Heart disease remains the most common cause of death in the developed world, with one in 10 patients still dying of a myocardial infarction (MI).

With the advent of assays to measure cardiac troponins (cTns), the diagnosis and prognostication of acute coronary syndromes (ACS), including MI, has greatly improved. Katus et al. were the first to describe the measurement of cTnT, followed by Bodor et al. describing the development of the cTnT assay, building on the work of Cummins et al. – both for the diagnosis of MI. After a number of studies, the cTns are now considered the ‘gold standard’ biochemical test for the diagnosis of acute MI (AMI).3

Structure and Biochemistry of Troponins

The troponin complex was first described in 1946 by Bailey in a letter to Nature. However, it was the work by Ebashi et al. that showed that the contraction of striated muscle but not smooth muscle was regulated by a special protein complex, now known as the troponin located on actin filaments. With the development of techniques such as site-directed mutagenesis, studies have yielded new details about the structure of the troponin complex.

The troponin complex consists of three sub-units:

• Troponin-C (TnC) is the component that binds calcium and regulates the activation of thin filaments during contraction by removing troponin-I inhibition. It has a molecular weight of 18 kilo Daltons (kDa).
• Troponin-I (TnI) is the sub-unit that inhibits the ATPase activity of actinomyosin. Its molecular weight is 22kDa and it is encoded for by chromosome 19q13.3.
• Troponin-T (TnT) plays a structural role. It binds the troponin complex to tropomyosin. TnT is also involved in activating actinomyosin ATPase activity. Its molecular weight is 37kDa and its gene is present on chromosome 1q32.

Kinetics of Release and Clearance of Troponins

The majority of cTnT and cTnI is found in the contractile apparatus and released as a result of proteolytic degradation. Six to eight percent of cTnT and 3–8% of cTnI is present in the cytoplasm.

cTnT has a biphasic release pattern with an initial peak at 12 hours after the onset of muscle ischaemia, followed by a plateau phase lasting 48 hours and a subsequent fall to undetectable levels after 10 days. Successful early reperfusion leads to a more rapid peak that is generally a marker of favourable prognosis. The duration of the elevation is determined by the size of infarction; with small infarctions, cTnT may remain elevated for as little as seven days and with large infarcts, it may remain detectable for as long as three weeks. cTnI, on the other hand, has a monophasic release pattern. The duration of elevation is typically five to 10 days, but depends greatly on infarct size.

Regarding clearance, there is a common misconception that the cTns have a long half-life. It has been clearly demonstrated that the half-life of cTnT in circulation is approximately two hours. The prolonged and continuous detection of the troponins is due to its release from the myofibrillar pool, as the contractile apparatus in the cell undergoes total degradation. The half-life of cTnI in dogs has been shown to be approximately 70 minutes.

Wu et al. have shown that there is little free TnI in blood and that the predominant form in blood is the binary troponin I–C complex. In contrast, following an AMI, free cTnT is released into circulation together with ternary troponin T–I–C complexes and other cTnT fragments. Labugger et al. have shown that there was modification of both the native cTnT and TnI over time in the bloodstream of AMI patients. It was suggested that these products were generated in the diseased myocardium itself and then subsequently released into circulation upon an infarction. Other studies of ischaemic reperfused rat hearts and human
post-ischaemic myocardium have shown that there are post-translational modifications, such as selective degradation, covalent complex formation and phosphorylation. Thus, the cTns found in circulation after an AMI shows modifications that reflect primary insult on the myocardium as well as changes arising after the release of troponin in the bloodstream.

**Clinical Significance of Measured cTns**

**Role in Diagnosis**

**ACS**

ACS is a term that encompasses a spectrum of clinical manifestations resulting from a common pathophysiological mechanism (see Figure 1). From early life, lipid-rich deposits containing macrophages and T-lymphocytes are laid as plaques in the coronary artery (fatty streaks). With increasing age, the lesions continue to enlarge and form a fibrous plaque that contains smooth muscle cells and may even start to become highly vascularised. It is important to note that the inflammatory process is also an integral part of atherogenesis.

