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ESC REPORT

Recommendations for the use of cardiac troponin measurement in acute cardiac care†

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The release of cardiomyocyte components, i.e. biomarkers, into the bloodstream in higher than usual quantities indicates an ongoing pathological process. Thus, detection of elevated concentrations of cardiac biomarkers in blood is a sign of cardiac injury which could be due to supply–demand imbalance, toxic effects, or haemodynamic stress. It is up to the clinician to determine the most probable aetiology, the proper therapeutic measures, and the subsequent risk implied by the process. For this reason, the measurement of biomarkers always must be applied in relation to the clinical context and never in isolation. There are a large number of cardiac biomarkers, but they can be subdivided into four broad categories, those related to necrosis, inflammation, haemodynamic stress, and/or thrombosis. Their usefulness is dependent on the accuracy and reproducibility of the measurements, the discriminatory limits separating pathology from physiology, and their sensitivity and specificity for specific organ damage and/or disease processes. In recent years, cardiac biomarkers have become important adjuncts to the delivery of acute cardiac care. Therefore, the Working Group on Acute Cardiac Care of the European Society of Cardiology established a committee to deal with ongoing and newly developing issues related to cardiac biomarkers. The intention of the group is to outline the principles for the application of various biomarkers by clinicians in the setting of acute cardiac care in a series of expert consensus documents. The first of these will focus on cardiac troponin, a pivotal marker of cardiac injury/necrosis.

Keywords Cardiac biomarkers • Cardiac troponins • Cardiac troponin I • Cardiac troponin T • Troponin assays • Myocardial infarction • Myocardial necrosis • Myocardial injury • 99th percentile decision level • Troponin assay impression

Introduction

Cardiac troponin (cTn) is the biomarker of choice for the diagnosis of myocardial necrosis because it is the most sensitive and specific biochemical marker of myocardial injury/necrosis available. Thus, cTn elevations in blood are integral to the diagnosis of acute myocardial infarction (AMI) and The Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction has advocated that the diagnosis of AMI be based on a rising and/or falling pattern of cTn in the appropriate clinical situation.[1] The National Academy of Clinical Biochemistry has developed nearly identical guidelines.[2,3] Unfortunately, there is a lack of understanding of many of the analytical and clinical issues that govern the use of this important marker. In 2001, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommended quality specifications for analytical and pre-analytical factors for cTn assays.[4] The goal was to establish uniform criteria to objectively evaluate the analytical and clinical performance of assays to ensure that all package inserts included the requested information on assay design, and pre-analytical and analytical performance characteristics. However, the heterogeneity of cTn assays and their lack of harmonization continue to result in analytical and

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interpretative challenges. Despite improvements in both sensitivity and imprecision, problems in cTn assay standardization, imprecision, interferences, and pre-analytical variability which influence their clinical use persist. This document will attempt in part to indicate which issues are of importance to clinicians and how they should think about them as part of their framework for interpreting the results of these assays.

Biochemical and pathophysiological background

The Tn protein complex is immobilized on the thin filament of the contractile apparatus of striated muscle. It consists of three distinct proteins encoded by separate genes.[5] The nomenclature of these proteins derives from their respective function in muscle contraction. The best studied function of the cTn complex is modulation of contractile function of the sarcomere in response to cytosolic calcium (Ca^{2+}) and protein phosphorylation (regulatory proteins of the sarcomere). Thus, the cTn complex plays a critical role in the regulation of excitation–contraction coupling in the heart. Cardiac troponin I (cTnI; molecular weight approximately 23 kDa) is a key regulatory protein in cardiac muscle contraction linking Ca^{2+}-TnC binding with the activation of cross-bridge reaction between the thin and thick filaments. cTnI inhibits actomyosin Mg^{2+}-ATPase and leads to muscle relaxation by interrupting the actin–myosin linkage. Cardiac troponin C (cTnC; molecular weight approximately 18 kDa) binds Ca^{2+} ions, which induces conformational changes that are transmitted by cardiac troponin T (cTnT) and cTnI phosphorylation to modulate cTnI inhibition. cTnT (molecular weight approximately 35 kDa) interacts with both cTnI and cTnC as well as tropomyosin to attach the cTn complex to the myofibrillar thin filament. The binding of cTnI with cTnC is tighter than the binding of cTnT with cTnC and cTnI. With triggered release of Ca^{2+} from intracellular stores at the onset of contraction, Ca^{2+} binds to the N-terminal Ca^{2+} binding site of cTnC, initiating a conformational change. This facilitates cross-bridge cycling and myocyte contraction, regulating the force and velocity of striated muscle contraction.

