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Short- and long-term biological variation of cardiac troponin I in healthy individuals, and patients with end-stage renal failure requiring haemodialysis or cardiomyopathy

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Abstract

Objectives: High-sensitivity (hs) cardiac troponin (cTn) assays can quantitate small fluctuations in cTn concentration. Determining biological variation allows calculation of reference change values (RCV), to define significant changes. We assessed the short- and long-term biological variation of cardiac troponin I (cTnI) in healthy individuals and patients with renal failure requiring haemodialysis or cardiomyopathy.

Methods: Plasma samples were collected hourly for 4 h and weekly for seven further weeks from 20 healthy individuals, 9 renal failure patients and 20 cardiomyopathy patients. Pre- and post-haemodialysis samples were collected weekly for 7 weeks. Samples were analysed using a hs-cTnI assay (Abbott Alinity ci-series). Within-subject biological variation (CV_I), analytical variation (CV_A) and between-subject biological variation (CV_G) was used to calculate RCVs and index of individuality (II).

Results: For healthy individuals, CV_I, CV_A, CV_G, RCV and II values were 8.8, 14.0, 43.1, 45.8% and 0.38 respectively for short-term, and 41.4, 14.0, 25.8, 121.0% and 1.69 for long-term. For renal failure patients, these were 2.6, 5.8, 50.5, 17.6% and 0.30 respectively for short-term, and 19.1, 5.8, 11.2, 55.2% and 1.78 for long-term. For cardiomyopathy patients, these were 4.2, 10.0, 65.9, 30.0% and 0.16 respectively for short-term, and 17.5, 10.0, 63.1, 55.8% and 0.32 for long-term. Mean cTnI concentration was lower post-haemodialysis (15.2 vs. 17.8 ng/L, $p < 0.0001$), with a 16.9% mean relative change.

Conclusions: The biological variation of cTnI is similar between end-stage renal failure and cardiomyopathy patients, but proportionately greater in well-selected healthy individuals with very low baseline cTnI concentrations.

Keywords: biochemistry; biological variation; myocardial infarct; troponin.

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Introduction

Cardiac troponin I (cTnI) and T (cTnT) are highly specific biomarkers of myocardial injury [1]. According to the *Fourth Universal Definition of Myocardial Infarction (2018)*, 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 [1]. The importance of monitoring serial changes in cardiac enzymes has long been recognised, and the current consensus statement provides significant change criteria as a guide for clinicians [1, 2]. If the initial cTn concentration is at, or below, the 99th percentile, then a 50–60% relative change may be suggestive of an acute myocardial

injury [1]. On the other hand, if the initial cTn concentration is above the 99th percentile, then a 20% change (based on three times the analytical variation [CV_A] of previous generation assays), may be suggestive instead [1, 3]. However, the magnitude of change constituting a significant rise and/or fall in cTn concentration continues to be a matter of debate due to limited evidence and conflicting data [4, 5].

In order to better define what constitutes a significant change, the normal variations in cTn results need to be quantified [5]. Such variations may occur due to pre-analytical, analytical and biological variation [6–8]. High-sensitivity cardiac troponin (hs-cTn) assays can be used to assess the within-subject biological variation (CV_I) of cTn, when serial testing at regular time intervals is performed under strictly controlled conditions [9]. These assays 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 [9]. In addition, the low CV_A of these assays means that random variation of cTn results due to analytical influences is low [6]. The assessment of CV_A and CV_I has enabled the calculation of reference change values (RCVs), which are assay-specific, and can be used to define a significant change [10]. Individuals with a change in cTn concentration that are within the limits of normal variation are unlikely to have experienced an acute myocardial injury.