The rupture or erosion of the atheromatous coronary plaque results in the formation of a thrombus, which may partly or completely obstruct the coronary artery. The clinical manifestation is dependent on the rupture of a plaque. The resultant intraluminal thrombosis may cause reduced blood perfusion, which usually leads to myocardial ischaemia and then overt myocardial necrosis. Thus, the spectrum ranges from unstable angina – which is associated with reversible myocardial ischaemia, where patients might usually be asymptomatic to ACS with variable degrees of myocardial necrosis – to frank MI, with large areas of necrosis causing left ventricular dysfunctions.

**MI**

An MI is defined as the necrosis of cardiac myocytes caused by prolonged ischaemia due to perfusion insufficiency. It is usually identified from a history of ischaemia-related symptomatology (chest, epigastric, arm, wrist or jaw discomfort/pain at rest or on exertion) and typical electrocardiogram (ECG) changes. For a specific review on AMI, refer to Boersma et al.

The cTns have been demonstrated to be the best markers for the definitive detection of MI and ACS, with better sensitivity and specificity than creatine kinase (CK) or CK-MB, and are now considered the ‘gold standard’ marker of myocardial necrosis.

**MI has traditionally been diagnosed according to the 1971 (revised 1979) World Health Organization (WHO) criteria. Briefly, MI was diagnosed by the documentation of two of the following three characteristics:**

- clinical symptoms (e.g. chest pain);
- increase in cardiac enzyme concentrations; and
- a typical ECG pattern, usually involving the development of Q waves.

With the advent of sensitive and specific assays that can detect very small infarcts, and improved imaging techniques, there was a need to reconsider the definition of MI. The diagnostic criteria have recently been reviewed and the current recommendations are based on the consensus document of the Joint European Society of Cardiology and the American College of Cardiology (ESC/ACC). The biochemical criteria for detecting myocardial necrosis according to the consensus document is that the maximal concentration of cTnT or cTnI must exceed the 99th percentile limit of a reference control group on at least one occasion during the first 24 hours.

A central theme in the consensus document is that any amount of myocardial necrosis caused by ischaemia should be labelled as an infarct, although the document does make it clear that the term MI should not be used without ‘further qualification’. Such qualifications include infarct size, context of the infarct (spontaneous or after a revascularisation procedure) and time of infarct (whether evolving, healing or healed). In addition, it has been recommended that the 99th percentile limit should achieve a total coefficient of variation (CV) of 10% or less. Currently, no cTnT or cTnI has met this criteria and it has been suggested that the level with...
a total CV of 10% or less should therefore be used as a cut-off rather than the 99th percentile limit.\textsuperscript{21,22}

The question is what the implications of the redefinition of MI are. First, a substantially increased proportion of patients with ACS will be classified as having had an MI. Thus, a patient previously diagnosed as having unstable angina might now be diagnosed as having had a small MI. This will have a profound effect on health services worldwide in terms of increased management and treatment costs. In one recent study, an analysis of cTnI in 1,719 ACS patients demonstrated an 85% increase in MI diagnosis when the 99th percentile reference limit (0.06\,\mu\text{g/litre}) for the cTnI assay (Dimension RxL, Dade-Behring) was used compared with the receiver operating characteristic (ROC) curve cut-off (0.6\,\mu\text{g/litre}).\textsuperscript{23} In the same study, using the concentration with a 10% CV, which was 0.26\,\mu\text{g/litre} instead of the ROC cut-off, produced a 26% increase in all cTnI-positive cases. The increase in positive cTn cases, whether true or false, will result in increased costs for laboratories offering the test and prompt further investigations that will also incur increased expenses for other hospital departments. However, it could be argued that the costs to society might be lower if it means that more patients are identified early and appropriate secondary prevention measures are instituted sooner.

