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# Biomarkers in Acute Myocardial Infarction

**M. Imteyaz Ahmad[1]* and Neera Sharma[2]**

[1]Department of Biochemistry, PGIMER & Dr. Ram Manohar Lohia Hospital, New Delhi, India
[2]Department of Laboratory Medicine, PGIMER & Dr. Ram Manohar Lohia Hospital, New Delhi, India

**Abstract**

Acute coronary syndrome (ACS) is a significant cause of morbidity and mortality worldwide. Patients can be stratified by symptoms, risk factors and electrocardiogram results but cardiac biomarkers also have a prime role both diagnostically and prognostically. The proper diagnosis of ACS requires reliable and accurate biomarker assays to detect evidence of myocardial necrosis. Currently, troponin is the gold standard biomarker for myocardial injury and is used commonly in conjunction with creatine kinase-MB (CK-MB) and myoglobin to enable a more rapid diagnosis of ACS. Other markers of myocardial necrosis, inflammation and neurohormonal activity have also been shown to have either diagnostic or prognostic utility, but none have been shown to be superior to troponin. The measurement of multiple biomarkers and the use of point of care markers may accelerate current diagnostic protocols for the assessment of such patients.

**Keywords:** ACS–Acute coronary syndrome; cTnT-Cardiac troponin–T; cTnI-cardiac Troponin I (MPO - Myeloperoxidase); PAPPA-Pregnancy associated plasma protein A

## Introduction

Cardiovascular disease (CVD) is a major global cause of mortality in the developed countries. Intravascular thrombogenesis, the main pathogenic mechanism of the coronary artery disease (CAD), is influenced by a complex interplay of procoagulant, anticoagulant, fibrinolytic, endothelial damage/ dysfunction and inflammatory processes [1]. The traditional theory for causation of CAD centers on a complex interplay between genetic and environmental, modifiable and non modifiable risk factors setting into motion an inflammatory cascade of monocyte migration, lipid oxidation and atheromatous plaque formation [2,3]. Therefore, the clinical management of the at-risk patient is conventionally directed toward the identification and attenuation of these provocative risk factors. Though clinical assessment and risk factor identification remain cornerstones in estimating the burden of coronary disease, they fail to both adequately predict CAD risk and risk of recurrent events.

The term biomarker is an abbreviation for "biological-marker" a phrase first introduced in 1989. In 2001, the definition of biomarker was refined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes or pharmacologic responses to a therapeutic intervention" [4].

## Cardiac specificity

The term "specificity" is generally defined as the quality or attributes relating to one particular thing. In biology and clinical biochemistry, one should distinguish the "analytical specificity", referring to the ability of an assay to measure in biological samples a well defined molecule or substance, i.e. an analyte, rather than others, from the "diagnostic specificity", statistically the percentage of individuals not having a given condition who are correctly identified by an assay as negative for that condition [5]. Focusing on laboratory testing, the analytical specificity of a biochemical marker depends not only on avoiding any methodological cross reactivity with other biologically related molecules, but also on biological characteristics of the marker as well, showing no other tissue sources, even in trace amounts or under pathological conditions, in addition to the anatomic or histologic target. For biochemical markers of myocardial injury, for which the target organ is the heart, the cardiac specificity is of pivotal importance, even crucial for their clinical application [6]. Although any molecule candidate to become a successful cardiac biomarker should have certain characteristics, cardiac specificity is the hallmark of the ideal biomarker, because it definitively improves all its diagnostic characteristics, from sensitivity for damage detection to earlier appearance in blood. A lower cut off, close to the detection limit of the method, can be introduced, thus improving diagnostic sensitivity at its best, because also the smallest quantity of analyte different from the analytical noise may reflect a myocardial damage. Furthermore, when the assay is sensitive enough to detect small amounts of the cardiac specific marker, it may give earlier information revealing as rapid as possible the occurrence of myocardial injury. Therefore, it is not surprising that along the story of the search of the "ideal" cardiac biomarker, we are often recording scientists searching for new and even more cardiac specific markers as powerful tools to rapidly and accurately define any myocardial injury (Table 1).