TnC is encoded by two genes, one specific for fast-twitch skeletal muscle and a second expressed in both slow-twitch skeletal and cardiac muscle.[5] Troponin T and TnI are encoded by different genes in cardiac, slow-twitch skeletal, and fast-twitch skeletal muscle. Consequently, in differentiated skeletal muscle, only the skeletal but not the cardiac isoforms are expressed. Thus, due to their cardio-specific isoform expression, cTnI and cTnT can be used, once they are released into the blood stream, as highly specific markers of myocardial damage. Initially, a small cytosolic pool (approximately 5% of total content) of both cTnI and cTnT was reported in myocardium based on solubility studies in human heart and membrane injury in a Langendorff model.[6,7] Considering the preparation protocols used and the poor solubility of both cTns in the hydrophilic cytoplasm, a better term is probably an 'early appearing pool'. These proteins appear to be more loosely bound to cellular components such as myofilaments during physiological conditions and thus are more easily released.

Proteolysis of cTnI and cTnT occurs in myocardium in response to ischaemia leading to post-translational modification that includes selective degradation, covalent complex formation, phosphorylation and/or oxidation, and N-terminal acetylation.[8–13] Thus, cTn found in the blood of patients is a heterogeneous mixture of free post-translational modified, degraded, and truncated forms. In human blood, cTnI exist as free and as complex forms with cTnC (the predominant form is the cTnI–cTnC complex) and, to a lesser extent, complexes with cTnT (cTnT–cTnI–cTnC complex).[8,9,11–13] Cardiac troponins are degraded by intracellular proteases present in myocardium (e.g. by calpain-I, caspases, matrix metalloproteinase-2) and in blood.[9,13] Thus, cTnI and cTnT are released from necrotic myocardium both as intact proteins and degradation products.[13] Time-related changes in the forms present in blood have also been reported.[12] Cardiac troponin T mainly circulates as a free form, but cTnT fragments also have been reported.[10] The extent of cTn degradation within the myocardium varies during the time course of AMI[12] and may influence the proportion of free and complex cTn in the blood.[9,12] In addition, the distribution of these forms of cTn may be related to the stimulus leading to cTn release but the details of these differences have not yet been well defined.

Currently, cTn assays do not discriminate between ischaemic or non-ischaemic aetiologies of myocardial damage but rather focus on trying to detect all major circulating forms equally (i.e. being equimolar). This approach optimizes analytical and clinical sensitivity. Circulating cTnI auto-antibodies and recently also auto-antibodies to cTnT have been described in human blood.[14,15] These antibodies can interfere with cTn detection especially when concentrations are low as the epitope targets of assay antibodies can be masked.[14] That has not been a clinical important issue with the present iteration of assays, but it could be a problem as assay sensitivity increases.

Cardiac troponin I has not been reported to be expressed outside of cardiac tissue. However, foetal isoforms of cTnT exist in diseased and regenerating skeletal muscle and were detected with the first generation cTnT assay. Subsequent generations of the assay have employed antibodies that avoid detection of these foetal cTnT forms and are hence highly specific for adult myocardial injury.[16–18]

Release of cTn occurs first from the early appearing myocyte pool and subsequently from the structural pool.[6] Release from the latter is the reason for the sustained elevations observed clinically and is a surrogate for irreversible break down of sarcomeric proteins. This has been used to argue that transient elevations of cTn can occur without cardiomyocyte death. However, evidence for this is controversial. A recent clinical study of exercise-induced ischaemia was unable to show cTnT release with stress-induced ischaemia despite the use of a novel high-sensitivity (hs) cTnT assay.[19] In contrast, recently, significant cTnI increases were described in association with scintigraphic documented ischaemia with a novel, very sensitive, and highly precise cTnI research assay.[20] However, the study did not determine the integrity of the cardiomyocytes that released the cTnI, so irreversible myocardial damage could have been present. Indeed, a recent study in cultured neonatal rat cardiomyocytes exposed to toxic metabolic inhibition demonstrated that the release of intact cTnI and cTnT

and their degradation products parallel each other and only occur after the onset of irreversible cardiomyocyte damage.[13] This issue remains controversial. There are convincing data that cTn concentrations on day 3 or 4 in patients with AMI reflect the mass of lost myocardium, i.e. infarct size.[21,22] On the whole, the understanding of the biochemical and structural nature of the cTn complex enhances the insight into the ways in which cTn measurements may be influenced by various clinical scenarios and analytical confounders.