Second, because all elevated levels of cTns have been shown to have an adverse outcome, the redefinition of any amount of myocardial necrosis as an MI might stimulate a physician into considering intensive long-term patient management that might not have been previously considered necessary. Thus, an increased diagnosis rate might mean better prognosis for individual patients in the long term.

Third, it must also be borne in mind that the new definition will affect the national health policies of a country and may necessitate the preparation of new clinical guidelines.

\textbf{Role in Prognosis and Risk Stratification}

Whereas the early 1990s saw the emergence of cTns as a diagnostic marker of MI, in the mid- and late-1990s, it was convincingly demonstrated that even minor elevations of the cTns showed poor short- and long-term prognoses. The first study to demonstrate the risk stratification potential of cTn was published in 1992 by Hamm et al.\textsuperscript{24} They showed that cTnT (Roche Diagnostics) was a more sensitive marker of myocardial injury than CK-MB and a useful prognostic indicator in patients with unstable angina. This study concluded that cardiac risk during hospitalisation could be estimated by measuring cTnT soon after admission to guide management. Apart from being a small study with 109 patients, and mostly male (80 out of 109), this study laid the foundation for the next wave of research in the clinical utility of cTns.

In the prospective study by Ohman et al. (which was a sub-study of the Global Use of Strategies to Open Occluded Coronary Arteries in Acute Coronary Syndromes (GUSTO) IIa),\textsuperscript{25} cTnT levels (Roche Diagnostics) above 0.1\,\mu\text{g/litre} measured as soon as possible after admission were shown to be associated with significantly higher mortality within 30 days in patients with AMI. When used in combination with CK-MB and electrocardiography, it allowed for further risk stratification. This study verified the findings of previous observations in smaller studies, and showed that the use of a single blood sample obtained early could be used for the stratification of risk. Importantly, it identified a lower threshold for increased risk (0.1\,\mu\text{g/litre}). Previous studies had used a cut-off of 0.2\,\mu\text{g/litre} for cTnT.\textsuperscript{24} The main limitations of this otherwise excellent study were several. First, the study was a highly selected, high-risk population with acute ischaemic syndromes, among whom 72% had diagnosed MI. Second, patients with suspected renal failure as defined by a serum creatinine >25\,mg/litre (>221\,\mu\text{mol/litre}) had been excluded, which may have therefore increased the prognostic value of cTnT in the study. Third, the study used the only commercially available (first-generation) cTnT assay in use at the time, the detection antibody of which had a 12% rate of cross-reactivity with skeletal–muscle TnT.\textsuperscript{2}

The retrospective study by Antman et al. (data was obtained from the Thrombolysis in Myocardial Ischaemia (TIMI) phase IIIb study) showed that, in patients with the ACS, cTnI (Stratus II, Dade) provided prognostic information that could permit the early identification of patients with increased risk of death.\textsuperscript{26} The mortality rate at 42 days was found to be significantly higher in the 573 patients with cTnI levels of 0.4\,\mu\text{g/litre} or higher (21 deaths, or 3.7%) compared with the 831 patients with levels lower than 0.4\,\mu\text{g/litre} (eight deaths, or 1.0%; \textit{p}<0.001). A statistically significant increase in mortality was also noted with increasing levels of cTnI. In addition, the prognostic potential of cTnI persisted even after adjustment for independent variables, such as an age of 65 years or older and ST segment depression on ECG, that are known to be significantly associated with an increased risk of cardiac events. Importantly, it was also found that patients with a CK-MB concentration in the normal range but with elevated cTnI levels had an increased risk of mortality.
One meta-analysis that included 2,847 unstable angina patients, with a median follow-up duration of 30 days, showed that, in patients with an elevated cTnT, the cumulative odds ratio (OR) for the risk of AMI and cardiac death was 2.7 (95% confidence interval (CI): 2.1–3.4; p<0.0001) whereas, for patients with increased cTnI, the cumulative OR was found to be 4.2 (95% CI: 2.7–6.4; p<0.0001).27