## The early and reliable diagnosis of AMI

Acute myocardial infarction (AMI) is the major cause of death and disability worldwide with an ongoing increase in incidence. The risk of death is highest within the first few hours from AMI onset. The term acute myocardial infarction (MI) should be used when there is evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischaemia. Under these conditions any one of the following criteria meets the diagnosis for MI: Detection of a rise and/ or fall of cardiac biomarker values [preferably cardiac troponin (cTn)] with at least one value above the 99th percentile upper reference limit (URL) and with at least one of the following:

Symptoms of ischaemia. New or presumed new significant ST-

***Corresponding author:** Dr. Imteyaz Ahmad, Department of Biochemistry, NursinghHome lab ,Room no.20., Dr Ram Manohar Lohia Hospital, Baba kharak singh marg, New Delhi, PIN 110001, India, Tel: +919953130525; E-mail: dr.imteyaz579@gmail.com

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| Characteristics of an ideal cardiac biomarker |
|---|
| **High sensitivity** |
| High concentration in myocardium after myocardial injury |
| Rapid release for early diagnosis |
| Long half-life in blood for late diagnosis |
| **High specificity** |
| Absent in non-myocardial tissues |
| Not detectable in blood of non-diseased subjects |
| **Analytical characteristics** |
| Measurable by cost-effective assay |
| Simple to perform |
| Rapid turnaround time |
| Sufficient precision and trueness |
| **Clinical characteristics** |
| Ability to influence therapy |
| Ability to improve patient outcome |

**Table 1:** Characteristics of an ideal cardiac biomarker.

segment–T wave (ST–T) changes or new left bundle branch block (LBBB). Development of pathological Q waves in the ECG.

Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

Identification of an intracoronary thrombus by angiography or autopsy. Rapid identification of AMI is critical to initiate effective evidence-based medical treatment and management. The 12-lead ECG and cTn are the diagnostic cornerstones and complement clinical assessments. In most patients with ST-elevation AMI, clinical assessment and the ECG provide a straight forward diagnosis and allow the initiation of revascularization within minutes. However, ST-elevation AMI represents only about 5% of consecutive patients presenting with acute chest pain [7]. The ECG by itself is often insufficient to diagnose an AMI since ST deviation may be observed in other conditions, such as early repolarisation patterns, acute pericarditis, left ventricular hypertrophy, left bundle brunch block, hyperkalemia and the Brugada syndrome [7-9]. Therefore, cTns have become a prominent role in the diagnosis of AMI. cTns, sensitive and specific biochemical markers of cardiomyocyte necrosis [10-15], are very helpful in clinical practice to identify patients with acute coronary syndromes at high risk, and to select those patients who will benefit from early coronary angiography and, whenever possible, percutaneous coronary intervention.

## Current Cardiac Biomarkers

### Biomarkers of myocardial necrosis

**Troponin**: The most widely established and useful biomarker for myocardial injury is cTn. The cTn complex is made up of 2 subunits—C, I, and T—which together control calcium mediated interaction of actin and myosin, leading to the contraction and relaxation of striated muscle [16].

Troponin I (cTnI) and troponin T (cTnT) are expressed only in cardiac muscle, which allows these biomarkers to achieve extremely high specificity for myocardial damage [17,18]. cTn subunits are detectable in the peripheral circulation when damage to the cardiac myocyte first leads to the release of cytoplasmic cTn, which accounts for 3% to 5% of cTnI and 7% of cTnT levels [19,20] the release of bound cTn subunits contributes to the continued rise in peripheral levels. After infarction, cTn remains detectable for days (4–7 days for cTnI and 10–14 days for cTnT), cleared from the circulation primarily by the reticuloendothelial system, and fragmented into molecules that are cleared renally. Although cTn elevation persists for days, initial detection is delayed after myocardial injury, as necrosis typically requires 2–4 h to occur in the setting of ischemia. Consequently, cTnT and cTnI are detectable only after this latency period following the onset of injury, and recommendations call for serial measurements to be drawn at presentation and again after 6–9 h from the onset of symptoms.