Critical clinical concepts

(1) Cardiac troponin is an important regulatory component of the myocardial contractile apparatus.
(2) Its release from the cardiomyocyte denotes serious and probably irreversible injury.
(3) The release of cTnI and cTnT is highly specific for myocardial injury in contrast to the cardiac biomarkers used in the past, i.e. creatine kinase and its MB isoenzyme, lactate dehydrogenase isoenzymes, and myoglobin.
(4) Any type of myocardial injury, not just ischaemic injury, can result in release of cTn into the blood.

Pre-analytical and analytical factors of cardiac troponin assays

Pre-analytical factors and characteristics need to be considered for both laboratory-based analyses and point-of-care testing assays. These considerations include how samples need to be collected, preserved, stored, and transported to the laboratory to ensure accurate measurements. Knowledge of these issues is essential. As these characteristics are method-dependent, they require separate definition for each commercially available cTn assay before this one is introduced into clinical practice. Clinicians should inquire about these issues for the assay they use locally. For clinical research, information on the long-term stability of cTn in frozen samples is important prior to the use of archived samples. To increase the rapidity with which values can be obtained in clinical routine, whole blood and plasma are the specimens of choice for the emergency laboratory to avoid delay due to sample centrifugation and clotting problems. There can be significant differences between serum and plasma concentrations for some analytical systems.[23] Ethylene diamine tetra-acetic (EDTA) acid influences the degree of cTnI complex formation and, therefore, differences between EDTA plasma and other sample types have to be carefully evaluated. The high doses of heparin used during sample collection can bind cTn and affect results, by masking specific epitopes resulting in a reduction in the measured concentration of cTn.[24] The effects of therapeutic doses of heparin also have to be carefully evaluated, because interference may occur in early samples from AMI patients although false negative results have not been reported. Furthermore, haemolysis may lower cTnT values and increase cTnI concentrations of some assays.[25]

Lack of cardiac troponin assay standardization

In contrast to cTnT, many cTnI assays are on the market. These cTnI assays are not standardized as yet and studies have documented substantial differences across methods.[26] The lack of comparable cTnI patient values and the inability to define common decision limits for cTnI have led to confusion among clinicians using data from different methods. It is important that a clinically relevant cardiac marker, such as cTn, is measured with standardized methods to achieve comparable results. A cTnI standardization subcommittee of the American Association for Clinical Chemistry in collaboration with the National Institute of Standards and Technology has developed a reference material (SRM no. 2921), which is a purified cTnICT complex recommended as a calibrator of commercial cTnI assays.[27] However, it is of limited value for cTnI assay harmonization because of instability in human serum and matrix effects in different assay platforms.

Apart from the lack of a commutable reference material, other factors contributing to quantitative differences between cTnI methods include the variable antibody immunoreactivity to different circulating cTnI forms and varying calibrators used in different cTnI assays. The proper way to achieve complete standardization for cTnI would be to exert pressure on the manufacturers to agree employing antibodies with similar epitope specificities for all commercial assays, as well as to overcome matrix effects with a serum-based common reference material for calibration.[28] However, that is a complicated matter and, evidently, the progress in the standardization and harmonization of cTnI assays is slow. Thus far, only one manufacturer has marketed a diagnostic cTnT assay but the recent commercially available hs-cTnT assay is not harmonized with the previous cTnT assay generation across the entire measuring range. At low concentrations, the hs-cTnT does not provide results comparable to the previous cTnT assay including at concentrations near the critical 99th percentile cut-off.[29] Thus, low decision limits obtained with the former cTnT assay generation cannot be extrapolated to the new hs-cTnT assay.

