Review: Troponin I: an Update on Clinical Utility and Method Standardization

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Abstract. Cardiac troponin I (cTnI) is now widely recognized as one of the preeminent biochemical markers for the diagnosis of myocardial injury. The biochemical specificity of this biomolecule for cardiac tissue has forced a reevaluation of the diagnostic criteria for non-Q-wave acute myocardial infarction, unstable angina, acute coronary artery disease, and minor myocardial injury. Further, its use by clinicians has revolutionized the way that chronic and acute heart diseases are both diagnosed and managed. Unfortunately, the standardization of cardiac troponin I assays is problematic. Up to 20-fold variation of serum cTnI mass determinations may be observed for a given patient sample when measured by different assay systems. As a result, significant ambiguity often exists in the clinical interpretation of serum cTnI concentrations. Recent efforts have been directed toward the biochemical standardization of cTnI assays. However, the heterogeneous nature and biochemical complexity of the serum forms of cTnI and differences of the epitope recognition by the various methods have hindered the harmonization of serum cTnI assays. (received 6 June 2000, accepted 8 August 2000)

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Introduction

The measurement of cardiac troponin I (cTnI) is now considered to be one of the most specific and sensitive methods for the detection of myocardial injury. Cardiac troponin I, as a test for the confirmation of an acute myocardial infarction, has replaced creatine kinase-MB (CK-MB) as the "gold standard" and is now acknowledged by many to be the definitive test. Unlike CK-MB, cTnI does not lack cardiospecificity in patients with skeletal muscle, thyroid, or renal diseases. Additionally, it is recognized that serum cTnI, appropriately sampled during the time associated with a clinical event, accurately confirms the absence of a related myocardial infarction. Recently, measurement of cTnI has gained importance in cardiac risk stratification, as increasing evidence suggests that elevated cTnI concentration in a patient with unstable angina signifies an increased risk for a future cardiac event.

This review discusses the role of troponin I in muscle contraction, the clinical utility of serum cTnI and other common diagnostic tests, and the difficulties encountered in standardization of cTnI methods.

Role of Troponin in Muscle Contraction

The contractile elements of muscle include many different proteins, the two most abundant of which are myosin and actin. Myosin comprises about 60% to 70% of muscle protein and is the primary component of the thick filament, while actin accounts for approximately 20% to 25% of muscle protein and comprises most of the thin filament [1,2]. The globular head section, or the working unit, of myosin contains both the ATPase activity responsible for the production of energy for the contractile process, and the actin binding site [3].

Troponin and tropomyosin are proteins associated with actin filaments. Troponin is composed of three dissimilar subunits with molecular weights of about 18 kDa, 21 kDa, and 37 kDa, designated troponin C (TnC), troponin I (TnI), and troponin T (TnT), respectively. Functionally, the TnT subunit binds to...
tropomyosin, the TnI subunit is involved in inhibition of actin binding to myosin under resting conditions, and the TnC subunit binds calcium [4,5].

During muscle contraction, calcium ions bind to the troponin C subunit of troponin, causing a conformational change that results in the exposure of the myosin binding sites on the actin molecule. Myosin binds to actin and the binary complex undergoes a conformational change that allows for binding of ATP. This binding of ATP, however, facilitates the dissociation of myosin from actin, as the ATP-myosin-actin ternary complex is much less stable than the actin-myosin binary complex. Further, hydrolysis of ATP by the myosin-associated ATP-ase activity and subsequent dissociation of products produces a myosin molecule that is again capable of binding actin. This cycle of myosin-ATP interactions and hydrolysis produces a series of conformational changes that allow the myosin molecule to travel along the actin molecule, resulting in sequential muscle contraction and relaxation [6].

When a muscle cell dies, the cellular components, including the troponins, are released into the bloodstream and are available for measurement. There are important structural differences between the subunits of troponin and between the troponin T and troponin I subunits that are present in skeletal and cardiac muscle [7]. The differences between the amino acid sequences of skeletal TnT and TnI and cardiac TnT and TnI have enabled the production of antibodies that are specific for the cardiac forms of these subunits. These antibodies form the basis of the immunochemical tests used in hospital laboratories today.