In an early study of patients with suspected ACS presenting to the emergency department (ED) within 24 h of symptom onset, the sensitivity of cTnI and cTnT at the initial presentation for AMI was 3.7% and 33.3%, respectively, which improved to 82% and 89% after 6 h and to 89% and 96% after 12 h [21]. Because of the recommendations to use only cTn assays which are reliable (<10% coefficient of variation) at the decision limit (99th percentile), there has been development of high-sensitivity troponin assays (hs-cTn) to increase the analytical, and thus clinical, sensitivity for detection of myocardial injury. Such an approach may identify more patients at risk and permit earlier diagnosis [22]. With conventional cTn assays, the recommended level of precision is often unachievable at a level representing the 99th percentile but rather at levels from 1.5 to 9 times higher [23]. Challenges with poor cTn sensitivity early in clinical presentation have inspired the development of a new generation of highly sensitive assays, with a 10- to 100-fold lower limit of detection [24]. These hs-Tn assays have allowed the diagnostic cutoff to be lowered to the level of the 99th percentile or lower while maintaining precision at a CV, 10% [25,26].

It has become evident that although elevations in cTn reflect myocardial damage, they do not indicate its mechanism. In addition to spontaneous AMI secondary to plaque rupture and acute coronary occlusion, AMI can be secondary to the ischaemia produced either by increased oxygen demand or decreased supply, e.g. coronary artery spasm, coronary embolism, anaemia, arrhythmias, hypertension or hypotension. Therefore cTn may be raised in coronary, noncoronary cardiac as well as non-cardiac conditions such as sepsis.

As AMI is not the only cause of myocyte necrosis, it is key to consider the absolute level as well as the change in cTn within 1–3 hours as important criteria in the differential diagnosis of the cause of cardiomyocyte necrosis, the higher the absolute value of high-sensitive cTn at ED presentation in patients with suspected AMI, the higher the probability that it is AMI [25-27]. The differential diagnosis of a small amount of myocardial injury and therefore mild elevation of cTn is broad and includes acute and chronic disorders. CTn has to be interpreted as a quantitative variable. The term "troponin-positive" should therefore be avoided. "Detectable" levels will become the norm and have to be clearly differentiated from "elevated" levels.

Secondly, the larger the rise in high-sensitive cTn within the first few hours in the ED, the higher the probability that it is AMI. Thereby, serial changes documented by a second measurement help to differentiate acute cardiac disorders (showing a rise and/or fall) from chronic cardiac disease which usually exhibit constant cTn levels. It is a matter of debate whether absolute or relative changes best separate acute from chronic cTn elevations, as well as AMI from other causes of cTn elevation. The National Academy for Clinical Biochemistry has recommended changes in cTn of ≥20% (i.e. greater than imprecision levels assuming up to a 10% CV when levels are around the 99th percentile) from elevated baseline values (i.e. those with pathological levels of cTn). They make no recommendation on a threshold change from normal initial levels [84]. However it is important to highlight that a detailed clinical assessment remains mandatory to differentiate AMI from the other potential causes of myocardial injury.

**Myoglobin:** Myoglobin is a small cytoplasmic heme protein found in all muscles. Myoglobin increases within 1 to 3 h in the setting of myocardial necrosis, usually peaks within 6 to 9 h, and may become normal in, 24 h (Figure 1) [28]. Of the conventional biomarkers currently in use, myoglobin is the earliest marker to rise after AMI (2 h from the onset of chest pain) because of its relatively small size and high cytoplasmic content [29]. Myoglobin has limited specificity for myocardial necrosis in patients who have renal insufficiency and skeletal muscle trauma [30]. In addition, the rapid increase and normalization of myoglobin after AMI may lead to normal values for patients who present 24 h after symptom onset [31]. A single myoglobin measurement at presentation has been shown to have a sensitivity of 70% and a NPV of 97.4% for predicting AMI among patients with suspected ACS. Because of the poor initial sensitivity of cTn for AMI, myoglobin should be used in conjunction with cTn for the early detection of AMI. Myoglobin, when compared with CK-MB, cTnI, or cTnT, may offer the best overall diagnostic performance in screening for AMI within 2 h of ED presentation.