CV_I, defined as the random fluctuation of an analyte around an individual's inherent "homeostatic set point", is usually studied in healthy individuals [11]. It has been proposed that cTn release may not always be pathological, as it can 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 [12, 13]. However, determining significant change criteria for individuals with chronic, but stable, renal or cardiac disease is also important, as healthy individuals may not be representative of those in whom cTn is measured in clinical practice and because underlying pathology may alter CV_I. In addition, chronic elevations in cTn concentration above the 99th percentile may be commonly seen in those with renal or cardiac disease [14, 15]. An elevated, but stable, cTn concentration on serial testing is more likely due to a chronic, rather than an acute, cause of myocardial injury. However, current knowledge of hourly CV_I remains insufficient in these disease states, as few studies have been performed [5, 16, 17]. The advent of hs-cTn assays has also led to the development of protocols for early measurements of cTn, even within 1 h, to facilitate rapid rule-in or rule-out of myocardial infarction for individuals presenting with chest pain [18]. This highlights the importance of examining normal hourly changes. The assessment of weekly or monthly CV_I may have implications with regards to cardiovascular risk stratification and chronic disease monitoring [19, 20].

Additionally, haemodialysis may have differential effects on the concentration of cTnI and cTnT [21–24]. The concentration of cTn may increase due to haemoconcentration following haemodialysis, or decrease due to clearance of cTn from the circulation or adherence of cTn to haemodialysis membranes [21–24]. This highlights the need to interpret serial cTn results with respect to the time of haemodialysis. However, the effect of haemodialysis on cTn concentration currently remains unclear.

The primary aim of this study was to determine the short-term (hourly) and long-term (weekly) CV_I of cTnI using a high-sensitivity cardiac troponin I (hs-cTnI) assay in healthy individuals, patients with stable end-stage renal failure requiring haemodialysis and patients with stable cardiomyopathy. A secondary aim was to explore whether cTnI concentrations changed from pre- to post-haemodialysis.

Materials and methods

Participant recruitment

Participants were prospectively recruited at Fiona Stanley Hospital, a tertiary hospital in Western Australia, during a two-year period. Ethical approval was obtained from the Human Research Ethics Committee (number: EMHS-2016-110). All participants provided written informed consent and the study was conducted according to the principles of the Declaration of Helsinki. Baseline clinical characteristics were obtained from the participant and review of the medical records.

Healthy individuals were recruiting using advertisements attached to noticeboards in hospital staff rooms and were eligible if there were no self-reported symptoms or history of cardiovascular disease. All healthy individuals underwent an electrocardiogram, transthoracic echocardiogram and baseline blood tests for brain natriuretic peptide, creatine kinase, creatinine and high-sensitivity C-reactive protein to confirm normal cardiac structure and function. The echocardiogram was performed by a certified cardiac sonographer and reported by a cardiologist. Patients with stable end-stage renal failure were recruited in the outpatient dialysis unit. Renal failure patients were eligible if they had been managed with haemodialysis for at least three consecutive months and were on individually optimised doses of medication. The method of dialysis used for patients enrolled in this study, who were well-established on dialysis, was haemodiafiltration. Patients with stable cardiomyopathy were recruited from the cardiology advanced heart failure outpatient clinic. Cardiomyopathy patients were eligible if they had a left ventricular ejection fraction of less than 45% for at least one year, were on individually optimised doses of medications and had New York Heart Association class I to III symptoms.

Exclusion criteria for the study included the inability to provide consent, age less than 20 years, pregnancy, alcohol consumption of more than 1 unit per day, non-adherence to medication, fluid restriction or haemodialysis, a history of acute myocardial infarction, pulmonary embolism, tachyarrhythmia/bradyarrhythmia, exacerbation of congestive heart failure, sepsis, pericarditis, myocarditis, endocarditis, aortic dissection or cardiac intervention within the last 6 months or during the study period, planned cardiac intervention such as percutaneous coronary intervention or coronary artery bypass grafting in the 6 months from time of recruitment, or awaiting renal or cardiac transplantation.