Clinical Utility of Troponin I

The clinical utility of a diagnostic test is directly related to the test's ability to reflect the underlying pathophysiology of the relevant disease. In the case of acute coronary syndromes, approximately three-fourths are believed to result from a ruptured plaque followed by clot formation. These syndromes also show a spectrum of severity, with partial arterial occlusion and intermittent obstruction commonly observed. This pathological process is not static, but rather is highly dynamic, with concurrent thrombotic and thrombolytic events. The process of inflammation has gained importance in acute coronary syndromes [8-10].

The multiple pathophysiologic stages of acute coronary diseases are generally manifested by different clinical disorders. Unfortunately, the presence of a continuum of disease states frequently makes it difficult to discriminate among the different resulting clinical conditions. We tend to view cardiac-related conditions categorically, with acute myocardial infarction (either Q-wave or non-Q-wave acute myocardial infarction, or AMI) and minor myocardial damage (either unstable angina or procedure-related ischemia and necrosis) being the most common. This review of the clinical utility of troponin I is limited to these two conditions.

As with all disease states, one would like to have a way to diagnose or exclude acute myocardial infarction definitely in all patients. Unfortunately, with current techniques, this is not always possible. Clinicians and cardiologists have, however, developed a set of criteria that must be satisfied before a diagnosis of AMI can be rendered. These criteria represent, for the most part, the "gold standard" of diagnosis. It should be evident that these criteria are not without shortcomings.

The World Health Organization has established its criteria for diagnosis of acute myocardial infarction [11]. These include the presence of chest pain and either diagnostic electrocardiographic changes or elevated cardiac markers. In practice, apparently conflicting data are often obtained, especially in patients with chest pain and low concentrations of serum biochemical markers. In the era of creatine kinase-MB assays, low levels of this enzyme were frequently dismissed by clinicians as insignificant or as evidence of ischemia and not infarction. However, with the advent of truly specific cTnl as a biochemical marker, the clinicians, seeking either to corroborate or refute the significance of the finding of elevated serum concentrations of troponin, often rely on the coronary arteriogram as the "gold standard" for diagnosing an AMI. This common practice places an overemphasis on the clinical utility of coronary arteriography to diagnose an acute coronary event.

Coronary arteriography, while offering a view of the overall anatomy of the vessel, can only detect lesions and provide a measure of the degree of stenosis. Further, coronary arteriography is sensitive to 50% to 70% narrowing of the lumen and is insensitive to the presence of a ruptured plaque. Thus, arteriography has little prognostic value for determining future acute
coronary events. Ambrose et al [10] reported that "serial angiographic studies performed prior to and following an acute event or angiographic studies performed early after a myocardial infarction indicate that the underlying plaque responsible for unstable angina and myocardial infarction was usually less than 50% narrowed prior to the acute event." This limitation was emphasized in a recent report by Bugiardini [12] in which he stated that "the site of the occluding thrombus causing infarction cannot be predicted by preinfarction angiograms, since thrombosis may occur at the site of mild stenosis whereas a severe stenosis remains unchanged on subsequent angiography." An important, but often overlooked, conclusion from these investigations is that a "normal arteriographic study" following a finding of a positive troponin concentration does not identify a "safe" patient who is not at risk for a subsequent event.

As previously mentioned, in the absence of elevated serum cardiac markers in a patient experiencing chest pain, the WHO criteria for the diagnosis of AMI require the presence of specific electrocardiographic changes. Contrary to popular belief, these S-T segment changes are not specific for an acute myocardial infarct. Rather, these changes commonly reflect localized asynergy rather than new injury. Possible clinical reasons for false positive S-T segment elevations include prior myocardial infarction, coronary artery spasm, electrolyte variations, non-infarction ischemia, ventricular aneurysm, and pericarditis [13]. The importance of this finding was recently evidenced by a publication of Karlson et al [14] in which they reported that "91% of all patients that would be treated (with S-T segment elevation) developed confirmed infarction." Additionally, electrocardiography lacks sensitivity. Reported sensitivities for the diagnosis of acute myocardial infarction range from 40% to 50%, resulting in non-diagnostic ECGs for 90% to 95% of the typical "chest pain triage group" [15-18].

While electrocardiography and coronary arteriography are both informative, especially when positive findings are correlated with clinical events, negative findings must be interpreted cautiously, as these methods may lack the sensitivity to detect the presence of life-threatening events. As a result, one needs to rely on serum markers to assist in the diagnosis of AMI.

