Mini-Review

Revisiting the Biological Variability of Cardiac Troponin: Implications for Clinical Practice

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Abstract
The diagnosis of acute myocardial injury requires a rise and/or fall of cardiac troponin (cTn) on serial testing, with at least one concentration above the 99th percentile value of a normal reference population according to the recently published *Fourth Universal Definition of Myocardial Infarction*.[1] However, the magnitude of change in cTn that constitutes a significant rise and/or fall was again not specified in detail. High-sensitivity cardiac troponin (hs-cTn) assays can measure ten-fold lower concentrations of cTn with more precision than older assays, and can accurately quantify cTn in more than 50% of healthy individuals with a coefficient of variation of less than 10% at the 99th percentile. These hs-cTn assays are also able to detect the normal variations in cTn results that are due to biological variability. Understanding and quantifying the normal variations in cTn is important as this would allow significant changes to be better defined. Numerous studies have sought to investigate the biological variability of cTn over the last ten years. Such studies are usually conducted in healthy individuals, however individuals with chronic cardiac disease or chronic renal failure have also been examined. These studies have yielded varying results in regards to significant change values for cTn. In light of the recent redefinition for myocardial infarction, the purpose of this mini-review is to revisit the biological variability of cTn. In particular, we outline concepts for determining a significant change value, review the results of previous studies on the biological variation of cTn and discuss potential considerations for clinical practice.

Introduction

Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are present almost exclusively within myocardial cells and are highly specific biomarkers of myocardial injury.[1] According to the recently published *Fourth Universal Definition of Myocardial Infarction*, the diagnosis of acute myocardial injury requires a rise and/or fall of cardiac troponin (cTn) on serial testing, with at least one concentration above the 99th percentile value of a normal reference population as shown in **Figure 1**.[1] For the diagnosis of acute myocardial infarction however, clinical evidence, including typical symptoms, new electrocardiogram changes or new cardiac imaging changes that are consistent with acute myocardial ischaemia, is also required.[1]

The majority of cTnI and cTnT forms part of the contractile apparatus within the myocardial cell with lower concentrations found in the cytoplasm.[2] Myocardial ischaemia can result in myocardial cell necrosis and the initial release of cTn from the cytosolic pool into the bloodstream within a few hours of the injury.[2] This is typically followed by a more prolonged and sustained elevation of cTn due to degradation of the contractile apparatus and which may also be a reflection of the infarct size.[2] However the release kinetics of cTn after myocardial injury can differ between individuals and is also dependent on myocardial blood flow.[1] It can also differ between cTnI and cTnT which are thought to have monophasic and biphasic concentration-time profiles respectively, and with the increase in cTnT tending to last for longer than that of cTnI.[1,2]

The importance of serial changes in cardiac-specific biomarkers for the diagnosis of myocardial infarction was recognised in reports as early as the 1970s by the World Health Organisation (WHO).[3] Around that time, the preferred biomarker was creatine kinase and its MB isozyme (CK-MB). Assays for cTnI and cTnT were not developed until the late

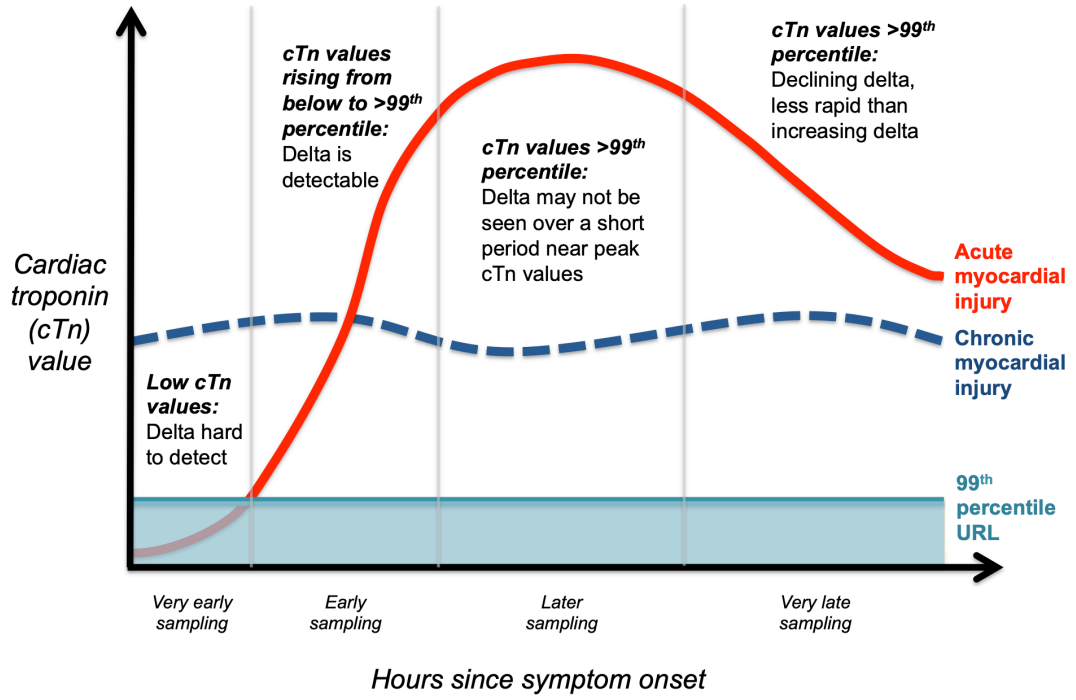

Figure 1. Early cardiac troponin kinetics after an acute myocardial injury (adapted from ref. 1).
Abbreviations: cTn, cardiac troponin; URL, upper reference limit.