**Heart fatty acid binding protein (hFABP):** H-FABP is a small low molecular weight (i.e., 15 kDa), 132 amino acid, soluble protein, with general characteristics resembling myoglobin. Heart-type fatty-acid-binding protein (H-FABP) is a cytosolic, low-molecular-weight protein involved in fatty acid transport and metabolism. Although it is expressed overwhelmingly in the myocardium, small quantities also can be found in the brain, kidney, and skeletal muscle [32]. H-FABP displays a very early raise after an AMI (i.e., increased concentrations can be detected as soon as 30 min after the onset of an ischemic episode), peaks in blood after ~6–8 h, and returns to baseline values after nearly 24–30 h It is unsuitable as a test for patients presenting >6 h from onset of symptoms due to rapid renal clearance [33-35]. H-FABP has also been shown to independently predict mortality in patients with ACS [36].

Although the kinetics of H-FABP after an AMI thus mirrors that of other early AMI biomarkers such as myoglobin, soluble CD40 ligand, ischemia modified albumin, pregnancy-associated plasma protein A (PAPP-A) and myeloperoxidase , this protein is reported to be more myocardium-specific [37], thus making it a promising biomarker to be used in combination with conventional troponin tests. Another advantage is represented by the availability of rapid bedside chromatographic as well as fully automated turbidimetric immunoassays, which allow rapid measurement and short turnaround time.

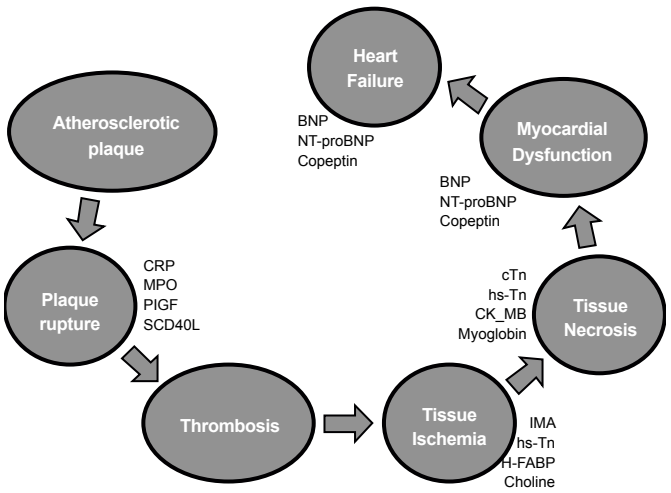


**Figure 1:** Schematic representation of the pathogenesis of MI and its consequences. The biomarkers implicated in each process are listed next to the bubble.

**Ischaemia modified albumin**: IMA is a biomarker for acute ischemia that is approved by the U.S. Food and Drug Administration. When exposed to ischemic conditions, the N-terminus of albumin is damaged, which makes it unable to bind metals and capable of being measured by an albumin cobalt-binding test [38]. Because its levels in the blood increase within minutes of the onset of ischemia and return to normal within 6–12 h, IMA has been implicated in the detection of acute ischemia prior to necrosis (Figure 1) One study of patients with suspected ACS found that IMA had a better NPV for ACS of 92% than the combination of CK-MB, myoglobin, and cTnT (86%), and the use of all 4 biomarkers together resulted in an NPVof 95% [39]. The sensitivity and specificity of elevated IMA for future mortality has been reported at 76% and 74%, respectively, which is of similar magnitude compared with cTnT. However, in this particular study, cTnT was not increased significantly in those with high IMA, which suggests that IMA may not have identified patients with eventual myocardial necrosis.