Antibody selection

For all assays, the epitopes recognized by the antibodies must be delineated. Selection criteria must take into account not only the specificity of cTn, but also binding affinities which determine detection limits and assay time. Cross-reactivity to other cardiac and skeletal Tns must be insignificant. Sample stability for cTn also depends on the specific epitopes recognized by the antibodies in a cTn test system. It is recommended that the antibodies selected bind cTn epitopes on stable parts of the molecule unaffected by complex formation or post-translational modifications.[30] The cTnI sequence located between amino acid residues 30 and 110 is the most stable region of cTnI and so this is the area most often targeted for antibody detection. Ideally, cTnI assays should recognize all major circulating forms of cTnI equally to allow monitoring of total cTnI present in samples. Manufacturers should provide information in cTnI package inserts about the immune reactivity with the major circulating cTnI forms. Antibody selection must also be optimized for cTnI to reduce influence by cTnI autoantibodies which bind to part of the central region (aa 87–91) that

may result in false negative test results at low cTnI concentrations.[14] Antibodies to the carboxyterminal end of the molecule should be avoided or the *in vitro* stability must be checked carefully since these epitopes are cleaved in myocardium and in blood. Cardiac troponin T assays should also measure total cTnT including all relevant degradation products to achieve optimal clinical sensitivity.

Detection limit and analytical imprecision

For clinical use, an important assay characteristic is the limit of quantification which is the lowest amount of cTn that can be quantitatively determined with clinically acceptable total error. Optimal discrimination between a small amount of myocardial injury and analytical noise requires assays that have a low detection limit and a high precision even at low cTn concentrations. Imprecise assays generally lack sensitivity. The analytical characteristics of commercially available cTn assays can be found at http://www.ifcc.org/pdf/scientificactivities/committees/c-smcd/ctn_assay_table_v091209.pdf. For some cTn assays, the literature gives higher 10% coefficient of variation (CV) concentrations and sometimes also different 99th percentile values.[31,32] This may reflect differences in sample size, or the reference population characteristics, but often assays perform better in a highly quality-controlled setting such as development laboratories than in the real world environment.[31,32] We endorse the statement of the Joint ESC/ACCF/AHA/WHF Task Force that cTn imprecision of 10% or less at the 99th percentile is desirable.[1] However, modestly higher CVs do not appear to lead to a statistically significant increase in false test results.[33–36] Thus, the analytical goal of imprecision at the 99th percentile represents a valuable quality specification, and cTn assays with substantial imprecision (a CV $>$ 20%) at the 99th percentile should no longer be used because of a significant risk of misclassification of patients and the fact that more precise routine assays are commercially available.[33]

Assay interferences

False positive and negative test results are rare but may occur in all immunoassays because of interferences from heterophilic antibodies or human auto-antibodies, which can mimic cTn by linking the capture and detection antibodies. Alternatively, they can prevent antibody binding to cTn in the blood sample. Most assay systems contain blocking antibodies to avoid these interferences but at times, antibody titres may be sufficiently high that they are not totally inhibited. Icteric, lipaemic, and haemolysed samples can also be a problem depending on the assay design. Accordingly, the lack of interferences in an assay system should be carefully documented. Interfering substances in general cause two types of patterns. The first is where the values are elevated and remain so chronically. Dilution of the samples often fail to cause changes in values until the interference (most often cross-reacting antibodies or antibodies to either the immunoglobulin used to make the assay antibodies or other constituents in the assay) is gone at which time, the values become markedly reduced. This artefact can be unmasked as well—if heterophilic antibodies are the cause—by the administration of additional blocking antibodies in heterophilic blocking tubes. Grossly abnormal cTn values which are inconsistent with a clinical presentation should alert the physician to an analytical interference. Low-level interferences are harder to detect, although constantly elevated cTn concentrations with little change not fitting the clinical presentation are highly suspicious. The second type of interference is due to fibrin strands which can cause transient false positive test results but usually in only one sample. In addition, though uncommon, false negative results which are difficult to recognize, especially when cTn increases are modest, can occur due to interfering heterophilic or cTn auto-antibodies[37,38] and in case of cTnT with haemolysis.[25]

Decision and reference limits

By general consensus, it is recommended that the decision limit for myocardial injury be the concentration that corresponds to the 99th percentile limit of the reference distribution in healthy people.[1] This cut-off value was chosen originally to minimize the number of false positive values that could/would potentially be included within the abnormal range which could potentially confound the diagnosis of AMI. To implement this sort of standard, the key characteristics for each commercial cTn assay should include determination of the distribution of cTn concentrations in a sex- and age-matched healthy reference population. This reference population should ideally have negative exercise stress tests and normal cardiac function as assessed by imaging. The effects of gender and ethnicity need to be evaluated as independent variables. The calculation of the 99th percentile is markedly affected by outliers, and consequently to reach the 95% probability that at least 99% of the population will fall below the highest observed cTn value, a sample size of at least 300 individuals per group is required.[39] It is difficult and costly to meet these requirements and most laboratories do not have the resources to perform cTn reference limit studies.