Plasma samples

Plasma samples for cTn analysis were collected from each participant by the hospital outpatient phlebotomy service into 3 mL BD Vacutainer® PST™ Lithium Heparin tubes using standard phlebotomy techniques, where local phlebotomists follow competency guidelines that are similar to that of the National Accrediting Agency for Clinical Laboratory Sciences [25]. Participants were asked not to perform strenuous exercise prior to sample collection, on the day of sample collection. To assess short-term CV_I, hourly plasma samples were collected from all participants for four consecutive hours, where no strenuous activity was allowed during this interval. For patients with end-stage renal failure, this was performed on a non-haemodialysis day. To assess long-term CV_I of cTn in the healthy individuals and in the patients with cardiomyopathy, weekly plasma samples were collected for a further seven consecutive weeks, on approximately the same day and time of the week. For patients with renal failure, weekly pre-haemodialysis samples were collected in the dialysis unit for a further seven weeks, on the same day of the week. Corresponding post-haemodialysis samples were also collected in the dialysis unit immediately after, to assess for a change in cTn concentration.

Blood samples were centrifuged to separate the plasma, which was then aliquoted into de-identified storage tubes, snap-frozen and stored at −80 °C in the hospital biochemistry laboratory by one of the study investigators on the day of collection. After closure of the participant recruitment period, plasma samples were mixed whilst thawed at room temperature, vortexed and re-spun before analysis with a hs-cTnI assay on the Abbott Alinity ci-series. The manufacturer stated 99th percentile for this assay is 34 ng/L for males and 16 ng/L for females (26 ng/L overall), and the limit of blank and limit of detection are 1.0 and 1.6 ng/L respectively. In order to minimize between-run variation, the analysis was performed in a single day by the same scientist, with one calibration and using two reagent packs from the same lot number. Quality control was performed before, during and after analysis (low: actual mean 18.5 ng/L vs. expected 20.0 ng/L, medium: actual mean 185.6 ng/L vs. expected 200.0 ng/L, and high: actual mean 14960.1 ng/L vs. expected 15000.0 ng/L).

To further characterise the cohorts, the initial baseline plasma sample collection for all participants included a separate 3 mL PST lithium heparin tube, and an additional 4 ml EDTA tube, which were analysed routinely for brain natriuretic peptide (Abbott ARCHITECT chemiluminescent immunoassay), creatine kinase (Abbott ARCHITECT enzymatic assay), creatinine (Abbott ARCHITECT enzymatic assay) and high-sensitivity C-reactive protein (Abbott ARCHITECT immunoturbidimetric assay) on the day of collection.

Statistical analysis

All statistical analyses were carried out using Microsoft Excel and SAS software. Baseline characteristics are described using median or mean with range where specified, or count with percentage. The Cochran test and Reed's criterion was used to identify outliers in cTnI results [11]. Within-subject and between-subject outliers were excluded from further analysis. Participants with non-quantifiable (zero) cTnI results for more than half of their samples were also excluded from further analysis. CV_A was determined from internal quality controls (Thermo Fisher Scientific, Bio-Rad Laboratories, and Innov Research) or pooled samples at cTnI concentrations corresponding to the mean values of our study participants. CV_I and between-subject biological variation (CV_G) was calculated using the methods described by Fraser and Harris [11]. Standard RCVs were calculated using the formula by Fraser and Harris: $RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$, where $Z = 1.96$ for a 95% confidence with a two-tailed p-value <0.05 [11]. We also evaluated RCVs after performing a log-normal transformation as described by Fokkema et al. [26]. The index of individuality (II) was calculated using the formula: $II = (CV_A^2 + CV_I^2)^{1/2}/CV_G$ [11]. Subsequently, due to lower than anticipated cTnI concentrations in the healthy individuals, the analysis was also performed after exclusion of individuals where mean cTnI concentration was less than or equal to the limit of blank, 1.0 ng/L. Paired *t*-test was used to compare the mean cTnI concentration pre- vs. post-haemodialysis. The relative change in cTnI concentration from pre- to post-haemodialysis was calculated for each session and an average change was calculated for each participant, and for all participants.