## Biomarkers of inflammation

Coronary artery disease is an inflammatory process. Atherosclerotic plaque formation begins with endothelial cell injury thought to be triggered by a range of factors including smoking, diabetes, hypertension and dyslipidaemia. Dyslipoproteinaemias such as elevated low density lipoprotein (LDL)-cholesterol, play a central role in atherosclerosis. An elevated LDL concentration is pro-atherogenic because LDL is intimately linked to oxidative and inflammatory processes in the arterial wall. Impaired endothelial cells respond by activating adhesion molecules and secreting pro-inflammatory chemokines [40,41]. These attract monocytes (which then multiply and mature into active macrophages) and T lymphocytes. Eventually a plaque is formed consisting of a large lipid core, a thin fibrous cap, and an active inflammatory cell infiltrate. AMI results from plaque rupture or superficial erosion, stimulating the secretion of pro-inflammatory and pro-coagulant substances which may trigger formation of occlusive thrombus. Inflammatory processes therefore not only promote initiation and progression of atheromas but also contribute to the precipitation of thrombotic complications.

**C-reactive protein:** CRP is an acute-phase protein produced by the liver that is upregulated in conjunction with the inflammatory response.[41]. CRP activates complement through the classic pathway, and in doing so, it binds to damaged cells including those in infracted myocardium. C-reactive protein (CRP), an acute-phase reactant produced by hepatocytes in response to stimulation by inflammatory cytokines, primarily IL-6, is the most widely used inflammatory marker. In the absence of transient acute disturbances in CRP in response to certain stimuli such as infections, injuries etc, CRP levels in individuals otherwise remain relatively constant. CRP, in itself, also has a pro-inflammatory effect by inducing the expression of adhesion molecules and other inflammatory cells. CRP has been implicated in vascular dysfunction and in the progression of atherosclerosis, and subsequently has been shown to predict future cardiovascular events, including first-ever AMI, stroke and development of peripheral arterial disease. As a prognostic tool, CRP may be useful in patients with ACS in which high CRP levels (10–15 mg/L) have been a strong indicator of long-term future cardiac events [42-45], although the evidence for

CRP as a predictor of short-term events is conflicting. In another study of patients with MI treated with thrombolysis, high CRP levels (226 mg/L) were associated with an increased risk of death within the first 6 months of the infarct event.

It has also been shown that CRP is raised in patients with unstable coronary syndromes, but specificity and sensitivity are not sufficient for use as a reliable diagnostic marker. It is, however, a significant predictor of poor outcome. In 2003, the American Heart Association (AHA) and the Centres for Disease Control and Prevention issued a scientific statement that suggested the use of high-sensitivity CRP as an optional risk factor measurement in patients with ACS.

**Pro-inflammatory markers:** The major pro-inflammatory markers include IL-6 and TNF-α. The immuno-inflammatory response to injury resulting from ischaemia and reperfusion of infarcted myocardium is associated with the induction of many cytokines including IL-6 and TNF-α. IL-6 is involved in inflammatory cell recruitment and activation, stimulates the liver to produce acute-phase proteins such as CRP and may also have a negative inotropic effect mediated through myocardial nitric oxide synthase [46]. TNF-α is a cytokine found in endothelial cells, smooth muscle cells and macrophages. TNF-α is a cardio-inhibitory cytokine that depresses cardiac contractility either directly or through induction of nitric oxide synthase.

**Markers of plaque destabilization:** Metalloproteinases are also markers of plaque destabilization. Myeloperoxidase, (MPO) is the most abundant metalloproteinase and is an enzyme produced by polymorphonuclear neutrophils and macrophages. It catalyzes the conversion of chloride and hydrogen peroxide to hypochlorite and is involved in the oxidation of lipids contained within LDL particles. It generates an array of reactive oxidants and radical species that contribute to the development of atheroma and subsequent plaque rupture [47-50]. MPO has been implicated not only as a potential biomarker in stable ischemic heart disease but also in acute myocardial injury, in which it serves as a marker of plaque instability, with increased levels indicating the activation of inflammatory cells around a vulnerable plaque.