Critical clinical concepts

(1) Clinicians must be aware of the analytical quality and limitations of the cTn assay used in their local laboratory. For acute cardiac care, it is important to focus on a high analytical quality of the assay. The goal for imprecision at the 99th percentile value of the reference population should be $\leq$10% CV. Assays with a CV $>$20% should not be used because of the risk of patient misclassification.

(2) Laboratories should report the 99th percentile value of the reference population and ideally also cut-off values for significant magnitude of changes in serial cTn testing which are related mainly to assay imprecision at a given cTn concentration with most assays presently in use.

(3) Clinicians must know that analytical interferences as well as improper handling of specimens can cause false-positive and false-negative cTn results. In the case where implausible results arise such as constantly elevated cTn concentrations without significant changes, investigations to exclude false-positive elevations should be initiated.

(4) Clinicians must be aware of the lack of consistency between cTnI assays and the defective progress in the standardization and harmonization of cTnI assays.

Interpretation of cardiac troponin test results

Multiple issues should be taken into consideration when interpreting cTn test results. cTn elevations are markers of myocardial damage but only clinical symptoms and further diagnostic evaluations can clarify the aetiology of the cTn release. Only if acute myocardial damage is caused by myocardial ischaemia should AMI be diagnosed. However, other pathologic conditions such as depicted in *Table 1* can also lead to myocardial damage and should not be confused with AMI.[1]

The recommended routine cTn cut-off limit is the 99th percentile limit of a healthy reference population. However, there is increasing body of evidence demonstrating that any detectable concentration of cTn with the contemporary assays is associated with an impaired outcome in various clinical settings.[40–43] With the increasing sensitivities of cTn assays, it has become important to differentiate acute from chronic myocardial damage by evaluating the rise and fall of cTn concentration in serially drawn blood samples, e.g. 3–6 h apart.[44] Acute processes usually manifest a rising pattern, whereas those that are more chronic, e.g. those associated with chronic renal failure, stable CAD, chronic heart failure, and severe left ventricular hypertrophy, generally do not show much change. This will be of particular importance as the further increasing sensitivities of cTn assays unmask more and more chronic elevations. It should be emphasized, however, that in some circumstances a significant increase will not be seen despite a recent acute event. For example, near peak values and on the long persistent elevations on the tail of the time concentration curve, a significant change in values may not be observed. That is why changes must be always interpreted in the clinical context in which they are found. Therefore, it is important to define a significant cTn concentration increase. The difference between the results of two consecutively drawn blood samples is based on the variation around these measurements. This variation includes analytical variability, biological variability, and potentially ongoing pathology. With less-sensitive cTn assays, significant troponin elevations have to occur before a positive result is obtained and biological variation is overshadowed by the changes occurring from myocardial damage. With novel hs-cTn assays, biological variation may become significant at concentrations within the reference interval or modestly increased cTn concentrations.[45,46] Biological variation is less likely to be an issue with markedly increased cTn concentrations because of the predominant effects of ongoing myocardial damage. For measuring cTn over a short period of time, assay lot-to-lot variation can be usually neglected as well. The degree of analytical variation can be determined by making repeated measurements of a single sample. The obtained results are distributed normally around the mean value. Statistically, if the follow-up sample is outside the mean $\pm$ 3 standard deviation (SD) range of the baseline sample, the difference is significant. The SD can be calculated from the CV of an assay at a given cTn concentration [SD = CV (%) $\times$ mean (μg/L]/100], and the formula for the calculation of a significant difference is $\pm 1.96 \times \sqrt{2} \times \text{SD} = 2.77 \times \text{SD}$ assuming that the variability is similar for both values being measured.[46] For example, a cTn increase of 50% from baseline (from 0.010 to 0.015 μg/L after 3 h) may be significant even including biological variation with some assays but not others. Just based on analytical variation with a cTn assay with a CV $\geq$ 20% at 0.010 μg/L, the increase is not significant, whereas it is with an assay having a CV of 15%. If cTn values are markedly elevated, a >20% change is usually significant since at higher concentrations, the CVs for most assays are 5–7%.[3] It should be appreciated that the degree of change required for any given value will vary depending on the cTn concentration.