1980s.[4,5] Due to the higher specificity and sensitivity of cTn, the European Society of Cardiology (ESC) and American College of Cardiology (ACC) redefined cTn as the preferred biomarker for the diagnosis of myocardial infarction in a joint guideline published in 2000.[6] The Global Task Force further reinforced the increasing dominance of cTn as the gold-standard biomarker in the *Universal Definition of Myocardial Infarction* in 2007.[7] Major diagnostic and management advances, such as the advent of more sensitive cTn assays, have led to further revisions of the definition in 2012 and more recently 2018, where the term 'myocardial injury' was officially introduced.[1,8]

Modern understanding of what constitutes a significant rise and/or fall of cTn has also evolved over time.[1] However the magnitude of change that constitutes a significant rise and/or fall in cTn was again not specified in detail in the *Fourth Universal Definition of Myocardial Infarction*.[1] This reflects both a lack of evidence and also the inability of previous assays to accurately measure biological variability in order to inform the consensus of this important part of the definition. As such the current definition uses arbitrary thresholds for cTn concentrations, which is a continuous variable. It is suggested that a relative change of 50–60% over 3–6 hours may be indicative of an acute myocardial injury when the initial cTn concentration is at, or below the 99th percentile value.[1,9] This 50–60% change is said to have been based on the combined biological and analytical variability of cTn.[1] On the other hand, if the initial cTn concentration is above the 99th percentile value, a 20% change may be indicative instead, as the analytical variation of the assay will be less at higher concentrations.[1,9,10] However this suggested 20% change is essentially arbitrary, as it is based on a calculation of three times the analytical variability of previous generation assays.[10,11]

High-sensitivity cardiac troponin (hs-cTn) assays are now becoming more widely used in clinical practice and are able to accurately quantify small fluctuations in cTn concentration in apparently healthy individuals.[12] Such variations may occur due to reasons including biological variability - the random fluctuation of an analyte around an individual's inherent 'homeostatic set point'.[12,13] Understanding the normal variations in cTn results is therefore important, as this would then allow a pathological rise and/or fall to be better defined, so that clinicians are better able to recognise an acute myocardial injury.

The purpose of this mini-review is to bridge the biochemical and clinical understanding and discuss implications of the

biological variability of cTn. In particular, we outline the challenges associated with utilising hs-cTn assays, reasons for cTn variation and basic concepts for determining a significant change value, review the results of previous studies on the biological variation of cTn, and discuss potential considerations for clinical practice.

High-Sensitivity Cardiac Troponin Assays

Hs-cTn assays can measure ten-fold lower concentrations of cTn with more precision than previous generation assays and are able to accurately quantitate cTn in more than 50% of healthy individuals with a coefficient of variation of less than 10% at the 99th percentile.[14] The Joint Consensus Statement for the Diagnosis of Myocardial Infarction published in 2000, played a major role in the advent of hs-cTn assays as no assay met the coefficient of variation criteria at the time.[6]

The term 'high-sensitivity' reflects the analytical characteristics of the assay itself and not the analyte that is measured.[14] The analytical sensitivity refers to the ability of an assay to detect a low concentration of a given analyte, where the lower the detectable concentration, the greater the sensivity.[15] This is distinct to that of diagnostic sensitivity, which refers to the ability of a test to correctly identify individuals with a given condition, and is calculated by dividing the number of true positive tests by the number of individuals with the condition in a given population.[15]

The detection limit of cTn has become significantly lower with hs-cTn assays such that concentrations are now reported as whole numbers in nanograms per litre (ng/L).[14] The introduction of these assays has resulted in a paradigm shift, as they can now support an earlier rule-in or rule-out of myocardial infarction following the onset of symptoms, and may therefore allow for earlier treatment or discharge.[16,17] Consequently there has been increasing research in emergency department protocols for early or single measurements of hs-cTn to facilitate the rapid diagnosis or exclusion of myocardial infarction for individuals presenting with chest pain.[18-22]

Numerous high-sensitivity assays are currently available to measure cardiac troponin I (hs-cTnI) and cardiac troponin T (hs-cTnT).[12,14] However, there are significant differences between these assays with regards to their characteristics, such as the limit of detection (lowest amount of analyte that can be detected in a sample with a given probability), analytical variability, and the antibodies that are used to detect various cTn epitopes.[14] A lack of assay standardisation and harmonisation makes it difficult to compare cTn results between assays, and, therefore between laboratories or hospitals.[12,14,23]

The *Universal Definition of Myocardial Infarction* requires at least one cTn concentration above the 99th percentile value of a normal reference population for the diagnosis of myocardial injury.[1] However there have been some concerns regarding the use of a 99th percentile threshold value for hs-cTn in this setting, as it is a very labile entity.[24] Firstly, the 99th percentile differs according to each assay.[14] Secondly, the 99th percentile differs according to reference population selection (age, gender, ethnicity and definition of healthy status), reference population size and the statistical method used to calculate it.[25-32] Some studies have found that elevations of hs-cTn are commonly seen in older adults, which may be independent of comorbidities.[26,28,30,33] As such, there is debate over whether older adults should have age-adjusted diagnostic thresholds for the diagnosis of acute myocardial injury.[31] Thirdly, detectable chronic elevations in cTn above the 99th percentile are commonly seen in conditions such as chronic renal or cardiac failure.[34-37] In addition, the improved analytical sensitivity of these assays has resulted in the detection of elevated cTn in numerous cardiac and non-cardiac conditions that cause myocardial cell necrosis, such as myocarditis, arrhythmia, cardiac procedures, cardio-toxic drugs, pulmonary embolism and sepsis.[1,12] Due to these challenges, international guidelines have sought to promote consistency by proposing recommendations for determining 99th percentiles.[38] It would therefore seem that the 99th percentile should not be the only metric for diagnosing acute myocardial injury.