MPO has demonstrated some value as a prognostic biomarker in predicting future adverse events. Although MPO can help assess the risk of CAD in healthy individuals, the relationship between MPO and CAD is stronger in patients with established or suspected ACS.

Pregnancy associated plasma protein A (PAPPA) is another metalloproteinase, originally discovered as a glycoprotein in pregnant women, produced by the syncytiotrophoblasts of the placenta. However, it is also produced by non-placental cell types, including fibroblasts, vascular endothelial cells, and vascular smooth muscle cells. It is responsible for the cleavage of insulin-like growth factor binding protein-4. The insulin-like growth factors are important regulatory proteins involved in cell proliferation and metabolism and have been implicated in atherosclerotic plaque progression and instability.

**Markers of myocyte rupture:** CD40L is a cytokine belonging to the TNF-α family and CD40 is its receptor. CD40L is up-regulated on platelets within fresh thrombus. Delivery into the peripheral circulation is thought to occur when activated platelets are released from the intracoronary thrombus that has formed at the site of the unstable/ruptured plaque [51,52]. sCD40L is a cellular ligand released from activated platelets and stimulated lymphocytes, and as a result it can be indicative of the activation of inflammatory and coagulant pathways. Platelet derived growth factor (PDGF) is a glycoprotein found in platelets, macrophages, smooth muscle cells and endothelial cells. Its main function involves wound repair by causing mitosis of smooth muscle cells and fibroblasts and attracting other cells such as monocytes, neutrophils and platelets [53,54].

## Novel cardiac biomarkers

**Choline:** Choline is an enzymatic product of phospholipase D. Phospholipase D, is involved in endothelial dysfunction, and is considered a marker of plaque instability, as well as a marker of severe myocardial ischaemia, and has been associated with elements of the metabolic syndrome. Choline is a water-soluble essential nutrient found in the head groups of phospholipids that make up cell membranes [55]. Choline is released into the blood after cleavage of phospholipids (Figure 1). It has potential use as a prognostic and diagnostic marker for ACS, ischemia, and necrosis. A recent study examining whole blood choline levels after hospital admission determined that choline is a strong predictor of cardiac arrest or death and may identify high-risk unstable angina patients who have not had an acute infarct event [56].

**F2 isoprostanes:** F2 isoprostanes are a biologically active product of arachidonic acid metabolism. The biosynthesis of F2 isoprostanes is thought to occur in many different cells involved in the formation of atherosclerosis, including monocytes. This has been supported by studies showing elevated F2-isoprostane levels in those who smoke and have dyslipidaemia and in the urine of those with unstable angina [57]. Studies have shown increased levels of free F2 isoprostane in those with ACS compared to those without. Increased free F2 isoprostane has also been shown to be predictive of a composite end-point of non-fatal myocardial infarction, development of heart failure, revascularization, and death.

**Growth-differentiation factor-15:** Growth-differentiation factor-15 (GDF-15) is a stress-responsive member of the transforming growth factor-b cytokine superfamily and is involved in regulating inflammatory and apoptotic pathways needed for development, differentiation, and tissue repair in various organs. Under normal physiologic conditions, the placenta is the only tissue that expresses significant amounts of GDF-15 but has been shown to up-regulate in a wide range of cancers and in many tissues following injury, ischaemia, and other forms of stress. In vitro experiments of cardiac myocytes have suggested that GDF-15 may play a role in cardiac injury and adaptation (Figure 1) [58], and GDF-15 may have both prognostic and diagnostic value for ACS. Diagnostically, serum GDF-15 levels increase after an ischemic event or reperfusion injury, although specificity is poor. As a prognostic tool, high levels of GDF-15 have been found to be an independent predictor for yearly mortality rate and the use of invasive strategy, and they add prognostic value to current cardiac biomarkers, including BNP, cTnT, and the thrombolysis in myocardial infarction score [59,60]. A high level of GDF-15 on admission of patients with ACS has shown to be a strong predictor of recurrent MI and to identify patients that would benefit from an invasive strategy.