Results

There were 20 healthy individuals, nine patients with stable end-stage renal failure requiring haemodialysis and 20 patients with stable cardiomyopathy who were eligible for participation and provided consent. All screening echocardiograms for the healthy individuals demonstrated normal cardiac structure and function. In the healthy cohort, one individual had a raised creatine kinase of 420 U/L and a right bundle branch block morphology on electrocardiogram. In the renal failure cohort, the predominant causes of renal failure were diabetic nephropathy (22.2%), hypertensive nephrosclerosis (22.2%) and glomerulonephritis (22.2%). In the cardiomyopathy cohort, the median left ventricular ejection fraction was 30.5% (range 23–40%) and the predominant causes of heart failure were ischaemic heart disease (30%), valvular heart disease (15%), idiopathic cardiomyopathy (15%), left ventricular non-compaction cardiomyopathy (10%) and myocarditis (10%). Baseline characteristics according to disease status are summarised in Table 1.

For short-term CV_I, there were 99 plasma samples obtained from healthy individuals, 45 from patients with renal failure and 100 from patients with cardiomyopathy. This represents an overall short-term follow-up of 99.6%,

Table 1: Baseline characteristics.

Characteristic	Healthy (n = 20)	Renal failure (n = 9)	Cardiomyopathy (n = 20)
Age, years	40 (22–70)	72 (54–90)	57.5 (47–69)
Male gender	10 (50.0)	3 (33.3)	17 (85.0)
Body mass index, kg/m^2	26.0 (16.4–31.6)	29.1 (13.7–46.3)	29.4 (18.3–39.0)
Current smoker	0	1 (11.1)	2 (10.0)
Diabetes mellitus	0	5 (55.6)	6 (30.0)
Hypertension	0	8 (88.9)	10 (50.0)
Known ischaemic heart disease	0	3 (33.3)	7 (35.0)
ACE-I or ARB use	0	3 (33.3)	17 (8.05)
Beta-blocker use	0	6 (66.7)	17 (85.0)
MRA use	0	0	12 (60.0)
Loop or thiazide diuretic use	0	4 (44.4)	12 (60.0)
Creatinine, µmol/L	68 (52–106)	476 (403–735)	100.5 (64–184)
CK, U/L	97.5 (41–420)	71 (17–164)	93 (27–506)
hs-CRP, mg/L	1.0 (0.1–5.2)	7.1 (0.5–217.9)	1.9 (0.3–15.2)
BNP, ng/L	12 (<10–39)	288.5 (85–1070)	192 (18–1330)
cTnI, ng/L	0.4 (0.0–2.7)	12.6 (6.2–28.9)	5.0 (1.1–102.6)

Data are expressed in median (range) or count (percentage), ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist; CK, creatine kinase; hs-CRP, high-sensitivity C-reactive protein; BNP, brain natriuretic peptide; cTnI, cardiac troponin I.

where only one blood draw was missed. In the renal failure cohort, one patient (participant 8) was hospitalised for sepsis prior to providing samples for long-term CV_I and was excluded from further participation. In the cardiomyopathy cohort, one patient (participant 4) was hospitalised for an acute exacerbation of congestive cardiac failure after providing one sample for long-term CV_I and was excluded from further participation. For long-term CV_I, there were 133 plasma samples obtained from healthy individuals, 51 from patients with renal failure and 126 from patients with cardiomyopathy. This represents an overall long-term follow-up of 90.4%.

The distribution of all cTnI results for each participant is shown in Figure 1. The percentage with a baseline cTnI concentration above the manufacturer stated gender-specific 99th percentile was 0% for healthy individuals, 22.2% for patients with renal failure and 10.0% for patients with cardiomyopathy. The median cTnI concentration, CV_I and CV_A data, calculated RCV, log-normal RCV and II results are presented in Table 2. The CV_A was determined to be 14.0% at a mean cTnI concentration of 3.2 ng/L (297 replicates) and 5.8% at a concentration of 12.8 ng/L (43 replicates), using internal quality controls and pooled samples.

