7 (2008)

97. Kaefer M, Piva SJ, De Carvalho JA et al.: Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. *Clin.Biochem.* 43:450-4 (2010)

98. Dahiya K, Aggarwal K, Seth S, Singh V, Sharma TK.: Type 2 diabetes mellitus without vascular complications and ischemia modified albumin. *Clin.Lab* 56:187-90 (2010)

99. Kurban S, Mehmetoglu I, Yerlikaya HF, Gonen S, Erdem S.: Effect of chronic regular exercise on serum ischemia-modified albumin levels and oxidative stress in type 2 diabetes mellitus. *Endocr.Res.* 36:116-23 (2011)

100. Polk JD, Rael LT, Craun ML, Mains CW, Davis-Merritt D, Bar-Or D.: Clinical utility of the cobalt-albumin binding assay in the diagnosis of intestinal ischemia. *J.Trauma* 64:42-5 (2008)

101. Gunduz A, Turedi S, Mentese A et al.: Ischemia-modified albumin in the diagnosis of acute mesenteric ischemia: a preliminary study. *Am.J.Emerg.Med.* 26:202-5 (2008)

102. Gunduz A, Turkmen S, Turedi S et al.: Time-dependent variations in ischemia-modified albumin levels in mesenteric ischemia. *Acad.Emerg.Med.* 16:539-43 (2009)

103. Dundar ZD, Cander B, Gul M, Karabulut KU, Girisgin S.: Serum ischemia-modified albumin levels in an experimental acute mesenteric ischemia model. *Acad.Emerg.Med.* 17:1233-8 (2010)

104. Bautista-Ortega J, Maxwell MH, Robertson GW, Bishop S.: Estimate of the heritability of plasma troponin T in broiler chickens. *Br.Poult.Sci.* 40 Suppl:S18-S19 (1999)

105. Jauniaux E, Hempstock J, Greenwold N, Burton GJ.: Trophoblastic oxidative stress in relation to temporal and regional differences in

maternal placental blood flow in normal and abnormal early pregnancies. *Am.J.Pathol.* 162:115-25 (2003)

106. Prefumo F, Gaze DC, Papageorghiou AT, Collinson PO, Thilaganathan B.: First trimester maternal serum ischaemia-modified albumin: a marker of hypoxia-ischaemia-driven early trophoblast development. *Hum.Reprod.* 22:2029-32 (2007)

107. van Rijn BB, Franx A, Sikkema JM, van Rijn HJ, Bruinse HW, Voorbij HA.: Ischemia modified albumin in normal pregnancy and preeclampsia. *Hypertens.Pregnancy.* 27:159-67 (2008)

108. Guven S, Alver A, Mentese A, Ilhan FC, Calapoglu M, Unsal MA.: The novel ischemia marker 'ischemia-modified albumin' is increased in normal pregnancies. *Acta Obstet.Gynecol.Scand.* 88:479-82 (2009)

109. Gafsou B, Lefevre G, Hennache B, Debarge VH, Ducloy-Bouthors AS.: Maternal serum ischemia-modified albumin: a biomarker to distinguish between normal pregnancy and preeclampsia? *Hypertens.Pregnancy.* 29:101-11 (2010)

110. Papageorghiou AT, Prefumo F, Leslie K, Gaze DC, Collinson PO, Thilaganathan B.: Defective endovascular trophoblast invasion in the first trimester is associated with increased maternal serum ischemia-modified albumin. *Hum.Reprod.* 23:803-6 (2008)

111. Ozdemir S, Kiyici A, Balci O, Goktepe H, Cicekler H, Celik C.: Assessment of ischemia-modified albumin level in patients with recurrent pregnancy loss during the first trimester. *Eur.J.Obstet.Gynecol.Reprod.Biol.* 155:209-12 (2011)

112. Gugliucci A, Hermo R, Monroy C, Numaguchi M, Kimura S.: Ischemia-modified albumin levels in cord blood: a case-control study in uncomplicated and complicated deliveries. *Clin.Chim.Acta* 362:155-60 (2005)

113. Iacovidou N, Briana DD, Boutsikou M et al.: Cord blood ischemia-modified albumin levels in normal and intrauterine growth restricted pregnancies. *Mediators.Inflamm.* 2008:523081 (2008)

114. Songul Sahinli A., Marakoglu K, Kiyici A.: Evaluation of the levels of oxidative stress factors and ischemia modified albumin in the cord blood of smoker and non-smoker pregnant women. *J.Matern.Fetal Neonatal Med.* 2011)