Biological variability has been difficult to measure since it requires cTn analysis of healthy people over time. At present, the only available measurement of conjoint analytical and biological within hour variation of cTnI in 12 healthy individuals suggests that it is in the range of 32–46% for one of the very high sensitivity assays in development (detection limit about 10-fold lower than the most sensitive currently available routine cTn assay).[45] However, this measure needs to be calculated for each assay and the value from one assay cannot be extrapolated to another.

Table 1 Elevations of cardiac troponin in the absence of overt ischaemic heart disease

Damage related to secondary myocardial ischaemia (MI type 2)
Tachy- or bradyarrhythmias
Aortic dissection and severe aortic valve disease
Hypo- or hypertension, e.g. haemorrhagic shock, hypertensive emergency
Acute and chronic heart failure without significant concomitant coronary artery disease (CAD)
Hypertrophic cardiomyopathy
Coronary vasculitis, e.g. systemic lupus erythematosus, Kawasaki syndrome
Coronary endothelial dysfunction without significant CAD, e.g. cocaine abuse
Damage not related to myocardial ischaemia
Cardiac contusion
Cardiac incisions with surgery
Radiofrequency or cryoablation therapy
Rhabdomyolysis with cardiac involvement
Myocarditis
Cardiotoxic agents, e.g. anthracyclines, herceptin, carbon monoxide poisoning
Severe burns affecting >30% of body surface
Indeterminant or multifactorial group
Apical ballooning syndrome
Severe pulmonary embolism or pulmonary hypertension
Peripartum cardiomyopathy
Renal failure
Severe acute neurological diseases, e.g. stroke, trauma
Infiltrative diseases, e.g. amyloidosis, sarcoidosis
Extreme exertion
Sepsis
Acute respiratory failure
Frequent defibrillator shocks

The reported intraindividual variation of cTnI was low (about 10%) compared with the interindividual variation (about 60%).[45] The biological variability of cTnI in healthy individuals cannot be extrapolated to cTnT and it is only recently that such data of a comparably higher biological short-term variability of cTnT over a period of a few hours in healthy individuals has become available when cTnT was measured with a hs-cTnT assay.[47] In general, good laboratories should be able to develop algorithms to determine if significant changes have occurred in serial testing and laboratory reports should state if significant changes have occurred. In particular, this is essential for low cTn concentrations in the range of the 99th percentile value. In this range even with very precise assays, based on the limited available data, only marked increases should be considered as being significant. This area is evolving rapidly so when additional biological, analytical, and clinical data are available, more robust recommendations may appear.

Critical clinical concepts

(1) cTn values should be interpreted in proper clinical settings.
(2) An assay-specific cTn decision limit (the 99th percentile value) is mandatory for an immediate management of the patient. However, any detectable cTn value measured with worldwide approved routine cTn assays should be taken into consideration as an indicator of higher risk.
(3) Scrutiny of the kinetics of cTn release in blood is essential to differentiate acute from chronic myocardial damage.
(4) The interpretation of changes cannot be done in isolation from the clinical situation and particularly issues relating to the timing of the event being evaluated.
(5) If the timing of sample acquisition is consistent, laboratories can and should report changes that exceed analytic, and if relevant for very high sensitivity assays, biological variation.

Measuring cardiac troponin for detection of myocardial infarction

The criteria of the universal definition of AMI is a rising and/or falling pattern of cTn concentrations with at least one value above the 99th percentile limit of the reference value distribution in the setting of a patient with clinical features of myocardial ischaemia.[1] The latter is indicated by symptoms of ischaemia, ECG changes indicative of new ischaemia, development of pathological Q-waves, or imaging evidence of the new loss of viable myocardium or new regional wall motion abnormalities.[1] When an increased cTn value is encountered in the absence of evidence of myocardial ischaemia, a careful search for other possible aetiologies of cardiac damage should be undertaken (*Table 1*). Serial measurements of cTn are necessary when cTn concentration is not elevated on admission as cTn values may not appear in blood within the first hours after myocardial injury. As the timing of symptoms may not be totally reliable, cTn must be measured on admission and 6–9 h later. In patients with an intermediate or high clinical index of suspicion who remain cTn negative, and in those with plausible recurrence of ischaemic symptoms, repeat testing at 12–24 h should be considered to increase diagnostic sensitivity. Given the rapid positivity of contemporary assays, some have advocated a sample at 3 h after admission as well since upwards of 80% of AMI patients will have elevations by that time.[48] The introduction of still more sensitive and precise cTn assays and the use of the 99th percentile value allows for an earlier more accurate diagnosis of AMI,[49,50] which questions the need for additional testing with 'early ischaemic biomarkers'.[51]