The increasing use of hs-cTn assays has required that, to aid in the diagnosis of acute myocardial infarction, clinically and statistically significant changes in cTn results on serial testing be established. In order to do this, an understanding of the normal changes in cTn concentration over time is necessary. The four main reasons why cTn results may change are sample integrity (e.g. pre-analytical variation), assay variation (e.g. analytical variation), biological variation and pathology.[39,40] It is only by understanding and quantifying the first three of these sources of variation that reliable data on the important pathological changes can be formulated.

Pre-Analytical Variability

Pre-analytical variability refers to factors that can influence test results prior to analysis.[41] For example, differences in how samples are collected, transported, handled and stored, can contribute to pre-analytical variability.[13,41] Individual factors such as fasting status, recent exercise and posture may also contribute to variations in test results.[13]

For cTn, pre-analytical factors such as differences in specimen collection tube, lipaemia, icterus, haemolysis, specimen storage duration and temperature, and microclots or debris, can be contributing factors.[40] However the variation in hs-cTn

results caused by these factors is likely to be relatively small in magnitude.[40,42,43] Patient factors including physiological stress to the myocardium, due to various forms of exercise or pharmacological stress testing, can result in the release of cTn into the circulation, even in normal hearts.[44-46] For example, one of the largest studies to date examining cTn post-exercise, in 482 marathon runners, found that 68% had an increased cTn concentration after the race.[47] Release of cTn post-exercise is currently thought to be physiologic rather than from myocardial necrosis, and may be influenced by factors such as exercise intensity, age, training experience, time of blood sampling and the assay used.[46] Additionally, physiological stress can occur in the surgical setting, with some studies showing post-operative increases in hs-cTn, even in young adults without cardiovascular disease undergoing non-cardiac surgery.[48,49] Changes in posture however, do not appear to cause significant variation in cTn results.[50]

Analytical Variability

Analytical variability (CV_A), also known as coefficient of variance, or imprecision, refers to the inherent variation of the assay.[13] The analytical variability can be determined by assaying test samples in duplicate to evaluate for variation in results.[13] Although every assay has intrinsic sources of variability and bias, these can be minimised by high quality laboratory practice and methodology.[13] Hs-cTn assays should have an analytical variability that is less than 10% at the 99th percentile of a normal reference population.[14] Such a low analytical variability means that random variation of cTn results due to analytical influences is low (i.e. there is less 'analytical noise').[13] Less common but important sources of analytical variability for cTn include instrument malfunction, calibration drift and the presence of interfering antibodies, which are further discussed below.[40,51,52]

Heterophilic antibodies and human anti-species antibodies can occasionally interfere with cTn immunoassay measurements and typically cause false positive results[51,52] The presence of interfering antibodies to cTn assays is unpredictable, with an estimated prevalence of up to 3.1% of individuals.[51] One should suspect such interference when test results do not fit the clinical context, thus highlighting the importance of communication between clinicians and the laboratory.[51] The presence of interfering antibodies can be further investigated by initially repeating the sample analysis on an alternative manufacturer's cTn assay, or by testing for non-linearity of cTn test results on serial dilutions, or testing for other biomarkers of cardiac injury.[40,51] Methods to remove or inactivate interfering antibodies include the use of heterophilic antibody blocking reagents, immunoglobulin-blocking reagents, immobilised protein A column or polyethylene glycol precipitation.[51,52]

In addition, anti-cTn antibodies may be directed towards cTnI or cTnT and can also interfere with cTn immunoassay measurements.[40] The presence of these antibodies is not as easily recognised as they usually cause false negative results by inhibiting the binding of assay antibodies to certain cTn epitopes, most commonly the stable mid-fragment of cTnI.[40,53] The estimated prevalence of anti-cTn antibodies was previously found to be 12.7% in 467 healthy blood donors and 9.2% in 510 individuals with suspected myocardial infarction.[54,55] Detection of these antibodies can be achieved by using immunoassays developed for this purpose.[53,55] However the presence of anti-cTn antibodies does not always result in cTn assay interference, as the specific epitope-binding sites targeted by assay antibodies vary widely between manufacturers.[40] It is possible that anti-cTn antibodies can increase the release of cTn and slow the clearance of cTn from the circulation after a myocardial infarction.[56] However the significance of anti-cTn antibodies and the correlation with long-term outcomes currently remains poorly understood.[55,56]

Macrotroponin occurs when cTnI is bound to a circulating antibody, resulting in a high molecular weight complex that is cleared more slowly from the circulation than free cTn.[40,57] It has recently garnered increased attention from the scientific community as this complex may be measured by some hs-cTn assays, particularly the Abbott Architect hs-cTnI assay.[40,57] Whilst anti-cTn antibodies typically cause false negative cTn results, macrotroponin can cause false positive results, largely manifesting as persistent low-level elevations in cTn concentration, which may not demonstrate a rise and/or fall pattern when acute myocardial injury is suspected.[40,57] The estimated prevalence of macrotroponin in one laboratory was found to be 4.7% of 1074 elevated cTn results.[57] Interference by a high molecular weight complex may be investigated further by re-analysis using an alternative assay, precipitation with an immunoglobulin-binding protein such as protein A or G, polyethylene glycol precipitation or gel filtration chromatography.[57] False positive cTn result may lead to further investigations that may be invasive in nature, thus further emphasising the need to be aware of analytical interference and the importance of interpreting laboratory results according to the clinical context.