The three biochemical markers commonly used in the diagnosis of an AMI (serum myoglobin, creatine kinase-MB, and troponin) all exhibit the classical temporal "rise and fall" pattern associated with acute myocyte necrosis and protein release. In general, myoglobin is released first, followed by CK-MB and troponin I. However, due to the presence of both cytosolic and myofibril-bound cTnI pools, serum troponin I concentrations typically remain elevated for several days following myocyte necrosis. This lengthy appearance in the serum allows cTnI to replace LDH isoenzymes as a late marker of myocardial infarction.

The results of four studies [19-22] of cTnI assays for the diagnosis of AMI are summarized in Table 1. Unfortunately, sample biases both in and among these studies makes their direct comparison difficult. Differences in diagnostic criteria and study populations (including comparison groups), inaccuracy in the reported time following the onset of chest pain, and demographic factors such as patient age, hospital location (urban or rural), and availability of emergency medical and hospital services all contribute to study

<table>
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<th>Authors</th>
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<th>Specificity (%)</th>
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<td>81</td>
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variability. The diagnostic criteria used to determine diagnostic sensitivity and specificity often differ among studies. Studies that correlate several cardiac markers are prone to produce results that differ from those that use the stricter WHO diagnostic criteria. Further, differences in pre-selected study populations also contribute to the observed variability.

Equally important is the relative degree of reliance on hospital presentation temporal data. Because chest pain onset times are often inaccurate, attempts to develop or interpret specific time–marker correlations should be approached with extreme caution. Rather, it is more prudent to develop relative time–marker correlations. That is, one should determine the relative time of onset of chest pain, and hence relative diagnostic sensitivities and specificities, from the relationship between the concentrations of multiple serum cardiac markers, like troponin I and myoglobin or myoglobin and CK-MB. Unfortunately, due to the direct testing costs involved, clinicians and laboratory workers are not always afforded this possibility.

The results summarized in Table 1 suggest, given the different study designs, that the diagnostic sensitivity and specificity indices for cTnl are relatively consistent, and that troponin I appears to be a very good biochemical marker to assist in the diagnosis of acute myocardial infarction. However, the presence of sample bias in these studies prevents rigorous calculations of confidence limits for diagnostic sensitivity and specificity. It is also evident that serum concentrations of troponin I are elevated in clinical conditions other than acute myocardial infarction. These studies, and others, indicate that cTnl levels are elevated in severe cases of unstable angina.

In the recent past, clinicians commonly considered unstable angina a purely clinical diagnosis. Frequently, patients were given a temporary diagnosis of unstable angina pending the availability of serum biochemical markers. Retrospectively, the patient’s diagnosis was changed to a non-Q-wave AMI if the subsequent rise and fall of the cardiac markers could be demonstrated. Recent reports indicate that with the advent of cTnl assays, this clinical classification, at least for high-risk cases, has been made easier. A new set of diagnostic criteria is being established for classifying patients with unstable angina, ie, the presence or absence of ischemic chest discomfort at rest, as well as ECG changes and biochemical markers of myocardial injury during and after ischemic episodes [23–25].

Troponin measurements are playing an increasing role in assisting the clinician in (a) diagnosing acute cardiac syndromes, (b) therapeutic intervention, and (c) risk stratification. Several investigators have reported that high-risk unstable angina patients, those likely to suffer an acute cardiac event in the near future, have concurrently elevated cTnl and normal CK-MB concentrations [26–34]. In 770 patients with acute coronary syndromes, GUSTO II investigators found that cTnl concentrations >1.5 μg/L on enrollment (assayed by the Dade Behring Stratus II analyzer) were correlated with increased mortality [26]. In 516 patients with unstable angina, researchers in the TRIM (Thrombin Inhibition in Myocardial Ischemia) study reported that cTnl concentrations >2.0 μg/L (assayed by the Dade Behring Opus analyzer) were associated with higher 30-day mortality [29]. Further, the TIMI (Thrombolysis In Myocardial Infarction) IIIB study examined 1,404 symptomatic patients with a diagnosis of unstable angina or non-Q AMI. Cardiac Tnl concentrations were obtained in each patient at enrollment and showed correlation with the 42-day mortality [30]. The results indicated that mortality at 42 days increased for patients with cTnl level ≥0.4 μg/L and elevated CK-MB concentration. Mortality at 42 days was also increased for patients with cTnl level ≥0.4 μg/L, without CK-MB elevation. It would be incorrect to infer that cTnl–positive and CK-MB–negative results are inclusive of all unstable angina patients. Rather, this set of clinical parameters likely identifies a "high-risk unstable angina" group that may benefit, as we shall see, from administration of the GPIIb/IIIa group of platelet antagonists and the low molecular weight heparins. It is now generally accepted that cTnl assays can aid in selecting cases for treatment, even when the coronary arteriogram is "negative."