Role in Guidance of Therapy and Interventions

As the determination of cTns provides an important prognostic tool for risk stratification, treatments and intervention decisions might logically be based on a measured cTn level. The translation of increased troponin levels to increased risk has been simultaneous with the development of new treatments. Due to the fact that these drugs can be relatively expensive, the identification of high-risk patients with biochemical markers is of great advantage. Testing for cTns has been shown to be appropriate for triage and decision-making with regard to hospital admission and interventional procedures, such as percutaneous coronary intervention (PCI).28

In the Fragmin in Unstable Coronary Artery Disease (FRISC) trial of Dalteparin (low-molecular-weight heparin), unstable angina patients were classified into two groups with cTnT (Roche Diagnostics) concentrations lower than 0.1µg/litre and 0.1µg/litre and higher.29 In this study, in the short term, all patients received subcutaneous dalteparin or placebo twice-daily for six days. In the long term, they continued with dalteparin or placebo once-daily for five weeks. It was found that dalteparin reduced the incidence of death or MI from 2.4% to 0% (p=0.12) and from 6% to 2.5% (p<0.05) in the two groups, respectively. During the long-term phase, there was an increasing difference between the placebo and dalteparin in those with cTnT levels of 0.1µg/litre or higher, but no beneficial effect of long-term treatment could be demonstrated in those with cTnT levels lower than 0.1µg/litre.

This study demonstrated that elevated cTnT identifies a subgroup of patients in whom prolonged antithrombotic treatment was beneficial. Similar results have now been found for placebo-controlled trials using platelet glycoprotein IIb/IIa receptor inhibitors (PGRI). The first study to demonstrate that cTnT was effective in predicting which patients would benefit from PGRI therapy was the trial using Abciximab.30

A recent meta-analysis of all major randomised clinical trials showed that PGRI reduce the occurrence of death or MI in patients with ACS not routinely scheduled for early revascularisation.31 These compelling data have prompted the inclusion of cTn measurements into guidelines for the identification and clinical management of high-risk unstable angina and MI patients.28,32

Regarding interventions such as PCI, it has been shown that even minor elevations of cTnT and TnI identify high-risk patients with unstable angina and non-ST elevation MI (NSTEMI) who can benefit from an early invasive strategy as opposed to a conservative strategy.33

Perioperative MI is the most common cause of morbidity and mortality in patients who have had surgery and ranges from 36% to 70%.34 Distinguishing cardiac injury due to the surgery from damage caused by MI itself is very difficult. One study demonstrated that the measurement of cTnI was a sensitive and specific marker for the diagnosis of perioperative MI.34 A study by Baggish et al. showed that post-operative cTnT could predict length of stay in the intensive care unit (ICU) following cardiac surgery.35

Conclusions

The cTns have been shown to have both diagnostic and prognostic utility. An elevated cTn level is predictive of a higher risk of adverse cardiac events, both during admission and follow-up. The use of cTns to guide therapy and management has been shown to translate into potentially more economical and effective strategies. Finally, the cTns are now considered the ‘gold standard’ marker in the diagnosis of ACS/MI.

Future Perspective

Several generations of research and assay refinements have validated the cTns as a diagnostic and prognostic marker for ACS. For the future, it is anticipated that more sensitive assays will emerge that will be able to achieve a precision of 10% total CV at the 99th percentile reference limit. It is anticipated that cTn assay manufacturers will continue to improve the sensitivity and precision of their respective assays. Perhaps only then will progress be made in finally delineating the frontiers between ischaemia and infarction. The reasons for the increase in cTn levels in conditions other than ACS, such as in renal failure and sepsis, will need to be elucidated. At the molecular level, the partial crystallisation of the core domain of the human cTn has yielded vital information regarding its overall architecture, and progress continues to be made.36
References