**Copeptin:** Preprovasopressin is the precursor peptide for anti-diuretic hormone, copeptin and neurophysin ll. Anti-diuretic hormone is involved in the regulation of the endogenous stress response via the hypothalamopituitary- adrenal axis and promotes renal water conservation and hence influences osmoregulation and cardiovascular homeostasis. Copeptin, the C-terminal portion of provasopressin, is a 39-amino acid glycopeptide of unknown function in the circulation. Anti-diuretic hormone has been shown to be elevated in heart failure and in different states of shock but is unstable with a short half life of

only 5 to 15 min, Copeptin levels have been found to be significantly higher in patients with AMI as compared with patients having other diagnoses but only in those presenting early [61]. Normal degrees of copeptin secretion mirror vasopressin secretion in maintenance of plasma osmolality. However, in severe diseases such as shock, sepsis, stroke, or cardiovascular diseases, the nonosmotic release of AVP is portrayed by a sharp increase in plasma copeptin, which carries diagnostic and prognostic value for myocardial injury.

The Leicester Acute Myocardial Peptide study was the first to investigate the prognostic potential of copeptin in patients admitted with AMI [62]. Plasma copeptin was the highest on admission and reached a plateau at days 3 to 5. Persistently elevated copeptin at this later time point was associated with death and readmission for heart failure, independent of established conventional risk factors, showing that copeptin can be used as a marker of death or heart failure in patients with AMI.

## The History of Cardiac Biomarkers

Aspartate transaminase (AST) was found to be elevated in patients with AMI in 1954 and was the first cardiac biomarker to be used in clinical practice. AST is found in the heart, liver, skeletal muscle, kidneys and brain and is currently used clinically as a marker for liver health [63-66]. As the use of AST became more widely used, its lack of specificity for cardiac tissue injury was appreciated.

Plasma creatine kinase (CK), an enzyme that catalyzes the transfer of high-energy phosphate from creatine phosphate to adenosine triphosphate, is rapidly released during muscle damage. In 1959, it was demonstrated that CK was an extremely sensitive index of skeletal muscle disease and one year later, it was also seen in patients with AMI [63-66]. In 1960, lactate dehydrogenase (LDH), an enzyme that catalyses the reversible oxidation of lactate to pyruvate, was discovered. However, LDH is found in all cells and, like AST, is very nonspecific. CK was found to be more specific than either AST or LDH because low levels of CK in the liver less confound results in those with hepatic dysfunction. In 1979, WHO recommended CK, AST and LDH as the biomarker components for diagnosis of AMI. Despite this, specificity remained a problem, especially in patients with muscle and hepatic diseases or injury.

Advances in electrophoresis allowed identification of more cardiospecific iso-enzymes of both CK and LDH. Cardiac muscle has higher CKMB levels (25–30%) compared with skeletal muscle (1%), which is mostly CKMM. The measurement of CKMB, CKMB fraction or CKMB/CKMM ratio was a more specific marker for AMI. Cardiac muscle is also particularly rich in LDH 1 (or HHHH) and 2 (or HHHM) compared with skeletal muscle, which contains primarily LDH 4 and 5. In the well-oxygenated heart, H subunits are more prominent but during infarction they become reduced, thus lowering relative ratios of LDH 1 or H subunits. Unfortunately these CK and LDH isoenzyme assays remained lacking in specificity [67-70].

However, the detection and measurement of biomarkers was revolutionized by the development of immunoassays (initially configured with polyclonal antibodies and then, in the 1980s, with monoclonal antibodies) as well as technical advances in automation. Monoclonal antibodies allowed measurement of CKMB mass. This enabled earlier and more rapid detection of myocardial damage and was also more sensitive and specific than the original CKMB activity assay. However, with further research it was realized that even CKMB mass was elevated in a variety of situations as a result of skeletal muscle injury as well as in non-ischaemic cardiac disease and certain malignancies [71,72].