Biological Variability

Biological variability (CV_I), also known as intra-individual variability or within-subject variability, is the random fluctuation of an analyte around an individual's inherent 'homeostatic set point', and is thought to be relatively constant in apparently healthy individuals or in individuals with chronic but stable disease.[58] It may occur secondary to circadian rhythm, monthly changes or seasonal changes.[12] The release of cTn into the bloodstream is not always thought

to be pathological, as it is postulated that cTn may be released due to normal myocardial cell turnover, apoptosis, cellular release of products, increased cell wall permeability with stress, and the production and release of membranous blebs containing cTn.[59] The biological variability of cTn can be accurately quantified under controlled conditions by serial testing of cTn in healthy individuals at regular intervals using hs-cTn assays.[14] These assays are able to accurately quantify cTn in healthy individuals and the normal fluctuations that occur, even at very low concentrations.[14]

The difference between the 'homeostatic set point' of individuals is termed the inter-individual biological variability (CV_G) or between-subject biological variability.[13] The ratio of the intra-individual biological variability to the inter-individual biological variability is termed the index of individuality (II).[58] The II is used to assess the utility of population-based reference intervals and can be calculated by the formula:[58]

$$II = (CV_A^2 + CV_I^2)^{1/2} / CV_G$$

where CV_A is analytical variation, CV_I is biological variation and CV_G is the inter-individual biological variability.

If the II is more than 1.4, then it may be more clinically useful to interpret a test result using population-based reference values.[58] On the other hand, an II less than 0.6 indicates strong individuality.[58] As such, conventional population-based reference values are of less value and the test may be better interpreted based on a change in serial results instead.[58] This is because a test result may lie within the normal population-based range, yet be highly unusual for a given individual due to the degree of change from a previous measurement.

Reference Change Values

Reference change values (RCV) are percentage changes which can be calculated by combining analytical and biological variation using the formula that was developed by Fraser and Harris:[58,60]

$$RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$$

where Z = 1.96 for a 95% probability or Z = 2.58 for a 99% probability, CV_A is analytical variation and CV_I is biological variation.

If the distribution of an analyte is non-parametric, the RCV may be expressed in terms of increasing or decreasing results after logarithmic transformation as described by Fokkema *et al.*[61] Although RCVs were initially described as percentage changes, they have recently been described using absolute numerical values.[62,63]

A change in serial test results that exceeds the RCV, suggests that the change is significantly different than that from intrinsic or normal sources of variation. Therefore determining RCVs would be useful for defining the rise and/or fall in cTn concentration that is required for the diagnosis of acute myocardial injury. However it must be noted that the formula by Fraser and Harris assumes that pre-analytical variation is minimised by strict sample collection and laboratory practice.[58] Furthermore, if a p-value of 0.05 is used to define statistical significance for the calculation of a RCV, then there is a 5% chance that a 'healthy' person may have fluctuations in cTn concentration that exceed this RCV.[64]

A variation of the RCV approach, where the Z-score is calculated instead, has been proposed.[65] Z-scores can be calculated using the following formula, which is a rearrangement of the RCV formula:[65]

$$Z = \Delta / (2CV_A^2 + 2CV_I^2)^{1/2}$$

where Δ is the difference in cTn concentration, CV_A is analytical variation and CV_I is biological variation.

Using a p-value of 0.05 to define statistical significance, a Z-score greater than 1.96 would mean that an observed difference in cTn concentration is unlikely to have occurred by chance.[65] The use of Z-scores has been shown to be comparable with absolute RCVs but superior to percentage change RCVs in terms of diagnostic performance.[65] In addition, using a Z-score of 1.96 has comparable diagnostic performance across different cTnI and cTnT assays with a range of analytical sensitivities.[65] In contrast to the RCV, which is assay-specific, the Z–score may provide a single cut-off value that can be used as a universal decision level for all cTn assays.[65]

Biological Variation Results from Previous Studies

Previous studies have sought to determine the biological variability of cTn in order to calculate RCVs, which can then be used to define significant changes in cTn concentration. Whilst this is a very important factor, it is only by comparing this statistically significant change to outcome data that pathological significance will be determined.

The biological variability of cTn has been evaluated in healthy individuals using a variety of hs-cTnI and hs-cTnT assays over the last 10 years. These studies generally recruit a modest number of between 10 to 20 participants whereby blood samples are collected at specific time intervals and designed such that pre-analytical variation is minimised by following strict specimen collection and handling protocols. However RCVs for a significant change in serial results have

been found to differ widely between studies. This may be due to disparities in study methodology, particularly participant selection and the definition of healthy status, time interval between serial samples, statistical methods and the use of different assays.[14,66] Furthermore, RCVs have, in general, been found to be higher for cTnT than cTnI, as summarised in **Table 1**.[11,32,64,67-75] Although there are gender-specific 99th percentile cut-off values for cTn, where cut-offs for women are generally lower, the biological variation may be similar between men and women according to some studies.[63,76] However the majority of studies do not report biological variability data stratified for gender, thus future studies are required to determine whether gender differences exist.