The use of cTnl to select patients for treatment with GPIIb/IIIa inhibitors has received much attention [34]. These agents inhibit platelet aggregation by blocking the binding of fibrinogen to the GPIIb/IIIa platelet receptors. Three common drugs of this class are abciximab (ReoPro®), eptifibatide (Integrilin®), and tirofiban (Aggrastat®). In a study of 2,222 patients with coronary artery disease and chest pain 24 hr prior to admission, baseline cTnl measurements were
obtained and the results showed correlation with adverse events, such as death, myocardial infarction, or recurrent ischemia, at 2, 7, and 30 days. In general, the adverse event rates were greater for patients with cTnI levels above the cut-off concentration. Additionally, the benefit of treatment with Aggrastat® (ie, decrease in death or myocardial infarction at 30-day follow up) was evaluated. The patients with cTnI levels ≥1μg/L who received Aggrastat® had lower hazard ratios than those with similarly elevated cTnI levels who received heparin [35].

In addition to its role in risk stratification, cTnI is now commonly used as an index of myocardial necrosis in procedures involving coronary stenting [36] and heart surgery [37]. Other studies have demonstrated its potential use in determining myocardial injury in sepsis [38] and for diagnosing advanced congestive heart failure [39].

**Method Standardization**

Several quantitative and qualitative assays for serum cTnI are currently available in the United States. The more common assay methods include those performed on laboratory-based random-access immunoassay analyzers and patient bedside testing devices [40,41]. Unfortunately, significant variability exists between these assay methods. Ten- to twenty-fold variations of cTnI concentrations in patient samples have been reported [29,42,43], resulting from a combination of biochemical and analytical factors.

Several investigators have reported that troponin assays are subject to the same analytical interferences that are commonly encountered with other immunoassays. Specifically, rheumatoid factors [44], hemolysis [45], hyperbilirubinemia [45], heterophile antibodies [46], fibrin [47], and heparin [48] have all been shown to affect troponin assays. Extensive and highly unpredictable variability has also been reported for troponin I control materials [49]. As control materials are frequently free from the interfering substances mentioned above, these observations suggest a potential biochemical source of assay variability. The significant lack of assay correlation reported for many cTnI assays provides further evidence of biochemically-associated variability.

Table 2 summarizes the results of five trials of troponin I assay correlation [21,41,45]. Ideally, the slope and intercept of any correlation study should be 1 and 0, respectively, indicating that the methods provide the same absolute concentration of troponin I in a given patient sample. It is evident, however, that

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<th>Authors</th>
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<th>Y-intercept of correlation line</th>
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<tr>
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<td></td>
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<td>Heeschen et al, 1999 [41]</td>
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<td></td>
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<td>0.34</td>
<td>0.06</td>
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<td></td>
<td>same assays, cTnI &gt;4 μg/L –</td>
<td>0.99</td>
<td>-1.72</td>
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substantial differences exist among these assays, as correlation line slopes vary from 0.97 to 4.1 and intercept values vary from 0.4 to -4.60. In the study reported by Heeschen et al [41], when all of the values across the assay range were correlated, a relatively desirable correlation line with a slope of 0.97 and an intercept of 0.4 was obtained. However, when one examines smaller ranges within this data set, that is, <4.0 μg/L and >4.0 μg/L, substantial differences appear between the subranges and the total range. The pattern suggests that a source of biological variability might be the physiological equilibrium between related biomolecules and the ability of the antibodies used in these assays to recognize these specific biomolecules.

Recently, the antigenic epitopes of cTnl have been described. Ferrieres et al [50,51] reported that the N- and C-termini of the human cTnl sequence are the two main antigenic regions of the molecule. It is not surprising, then, that the common immunoassays recognize either the N- or C-terminus of cTnl. Shi [49] determined the relative position of the epitopes recognized by four commonly used troponin I assays (Dade Behring Stratus, Beckman Coulter Access, Abbott AxSYM, and Dade Behring Opus). While all four assays recognized the intact cardiac troponin molecule, the Stratus, Opus, and AxSYM assays recognized the N-terminal portion of the molecule, and the Access assay recognized the C-terminal portion of the molecule. While these differences in epitope recognition may account for some of the observed variability, the relative importance of this differential recognition in understanding the sources of assay variation can only be addressed when the degree of sequence homology among the various forms of troponin present in serum is known.