Elevated cTn values in patients with acute ischaemic presentations are related to more extensive CAD, pro-coagulant activity, and lower coronary perfusion. As such, they mark patients at higher risk for the development of cardiac events during short- and long-term follow-up.[52,53] The risk is also related to the magnitude of elevations in patients with and without ECG changes. While the short-term outcome is closely associated with the higher acute thrombotic risk of the underlying unstable plaque, long-term prognosis most likely reflects the higher prevalence of a more severe and complex coronary anatomy.[54] Meta-analyses suggest a comparable prognostic performance of cTnT and cTnI assays in most clinical settings except for patients with end-stage renal disease.[53,55–57] It is also worthy of note that some patients, particularly women can have AMI in the absence of severe CAD. This is likely the reason for finding the contrast-enhanced cardiac magnetic resonance imaging diagnosis of AMI in patients with classic presentation but putatively normal or near normal coronary arteries.[58–61]

Critical clinical concepts

(1) Cardiac troponins are the preferred biomarkers for the diagnosis of myocardial necrosis, and for risk stratification of patients with AMI. Meta-analyses suggest a comparable performance of cTnT and cTnI assays in most clinical settings.
(2) Diagnostic cut-off values must comply with the universal definition of AMI, i.e. the employment of the 99th percentile upper reference value as decision limit.
(3) A rising pattern is important when patients present early. This criterion may not be met in the late phase after the onset of acute myocardial damage.
(4) If the first blood sample for cTn is not elevated, a second sample should be obtained after 6–9 h, and sometimes a third sample after 12–24 h is required. This may change with novel higher sensitivity assays that are being developed.
(5) In AMI and other clinical conditions, elevated cTn values signal a higher acute risk and an adverse long-term prognosis.

Other causes of cardiac troponin release

Table 1 summarizes other causes of myocardial damage, which can be separated into causes of secondary myocardial ischaemia (AMI type 2), diseases not associated with myocardial ischaemia, and conditions where the exact mechanisms are uncertain or multifactorial. Elevations of cTn, related to putative supply–demand abnormalities (ischaemia due to increased myocardial work in the absence of a significant structural or functional abnormality in a coronary artery), should be labelled as cardiac damage but not necessarily AMI even if there are ST and/or T wave changes. Other frequent mechanisms of troponin increases not related to

ischaemia include myocardial damage due to inflammatory processes or toxic agents or trauma.[62] Patients with elevated cTn values should be followed closely since these elevations in almost all situations are associated with an adverse prognosis.[52,55–57,63,64] Some of these patients if they manifest acute presentations may have a rising and/or falling pattern of cTn values. Patients without a changing pattern (see caveats above in regard to the timing of the evaluation) should not be diagnosed as having AMI or other acute reasons for the elevation. Some patients with stable CAD, chronic renal failure, chronic heart failure, and severe left ventricular hypertrophy can have chronic elevations of cTn which may or may not change markedly over the short term.[65–68] These individuals including those with values above the 99th percentile limit should not be diagnosed as having AMI or other acute aetiologies for the cTn elevations in the absence of significant changes in values over time. However, the higher the cTn values, the higher is the likelihood of an AMI.

Elevated cardiac troponin in heart failure

Patients admitted with acute onset or worsening of heart failure require particular attention. Often the substrate for heart failure is CAD. However, even individuals with dilated cardiomyopathy can have elevated cTn values with or without imaging evidence of cardiac injury. Some of these elevations could be related to subendocardial supply–demand abnormalities since wall stress is an important determinant of subendocardial blood flow. It may be that some of these elevations are related to coronary endothelial dysfunction which is known to occur in heart failure patients,[68] but cardiomyocyte injury may also be due to acute left ventricular stretch which may cause proteolysis and release of cTn.[69] It is evident that such events, both acutely and chronically, are associated with adverse short- and long-term outcomes. However, coding them as AMI despite comparable pathophysiology may in some cases require further delineation of the cause of the elevated cTn values by invasive and/or non-invasive evaluation. Nevertheless, the vast majority of these patients should probably be designated as having heart failure-related myocardial injury.