The weekly and monthly biological variability of cTn have also been studied, as this variability may have future utility in cardiovascular disease risk stratification, chronic disease monitoring and in the detection of myocardial damage from long-term cardio-toxic drug use.[11,12] It can also be seen in Table 1 that RCVs for serial measurements of cTn over the long-term (weeks to months) can vary considerably, and are usually higher than those for short-term (hours to weeks) measurements. In healthy children aged 8, 10 and 12 years, the long-term biological variability of cTn using a hs-cTnI assay has also been studied (see Table 1).[32] A large range of results for biological variability was seen, ranging from 0 to 136% (median 33%).[32] Interestingly, this study found that cTn was detectable in nearly all children, where concentrations increased with increasing age and left ventricular mass, thus supporting the notion that cTn release is not always pathological.[32]

In addition, it has recently been demonstrated that cTn may exhibit diurnal variations.[71,77,78] One study noted that cTnT concentrations exhibited a decreasing trend between 0830 hours and 1430 hours for healthy individuals and individuals requiring haemodialysis.[71] For cTnI concentrations, a decreasing trend during these hours was also noted in individuals requiring haemodialysis, however the pattern was not apparent in healthy individuals.[71] Furthermore another study in men with type 2 diabetes found that cTnT decreased during the day and then increased during the night, with peak concentrations in the morning at 0830 hours.[77] This was further confirmed in another study of healthy individuals, where cTnT exhibited diurnal variation but cTnI did not.[78] These findings may have implications for the diagnosis of myocardial infarction and also the determination of biological variability because of differences in blood sampling times.[78,79] Although there may be associations between circadian rhythm and acute myocardial infarction, the significance of diurnal variation in cTn concentration is yet to be established and further larger studies are needed.[77,80]

Although biological variability studies are normally conducted in healthy individuals, determining RCVs for cTn in older individuals or in individuals with chronic but stable renal or cardiac disease is also important. Furthermore, there have also been studies examining RCVs in patients presenting to the emergency department for assessment of acute coronary syndrome.[62,76] This is because healthy individuals may not be entirely representative of those in whom cTn is measured for the diagnosis of acute myocardial injury in clinical practice. In addition, elevations in cTn above the 99th percentile value are commonly seen in chronic renal or cardiac disease, due to reduced clearance of cTn from the circulation and chronic myocardial injury, amongst other reasons.[34-37] However, elevated but stable cTn concentrations on serial testing are more likely to be due to a chronic rather than an acute cause of myocardial injury. Current understanding of cTn variability is insufficient in these cohorts, as relatively few studies have been performed in individuals with renal or cardiac disease, as summarised in **Table 2** and **Table 3** respectively.[36,37,71-73,75,81-85]

Interestingly, the biological variability of cTn has been found to be fairly similar between healthy individuals and individuals requiring dialysis.[36,71,73] Furthermore, the biological variability of cTn in individuals with stable coronary artery disease or cardiac failure has also been found to be comparable to that in healthy individuals.[75,82,83] However RCVs are generally higher in healthy individuals due to a higher analytical variability at lower cTn concentrations.[36,71,73] Very few studies have assessed the biological variability of cTn in individuals with renal or cardiac failure over 3–6 hours.[71,82] One study in individuals on haemodialysis has shown that RCVs for hs-cTn are usually less than 20% over 6 hours.[71] As the diagnosis of acute myocardial injury typically relies on serial measurements over 3–6 hours, further studies on the short-term biological variability of cTn in these cohorts are required.

Overall, the majority of studies have found substantial inter-individual biological variation for cTn, thus resulting in a low II (less than 0.6). Therefore, for interpreting hs-cTn results, observing for a change in serial tests in an individual is more useful than comparing single values against population-based reference intervals. This further highlights the need to understand biological variability and for determining RCVs to aid in the diagnosis of acute myocardial infarction.

Clinical Considerations

Studies that have sought to determine the biological variability of cTn have yielded varying results for RCVs. It is widely recognised that cTnI and cTnT are released and/or cleared via different mechanisms, which is further supported by the differences seen in biological variability data. As such, there is no universal RCV for the diagnosis of acute myocardial

Table 1. Biological variation studies in healthy individuals. Table shows analytical variation (CV_A) and biological variation (CV_I) data for a number (n) of study participants in a particular time frame. The CV_A and CV_I are used to calculate reference change values (RCV) using the formula by Fraser and Harris as described in the main text and may be expressed as increasing and decreasing values after logarithmic transformation. The inter-individual biological variability (CV_G) is used to calculate the index of individuality (II) as described in the main text. CV_A, CV_I, RCV and CV_G are expressed in percentages.

Author	Assay	Time frame	n	CV_A	CV_I	RCV	Log-normal RCV	CV_G	II
hs-cTnI									
Wu et al.[11]	Singulex	4 hours	12	8.3	9.7	-	+46/-32	57	0.21
		8 weeks	17	15	14	-	+81/-45	63	0.39
Apple et al.[67]	Abbott Architect	Short*	*	13.8	15.2	50.1	+69.3/-40.9	70.5	0.22
		-	-	-	-	-	-	-	-
	Beckman Coulter	Short*	*	14.5	6.1	44.5	+63.8/-38.9	34.8	0.46
		-	-	-	-	-	-	-	-
	Siemens Dimension	Short*	*	13.0	12.9	47	+57.5/-36.5	12.3	0.11
		-	-	-	-	-	-	-	-
Goldberg et al.[68]	Abbott Architect	Short*	*	16.9	37.1	113	-	179.2	0.23
		Long*	*	16.9	117	328	-	179.2	0.66
Vasile et al.[69]	Beckman Coulter	4 hours	20	3.5	3.4	-	+45.2/-15.8	45.3	0.1
		8 weeks	20	2.7	2.6	-	+14/-10.6	41.6	0.1
Wu et al.[70]	Singulex	9 months	17	15	28	-	+98/-49	71	0.45
Aakre et al.[71]	Abbott Architect	6 hours	17	17.3	5.0	-	+64/-39	37.7	0.48
		10 weeks	15	13.8	15.6	-	+77/-44	25.9	0.80
Schindler et al.[72]	Abbott Architect	3 weeks	20	4.8	14.5	37	+53/-34	44.0	0.3
		3 months	20	4.8	14.7	36	+53/-35	56.7	0.3
van der Linden et al.[73]	Abbott Architect	24 hours	18	10.0	8.6	36.7	+44.0/-30.6	49.4	0.27
Koerbin et al.[32]†	Abbott Architect	4 years	453	*	33	-	+147/-59	106	0.36
hs-cTnT									
Vasile et al.[74]	Roche E170	4 hours	20	53.5	48.2	-	+84.6	85.9	0.84
		8 weeks	20	98	94	-	+315	94	1.4
Frankenstein et al.[64]	Roche Elecsys	4 hours	20	9.7	21	62	+90/-47	-	-
		4 weeks	17	9.7	30	86	+135/-58	-	-
	Roche E170	4 hours	20	7.8	15	47	+64/-39	-	-
		4 weeks	17	7.8	31	87	+138/-58	-	-
Aakre et al.[71]	Roche Modular E	6 hours	15	9.9	1.2	-	+22/-34	32.6	0.31
		10 weeks	14	9.7	8.3	-	+42/-30	26.8	0.48
Corte et al.[37]	Roche E411	5 weeks	11	5.1	5.9	21.6	+35.5/-26.2	30.4	0.20
Meijers et al.[75]	Roche Modular	4 months	28	1.5	16.0	44.9	+83.4/-27.0	51.2	0.3