Troponin I contains 2 cysteine residues (Cys-80 and Cys-97) that can be oxidized and 2 serine residues (Ser-23 and Ser 24) that can be phosphorylated. The oxidation of thiol groups on the cysteine residues affects the interaction of troponin I with other troponin components [43], and the phosphorylation of the serine residues by cAMP-dependent protein kinase, protein kinase C, or protein kinase A changes the conformation of troponin I [52,53]. Additionally, TnI is highly susceptible to proteolysis, and is often complexed with other troponin subunits. There is a debate about the relative percentages of free and complexed TnI, and the nature of these free and complexed forms. Katrukha et al [54] reported that serum troponin I exists largely as a TnI-TnC binary complex with a small amount of free TnI present. Morjana [55] reported that serum troponin I exists predominantly as a ternary TnI-TnC-TnT complex. Other investigators indicated that troponin I exists in serum primarily as a TnI-TnC binary complex, and that free TnI, the binary complex TnI-TnI, and the TnI-TnC-TnT ternary complex are seldom present, and when present, exist in small quantities compared to the binary TnI-TnC complex [56].

Using a rat model for myocardial ischemia, McDonough et al [57] found evidence of covalent complexes between TnI and TnC, TnI and TnT, and TnT and TnC. Specifically, troponin fragments in these covalent complexes consisted of TnI fragment 1-193, TnC fragment 1-94, and TnT fragment 191-298, and were likely the result of isopeptide bond formation by the enzyme transglutaminase between the C-terminal lysine (amino acid residue 193) of TnI and the N-terminal glutamine (amino acid residue 191) of TnT or with an internal glutamine residue of TnC. Similar troponin degradation products may exist in human serum following myocardial injury [58].

The impact of troponin degradation products on method standardization remains to be fully elucidated. There is indirect evidence that the degradation of troponin I occurs through the up-regulation of two processes by increases in intracellular calcium: the protease activity of calpain and covalent cross-linking [57-60]. These studies indicate that while both the C- and N-termini of free TnI are subject to proteolysis, the C-terminal portion is preferentially degraded. Moreover, complexed troponin I is similarly degraded, but more slowly than free TnI.

Many investigators have reported that the extent of degradation may be related to the degree of ischemia. That is, the greater the ischemia, the greater the degradation. Mild ischemia likely produces the initial C-terminal degradation product (amino acid residues 1-193), while severe ischemia results in the formation of secondary degradation products in which both the C- and N-termini have been removed (amino acid residues 63-193 and amino acid residues 73-193) [54,55,57]. There is general belief that physiological roles for these degraded forms exist, and that the extent
of TnI degradation directly correlates with contractile dysfunction [53,60-62].

The extent of serum troponin I degradation in patients appears to be highly variable, and this variability contributes, at least in part, to the standardization issues associated with troponin I assay methods. Shi et al [49] proposed that due to the mechanism of troponin I degradation, assay methods that recognize the C-terminal portion of the molecule tend to yield much lower apparent troponin concentrations than those assays that recognize the N-terminal portion. These investigators, and others [43,63], have reported that the current immunoassays differentially recognize free and complexed forms of TnI.

The American Association for Clinical Chemistry has launched a program for standardization of cTnI assays. The initial phase of this program is to develop a consensus international reference material for cTnI. Currently, several protein preparations are being evaluated by the AACC committee [64]. Given the different forms of troponin and the variable concentrations of these forms likely present in patients’ serum, the selection of a single protein that accurately reflects the biological forms of this analyte seems highly unlikely. As shown by the recent report of Newman et al [65], the selection of a common cTnI standard will potentially reduce, but not eliminate, inter-assay variability. Equally important in the standardization of these assay methods is the selection of antibodies that recognize conserved epitopes of troponin I.

Conclusions

Differences in troponin I epitopes that are recognized by the current assays, coupled with variable concentrations of troponin I degradation products in serum, probably account for much of the analytical variation that is commonly observed with patient serum samples. Standardization and correlation issues, however, do not directly affect the clinical utility of this biochemical marker. Currently, cTnI is the most sensitive and specific marker for acute myocardial infarction and related forms of myocardial tissue damage. It plays vital roles in the diagnosis, risk stratification, and therapy of cardiac patients.

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