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Review

# Cardiac biomarkers in acute myocardial infarction

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ABSTRACT

Each year, a large number of patients are seen in the Emergency Department with presentations necessitating investigation for possible acute myocardial infarction. Patients can be stratified by symptoms, risk factors and electrocardiogram results but cardiac biomarkers also have a prime role both diagnostically and prognostically. This review summarizes both the history of cardiac biomarkers as well as currently available (established and novel) assays. Cardiac troponin, our current "gold standard" biomarker criterion for the diagnosis of myocardial infarction has high sensitivity and specificity for this diagnosis and therapies instituted in patients with elevated troponin have been shown to influence outcomes. 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.



## 1. The history of cardiac biomarkers

Biochemical markers of ischaemic cardiac damage, used to diagnose AMI, have been used for over half a century. 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 catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. AST is found in the heart, liver, skeletal muscle, kidneys and brain and is currently used clinically as a marker for liver health [1–4]. In 1959, the world health organization (WHO) produced a definition for AMI, defined as at least 2 out of; symptoms suggestive of cardiac ischaemia, ischaemic electrocardiogram (ECG) changes and elevated cardiac biomarkers, with AST as the biomarker of choice [5]. As the use of AST became more widely used, its lack of specificity for cardiac tissue injury was appreciated [1–4].

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 [1,2,4]. 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 [1,2,4].

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 [1,2,6–9].

Electrophoretic assays were first developed in 1966 but lacked sensitivity. This improved with advances in chromatography in 1974 and the production of quantitative assays by the close of the 1970s [1,6,7,9–11]. 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 [1,6,7,9–11]. 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 [1–3,12,13].

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Recognition of the lack of specificity of CKMB for AMI underpinned the search for a test with superior performance. The contractile proteins of the myofibril include myosin, actin, tropomyosin and the troponin complex. When cardiac myocytes are acutely damaged and the integrity of the cell membrane is lost, myosin fragments are released into the circulation from a soluble cytoplasmic pool of myosin light chains. Myosin light chain release was thought to be a potential marker for AMI [14,15]. In itself, this discovery was disappointing, as peak levels of myosin light chains did not significantly vary between patients presenting with AMI, unstable angina or non-cardiac chest pain. However, this improved understanding lead on to a pivotal breakthrough, the discovery of troponin.

Troponin has three subunits. Troponin C (TnC) binds to calcium ions to produce a conformational change in troponin I (TnI), troponin T (TnT) binds to tropomyosin, interlocking them to form a troponin–tropomyosin complex and TnI binds to actin in thin myofilaments to hold the troponin–tropomyosin complex in place [16–19]. 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 [20]. 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. Early studies showed that cTn was raised in AMI (as diagnosed by WHO criteria) [7,20–27] with high sensitivities and specificities [7,12,17,22,24,25,28–38] and had the advantage over CKMB in differentiating cardiac from skeletal muscle injury [7,39]. Studies in the 1990s also showed that significant numbers of patients classed as unstable angina (as opposed to AMI) by conventional WHO criteria, had elevated cTn levels [25,26,36,38–44]. Furthermore, cTn positive patients exhibited an increased risk of subsequent death [29,36,38,45–50], AMI [21,38,43,44,47,51–53], need for revascularization [40,45,46,48,54] and readmission [36] than cTn negative patients, even though other baseline characteristics and symptoms appeared matched [40,52]. Those with "unstable angina" and cTn elevation were thought to have unstable plaque with subsequent platelet emboli leading to 'micro' infarcts, as opposed to stable plaque in those without elevations [21,27,33,39]. Subsequent studies showed that various medical and interventional techniques already instituted into practice or under investigation, were refined as the relationships with cTn and outcomes became apparent. Such interventions included low molecular weight heparin [18,55–60], glycoprotein IIbIIIa inhibitors in patients with refractory angina and in patients undergoing PCI [18,56–58,60,61], antiplatelet therapy [62], 24–48 h of telemetry [60], angiography as the preferred investigation [56,57] and revascularization [46,49,57,63].

In 2000, guidelines for the diagnosis of AMI were changed with the new definition suggesting cTn as the preferred biomarker [64]. 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. This change in guidelines had follow through effects. There was an increase in incidence of diagnosed AMI [65–70] although a small proportion of patients fulfilling WHO criteria for AMI were no longer considered AMI by the new definition [69]. There was an increase in coronary care unit admissions [68], an increase in number of angiograms performed [68] and a reduction in length of stay in patients without AMI [68]. Long term, there have possibly been decreases in post-AMI mortality [70,71] and heart failure admissions as the primary diagnosis [71]. The use of cTn as the biomarker of choice in AMI was further endorsed in 2007, by the World Heart Federation Task Force for the Redefinition of Myocardial Infarction [72].

There was and is only one manufacturer for cTnT (Boehringer Mannheim, acquired by Roche Diagnostics the late 1990s, and test platform Elecsys) and therefore the assay is standardized [73–75]. There are many manufacturers of cTnI assays which vary from each other by assay format, antibodies used, specificity to different epitopes of complexed, free and modified cTn, types of indicator molecule and detection technique (spectrophotometric, fluorescent, chemiluminescent or electrochemical) [76]. They may also have varying interference from pre-analytical variables such as haemolysis, icterus, lipaemia, anticoagulant, ascorbic acid levels, biotin levels, the use of streptokinase or ruthenium, heterophile antibodies and autoantibodies, which can lead to both false negatives and false positives [76–78]. This leads to differences in analytical sensitivity between assays and therefore different levels at which there is a <10% coefficient of variation and different limits of detection (LOD) [76–78]. The lack of standardization has lead to discrepancies in cut-point values [74,75,79,80] with over a 30–40 fold differences documented [81,82] and in the past have been notorious for poor performance at the lower end of the reference range. However, later generation assays are much improved and now many meet or are near to meeting, precision guidelines [81,83–85]. The early cTnT assay had slightly limited specificity in patients with skeletal muscle disease because of cross reactivity of the signal antibody with skeletal muscle and re-expression of foetal forms of cTnT (cTnI isoforms are not present in foetal skeletal muscle) in conditions such as rhabdomyolysis and chronic skeletal muscle diseases such as muscular dystrophy and myositis. These isoforms are not detected by the assay used today [73,74,79,80,86,87].

There have been many studies comparing troponin assays [74,75,79,86,88–92] demonstrating that correlation and concordance is variable [44,74,90–93], however, all have been shown to be sensitive and specific tests for the diagnosis of AMI.

## 2. Current cardiac biomarkers

### 2.1. Biomarkers of myocardial necrosis

#### 2.1.1. Troponin

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 [83,94–101]. This may allow more rapid triage to intensive and invasive treatment strategies in those with elevations in hs-cTn and possibly earlier stress testing or even discharge without such testing in those without elevations [97,102]. In the FRISC II subgroup analysis, comparing the results of several cTnT and cTnI assays, 10–12% of patients with a poor prognosis at 1-year follow-up were identified only by the cTn assay that had the highest analytical sensitivity [103]. The use of lower troponin cut–off concentrations also better separated the rate of clinical events at 1 year between groups receiving invasive versus non-invasive treatment. Other studies confirm this prognostic utility [85,104–106].

The hs-cTn assays also allow detection of circulating cTn in healthy individuals, and therefore definition of a true normal range [99,100]. However, although hs-cTn assays will allow refined definition of the upper limit of normal, their clinical application will also require revisiting specificity as the upper 1% of the normal range and non-coronary causes of cardiac injury may now more frequently confound the effort to rule out AMI [100].