* Specific details not available.

† Conducted in children aged 8, 10 and 12 years.

Table 2. Biological variation studies in individuals with renal disease. Table shows analytical variation (CV_A) and biological variation (CV_I) data for a number (n) of study participants in a particular time frame. The CV_A and CV_I are used to calculate reference change values (RCV) using the formula by Fraser and Harris as described in the main text and may be expressed as increasing and decreasing values after logarithmic transformation. The inter-individual biological variability (CV_G) is used to calculate the index of individuality (II) as described in the main text. CV_A, CV_I, RCV and CV_G are expressed in percentages.

Author	Assay	Time Frame	n	CV_A	CV_I	RCV	Log-normal RCV	CV_G	II
hs-cTnI									
Aakre et al.[71]	Abbott Architect	6 hours	15	6.2	3.3	-	+17/-22	148.1	0.05
		10 weeks	14	5.8	14.3	-	+53/-35	126.7	0.12
Skadberg et al.[85]	Abbott Architect	2-3 days	16	5.3	13.2	-	+48.2/-32.5	142.4	0.10
van der Linden et al.[73]	Abbott Architect	24 hours	18	5.6	7.7	26.4	+30.2/-23.2	62.4	0.15
hs-cTnT									
Aakre et al.[71]	Roche Modular E	6 hours	18	1.4	1.9	-	+1/-12	110.0	0.02
		10 weeks	15	1.7	8.3	-	+26/-21	101.5	0.08
Corte et al.[37]	Roche E411	5 weeks	18	6.0	14.7	44.1	+76.7/-43.4	77.8	0.20
Fahim et al.[81]	Roche E170	5 weeks	42	3.1	7.9	-	+33/-25	83	0.10
		4 months	39	2.4	12.6	-	+58/-37	79	0.16
Mbagaya et al.[36]	Roche E411	10 weeks	16	2.1	10.5	28.1	+34.4/-25.6	64.2	0.17
Skadberg et al.[85]	Roche Modular E	2-3 days	17	1.6	7.3	-	+23.0/-18.7	94.4	0.08

Table 3. Biological variation studies in individuals with cardiac disease. Table shows analytical variation (CV_A) and biological variation (CV_I) data for a number (n) of study participants in a particular time frame. The CV_A and CV_I are used to calculate reference change values (RCV) using the formula by Fraser and Harris as described in the main text and may be expressed as increasing and decreasing values after logarithmic transformation. The inter-individual biological variability (CV_G) is used to calculate the index of individuality (II) as described in the main text. CV_A, CV_I, RCV and CV_G are expressed in percentages.

Author	Assay	Time Frame	n	CV_A	CV_I	RCV	Log-normal RCV	CV_G	II
hs-cTnI									
Nordenskjöld et al.[82]	Abbott Architect	24 hours	24	8	13.5	49	+54/-35	187	0.08
		4-58 days	24	8	23.6	69	+97/-49	163	0.15
Schindler et al.[72]	Abbott Architect	3 weeks	39	4.8	8.5	33	+31/-24	99.3	0.1
		3 months	39	4.8	9.6	50	+34/-26	100.1	0.1
Peeters et al.[84]	Abbott Architect	1 year	16	14.9	9.2	-	+73.1/-42.2	35.0	0.5
hs-cTnT									
Frankenstein et al.[83]	Roche Elecsys	2 weeks	41	1.5	7.2	20.1	+8.8	-	0.07
		3 months	38	1.5	15.7	43.3	+9.7	-	0.19
Nordenskjöld et al.[82]	Roche Elecsys	24 hours	24	4	7.3	23	+26/-21	70	0.12
		58 days	24	4	11	32	+37/-27	65	0.18
Meijers et al.[75]	Roche Modular	6 weeks	83	1.5	11.1	31.4	+42.6/-22.1	96.6	0.1
Peeters et al.[84]	Roche Cobas 6000	1 year	16	3.7	11.2	-	+43.1/-30.1	57.2	0.2

injury. Previous studies have found that using a relative change of 20% after 2 hours for hs-cTnT or 30% after 3–6 hours for hs-cTnI after emergency department presentation for chest pain can significantly improve the diagnostic specificity, but reduce diagnostic sensitivity.[86-88] Another study using hs-cTnT found that large relative changes of greater than 117% over 3 hours or 243% over 6 hours yielded a specificity of 100% but a sensitivity of 69% and 76% respectively in individuals with acute coronary syndrome and an initial negative cTn concentration.[89] However the short-term RCVs for hs-cTnI in healthy individuals are generally in the order of 50–60%. RCVs for hs-cTnT have been found to be greater than 50–60%, with one study advocating RCVs of 85% over a 6-hour period.[74] Therefore, it is possible that false positive diagnoses of acute myocardial injury may occur if a 50% change is defined as significant, particularly for hs-cTnT.