Recognition of the lack of specificity of CKMB for AMI underpinned the search for a test with superior performance. However, this improved understanding lead on to a pivotal breakthrough, the discovery of troponin.

Troponin is found in both skeletal and cardiac muscle but cardiac TnI (cTnI) and TnT (cTnT) isotypes have additional residues on the N-amino terminal and can therefore be readily identified as cardiac type [73]. TnC cannot. Troponin, as a constituent of the muscle myofibril, was discovered in the 1970s but sensitive radioimmunoassays for cardiac troponin (cTn) were not developed until the late 1980s. cTn was proposed as a specific marker of myocardial necrosis but the high sensitivity of cTn compared with CK and CKMB had not been envisaged. In 2000, guidelines for the diagnosis of AMI were changed with the new definition suggesting cTn as the preferred biomarker [74]. Initial scepticism, due to a significant increase in the 'positive' rate, a lack of assay standardization and a lack of confirmed correlation between cTn and histopathology, was eventually replaced by widespread acceptance. Assay variability was acknowledged. This led to recommendations for only cut-off values with a coefficient of variation (CV) of <10% to be employed. The recommended cut-off value was now suggested at the 99th percentile, much lower than values previously used in practice. Many assays were not able to meet precision guidelines at this level [75-80].

## Point of Care Cardiac Markers

There are many commercial point of care (POC) kits for measurement of biomarkers including cTn, CKMB, myoglobin and BNP/NT-proBNP both individually as well as in multi-marker panels. POC devices have been shown to reduce turn-around times compared with standard testing due to elimination of sample transfer time, no or minimal sample preparation (analysers customarily use whole blood), and immediate availability of results. It has been recommended that if standard laboratory testing exceeds amaximum 60-minute turn-around time (the average being 65–128 min) or 25% of decision time, then a POC device (with an average turn-around time of 15–26.5 min) should be implemented [81-85].

## Multi-Marker Testing

The future of biomarkers in the detection of myocardial injury may call for a multimarker approach for diagnosis and prognosis (Table 1). One study using cTnT, CRP, and NT-proBNP showed that elevations in 2 or 3 of these biomarkers predicted worse outcomes than those with 1 biomarker alone [86]. Current AHA guidelines for cTn measurement recommend testing on presentation and again at 8–12 h post symptom onset [87] and National Academy of Clinical Biochemistry recommends an early marker at 0–6 h and a definitive marker at 6–9 h post-presentation [88]. With current international guidelines recommending disposition of 95% of Emergency Department patients at ≤4–6 h, the requirement for follow-up cTn measurement at these time points means patient admission to in-patient hospital care or observation units. Multiple markers analysed on presentation and/or repeated within a shorter time interval to comply with Emergency Department guidelines, may be sufficient for appropriate risk stratification [8]. Studies have shown sensitivities for AMI of 80–100% for CKMB/ myoglobin up to 4 h post-presentation , 94–96.7% for cTnI/ myoglobin up to 1.5 h post-presentation [89], 92.6% for cTnI/ CKMB up to 8 h post-presentation [90] and 100% for cTnI/

CKMB/ myoglobin up to 2 h post-presentation [91] compared with sensitivities for cTn alone of 86 92.3% .These studies used the original WHO criteria/CKMB as the gold standard for the diagnosis of AMI which may over-estimate test performance. However, the emerging generation of hs-Tn assays may obviate the need for these other assays, particularly myoglobin and CK-MB, as elevations may precede clear-cut myocardial necrosis. As we have begun to learn from hs-Tn assays, an adequate study of a multitude of assay considerations including biologic variability for emerging biomarkers will be necessary prior to widespread adoption into routine clinical practice. Regardless, the future undoubtedly will call for a more discerning clinician to interpret elevations in biomarkers, particularly hs-Tn, in an appropriate clinical context.

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