Elevated cardiac troponin in renal disease

Patients with severe or end-stage renal failure have often elevations of cTns and especially of cTnT. However, no satisfying generally accepted explanation of that has been found yet despite evidence of diffuse myocardial injury.[56,70,71] The mechanisms involved may be similar to those observed in heart failure patients or could be related to renal failure metabolic milieu which may cause skeletal muscle myopathies as well. As far as we know, cTnI is not expressed in skeletal myopathies. On the other hand, cTnT isoforms may be expressed in skeletal myopathy, but according to the available literature these isoforms do not cross-react with the antibodies used in the commercially available cTnT assay.[17] Thus, if cTnI and cTnT increase in skeletal myopathy, cardiac involvement must be suspected. However, these individuals usually have chronic elevations and should only be diagnosed as having AMI when they present with compatible symptoms, ECG or imaging findings, and a rising pattern of cTn values. Regardless of the mechanisms, the risk of death in end-stage renal failure patients increases directly with the measured cTnT concentration.[56] In several trials[56,70] of patients undergoing chronic haemodialysis, it has been found that the proportion of patients with positive cTnT (18–75%) is greater than that with a positive cTnI (4–17%). Although the risk associated with an elevated cTnI value is similar, because there are so few elevations, cTnI failed to predict prognosis overall for groups of patients in most studies of patients in end-stage renal disease.[56,70]

Critical clinical concepts

(1) In clinical settings other than AMI, the detection of elevated cTn values should prompt a careful search for other possible aetiologies of cardiac damage. In the absence of evidence of plausible myocardial ischaemia, these elevations should not lead to a diagnosis of AMI.
(2) Patients with heart failure often have cTn elevations and they can have a rising pattern of values. It can be difficult to distinguish these events from AMI on biochemical basis only.
(3) Patients with end-stage renal failure often have cTn elevations, particularly of cTnT which are of prognostic importance. In these individuals, AMI should only be diagnosed based on rising values from an elevated baseline in an appropriate clinical setting.
(4) Elevations of cTn values in critically ill patients should be evaluated in the context of their underlying disease and known cardiac and non cardiac co-morbidities.
(5) The higher the cTn values, the higher is the likelihood of an AMI.

Outlook: the highly sensitive cardiac troponin assays in development

Several advanced hs-generations of cTn assays are now being developed. Some would call these ‘high sensitivity’, ‘ultra sensitive’, or ‘novel highly sensitive assays’, but at the present time there is no consistent terminology. However, the hs-assays appear to be capable of measuring cTn concentrations in almost all healthy subjects,[20,29,72–74] and it has been suggested that this ability be used to define these assays regardless of the nomenclature.[74,75] The hs-assays will increase the ability to detect AMI at an even earlier phase as recently documented when a more sensitive contemporary assay substituted a former less-sensitive iteration or when the recommended 99th percentile decision limit is used rather than higher values.[49,50,76] Even these minor elevations found with high performance novel research assays might be able to identify myocardial ischaemia in exercise stress tests as suggested by Sabatine *et al.*[20] who demonstrate minute changes of cTnI associated with the presence and severity of perfusion defects in nuclear stress tests of patients with possible myocardial ischaemia. Whether the level of imprecision and the diagnostic accuracy are sufficient to make this observation in less selected individual patients is uncertain, it may be that this can only be documented for different groups of patients.[20,45] Therefore, before applying these results in clinical practice, further data are necessary. It appears that the hs-assays also may be troubled by

difficulties in accuracy at low concentrations.[77] At these low concentrations, biological variability of baseline values becomes an issue.[45–47] In addition, the determination of reference values will depend critically on what is selected as a reference population and, furthermore, the possibility of analytical confounders is higher with this level of sensitivity.[78] Testing the assay performance in unselected chest pain patients in emergency departments will be crucial before we begin utilization of these 'high performance' assays in clinical routine.

Critical clinical concepts

(1) The advent of highly-sensitive cTn assays will herald an era where there will be increased diagnostic information but also additional challenges in the interpretation of test results.
(2) Current data highlight a variety of still unresolved issues. These demand scientific scrutiny and have to be resolved before single or serial cTn values from these 'high performance' assays can be taken as proof of myocardial injury or necrosis and/or the routine use of these assays.

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