The wide variety of RCVs that have been previously reported by investigators makes defining an appropriate diagnostic cut-off percentage for a significant rise and/or fall in cTn concentration extremely challenging. In order to rule-out acute myocardial injury, the change in cTn concentration would need to be smaller than the RCV. However no RCV will be perfect for every situation. The improved analytical sensitivity of hs-cTn assays means that elevated cTn concentrations can be seen in numerous conditions that cause myocardial cell necrosis.[1] In other words, cTn is organ specific but not disease specific. If one were to select a smaller RCV for the diagnosis of acute myocardial injury, this would result in an increased diagnostic sensitivity but decreased specificity.[38] On the other hand, the use of a greater RCV would result in a decreased diagnostic sensitivity but increased specificity.[38] Therefore, optimal RCVs are likely to be different for rule-in or rule-out strategies.[90] As RCVs are dependent on a multitude of factors such as the assay used, serial sampling time interval and reference population, it may be that further larger studies should aim to determine assay-specific RCVs using strict criteria for laboratory methods, sampling intervals, participant selection and statistical analyses.[38]

Furthermore, defining absolute change rather than relative change criteria may also prove to be valuable in the early rule-in or rule-out of acute myocardial injury. One study found that at 2 hours after presentation with symptoms suggestive of acute myocardial infarction, early absolute changes in cTn concentration showed superior diagnostic accuracy compared to relative changes.[63] The superiority of absolute changes was further demonstrated in another study, which compared absolute changes to a 20% relative change within 3–6 hours of presentation.[62] The finding was that an absolute change of 9.2 ng/L in hs-cTnT yielded a very high negative predictive value, but absolute changes were superior only in individuals with low (less than 14 ng/L) or high (more than 100 ng/L) baseline hs-cTn concentrations.[62] A more recent study using hs-cTnI demonstrated that an absolute change less than 10 ng/L identified nearly all patients who did not have an acute coronary syndrome when the initial cTn concentrations were less than 40 ng/L.[76] The superiority of absolute changes may be because of several potential reasons in which the utility of percentage RCVs may be reduced. For example, large relative changes in cTn concentration of 50% or more can be seen in some individuals with low baseline cTn concentrations (i.e. a change from 4 ng/L to 6 ng/L) despite the absence of acute myocardial injury. In addition, large changes in cTn concentration may not be observed in individuals who present several hours after the onset of symptoms as cTn concentration may have plateaued.[62,63] However it remains debatable whether absolute or relative change should be used for the diagnosis of acute myocardial injury and current recommendations are that both should be calculated in future studies.[90,91]

It is therefore also important to consider the time since onset of symptoms when interpreting a change in cTn concentration.[1,38] As seen in Figure 1, there may be very little change in cTn concentration near peak cTn concentration or late after an acute myocardial injury.[1] In addition, the increase in cTn concentration may be more rapid than the decline in cTn concentration.[1] Clinical judgement remains of paramount importance and RCVs must therefore also be interpreted according to the clinical context, where information is incorporated from the history, physical examination, electrocardiogram and other investigation results. Furthermore, there may be a potential role for the clinical laboratory to provide interpretative comments or reports to improve clinician interpretation of serial results.

Protocols to assist clinicians in interpreting serial changes in cTn for the rapid rule-in and rule-out of acute myocardial infarction have been proposed. The European Society of Cardiology (ESC) currently recommends the use of a 0 and 3 hour serial testing algorithm, which requires the use of assay-specific 99th percentile values.[92] An alternative is the 0 and 1 hour algorithm, which uses assay-specific cut-offs for absolute changes in cTn.[92] Algorithms for 0 and 2 hours have also been proposed.[93] Ruling-out acute myocardial infarction is accomplished using these algorithms based on initial low concentrations of cTn or small changes in early serial measurements. However these algorithms use small absolute change values, which have raised concerns that analytical variation may exceed the suggested delta change at low cTn concentrations.[91] Numerous studies have developed and validated algorithms using either a relative change approach or an absolute change approach, with different

time intervals between tests.[93-96] Algorithms with combined absolute and relative change criteria have also been proposed. For example, a 7 ng/L change in cTn values below 70 ng/L or a 10% change in values above 70 ng/L have been reported to improve clinical sensitivity and specificity using a hs-cTnT assay.[97]

We have further illustrated this combined absolute and relative change concept in **Figure 2A**, using the Abbott Architect hs-cTnI assay as an example. This assay has previously been reported to have an analytical variation (blue line) of 10% at 6 ng/L.[98] Supposing that biological variation is 10%, the total variation (CV_T) can then be calculated using the formula CVT = $(CV_A^2 + CV_I^2)^{1/2}$, where any variation below the CVT (purple line) is likely to be non-significant. As cTn concentrations increase, CV_T approaches the value of biological variation (10% in this case), as analytical variation becomes smaller. For this example, we conservatively defined total variation as a 4 ng/L change in cTn values below 40 ng/L or a 10% change in values above 40 ng/L. RCVs can then be calculated with 95% or 99% probabilities using the formula by Fraser and Harris, as discussed previously (see **Figure 2** legend).[58] **Figure 2B** illustrates these RCVs (orange line for 95% and light blue line for 99%), which were modified to take into account the proposed absolute variation criteria of 4 ng/L for values below 40 ng/L. As such, for a cTn value of 5 ng/L, a 220% proportional increase (i.e. increase from 5 ng/L to 16 ng/L) would be required to be 95% confident that a significant change has occurred. For values above 40 ng/L, a 28% increase would be required to be 95% confident that a significant change has occurred. However combined absolute and relative change criteria for cTn currently remains a concept that is not widely used.

Further prospective studies are required to incorporate diagnostic protocols into routine clinical practice to facilitate optimal use of hs-cTn assays.[99] Such studies may also help to confirm diagnostic thresholds for significant change according to other factors such as gender, age group, time of symptom onset and comorbidities such as chronic cardiac or renal failure.[100] For example, a recent study in individuals with chronic kidney disease proposed algorithms where the relative change cut-off for hs-cTnI was 280% and for hs-cTnT was 250%, which are vastly different from the 20% cut-off defined by international recommendations.[101]

Serial measurements of cTn over the long term may also be useful in providing prognostic information.[102] Measurable concentrations of cTn using hs-cTn assays in the general population have been associated with structural cardiac disease, cardiac failure, and an increased risk of both cardiovascular and all-cause mortality, even at concentrations well below

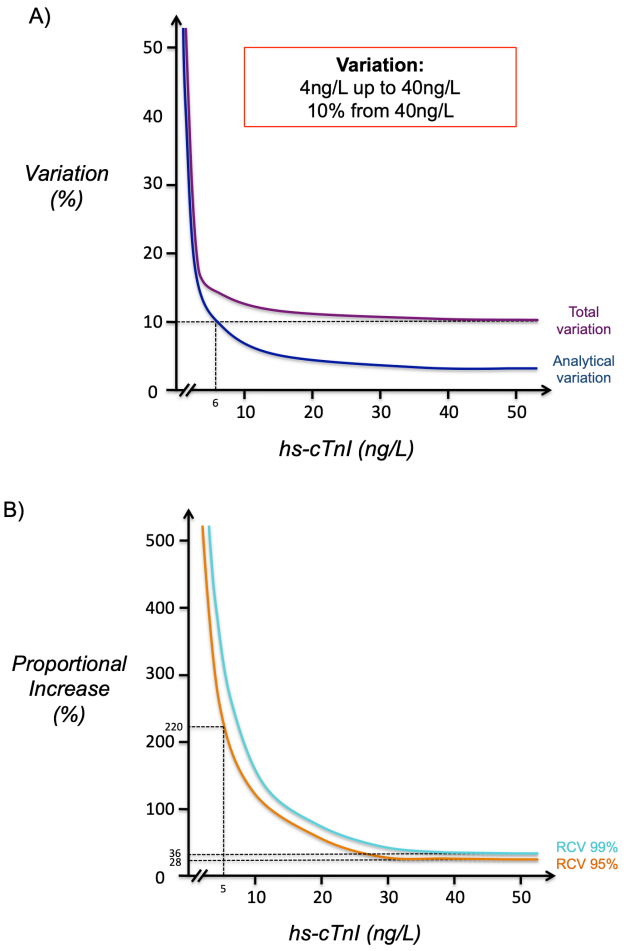

Figure 2. Example graphs illustrating the concept of combined absolute and relative change criteria using a high-sensitivity cardiac troponin I (hs-cTnI) assay. Graph A shows analytical variation (CV_A) and total variation (CV_T), assuming a biological variation (CV_I) of 10%. Graph B shows corresponding proportional increases with 95% or 99% significance using the fixed increase of 4 ng/L, if the troponin value was less than 40 ng/L or reference change values (RCV) for troponin values greater than 40 ng/L. Significant change was calculated using either the standard RCV equation [RCV = $2^{1/2}$ x Z x CV_T where Z = 1.96 for 95% or 2.58 for 99% probabilities and $CV_T = (CV_A^2 + CV_I^2)^{1/2}$]. The RCV equation was modified for the fixed increase of 4 ng/L, thus RCV = $2^{1/2}$ x Z x 4, to give an absolute increase which was in turn expressed as a percentage for the figure (i.e. for a troponin of 5 ng/L, the 95% probability increase = 1.41 x 1.96 x 4 = 11 ng, thus the increase as a proportion was 11/5 = 220% increase).

the 99th percentile value.[103-107] For individuals who have coronary artery disease, hs-cTn may be useful in predicting the risk of future myocardial infarction and cardiovascular mortality.[108,109] Numerous studies have also demonstrated that temporal changes in cTn over several years are independently associated with risk of coronary artery disease, cardiac failure

and cardiovascular mortality, concordant with the trajectory of change in cTn.[102,110-112] Therefore, serial measurements of cTn using hs-cTn assays over the long term may play an adjunctive role in prognostication, with higher-risk individuals identified by rising concentrations of cTn over time. However further long-term cTn biological variation studies to improve understanding are needed in this area to better define the potential role of monitoring long-term measurements for clinical decision-making.

Conclusion

Hs-cTn assays have revolutionised the diagnosis of acute myocardial injury. This review has revisited the short- and long-term biological variability of cTn and has highlighted some of the challenges in defining what constitutes a significant rise and/or fall in cTn concentration. Studies that have sought to answer this question have yielded varying results, largely due to vast differences in study methodology. Current guidelines for the diagnosis of acute myocardial infarction have suggested significant change values on serial testing as a guide for clinicians, but these remain poorly defined. Further rigorous studies on the biological variability are needed to improve clinical interpretation of serial cTn results. Moreover, studies incorporating RCV and troponin concentration thresholds guiding therapy with hard clinical outcomes would be the optimal proof of this concept. These studies will help to assist in the development of clinical algorithms and help refine and strengthen current recommendations for the diagnosis of acute myocardial infarction.

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