Pre- and post-haemodialysis plasma samples were obtained from eight patients with renal failure over 50 haemodialysis sessions. The mean cTnI concentration was lower post-haemodialysis compared to pre-haemodialysis (15.2 vs. 17.8 ng/L, $p < 0.0001$). The relative change results for each patient are presented in Table 3. Overall, the mean

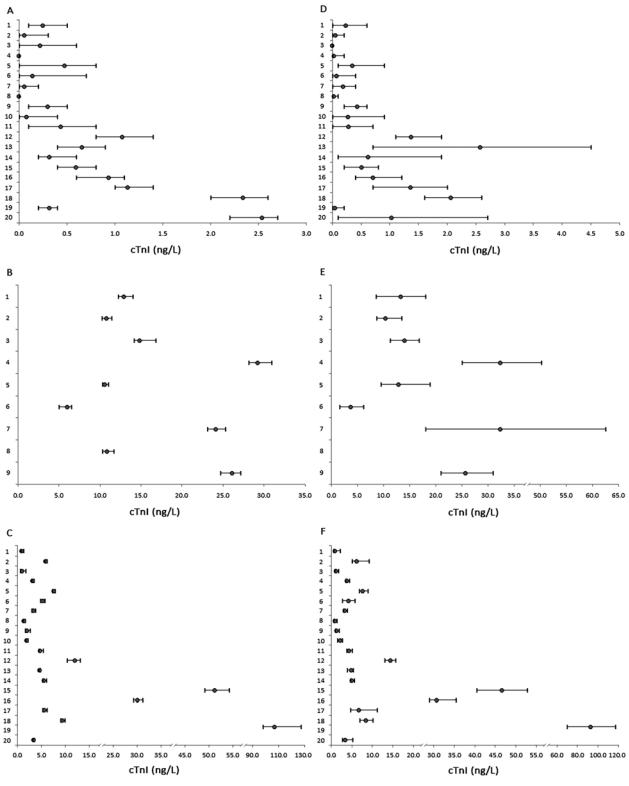

Figure 1: cTnI concentration results.
Data are presented as mean and range. Short-term variation (hourly) is shown for (A) healthy individuals, (B) patient with end-stage renal failure and (C) patients with cardiomyopathy. Long-term variation (weekly) is shown for (D) healthy individuals, (E) patient with end-stage renal failure and (F) patients with cardiomyopathy.

Table 2: Short- and long-term biological variation for cardiac troponin I.

Variable	Healthy (n = 20)				Renal failure (n = 9)		Cardiomyopathy (n = 20)	
	Short[a]	Long[a]	Short[b]	Long[b]	Short[a]	Long[a]	Short[a]	Long[a]
Number of individuals	14	11	4	5	9	4	17	15
cTnI, ng/L[c]	0.6 (0.1–2.9)	0.4 (0.1–2.6)	2.2 (0.8–2.9)	1.6 (0.1–4.5)	12.6 (5.0–30.9)	12.4 (8.6–18.9)	4.6 (0.6–13.1)	3.6 (0.5–10.1)
CV_I, %	23.6	47.9	8.8	41.4	2.6	19.1	4.2	17.5
CV_A, %	14.0	14.0	14.0	14.0	5.8	5.8	10.0	10.0
CV_G, %	92.7	96.1	43.1	25.8	50.5	11.2	65.9	63.1
RCV, %	76.1	138.5	45.8	121.0	17.6	55.2	30.0	55.8
RCV+, %[d]	+111.2	+269.9	+57.6	+218.4	+19.2	+72.8	+34.9	+73.7
RCV–, %[d]	−52.6	−73.0	−36.6	−68.6	−16.1	−42.1	−25.9	−42.4
II	0.30	0.52	0.38	1.69	0.13	1.78	0.16	0.32

cTn, cardiac troponin; CV_I, within-subject biological variation; CV_A, analytical variation; CV_G, between-subject biological variation; RCV, reference change value; II, index of individuality. [a]Analyses performed after exclusion of outliers using the Cochran test and Reed's criterion, those with cardiac troponin of zero in over half of samples (6 healthy participants) and those lost to long-term follow-up (1 renal failure patient). [b]After further exclusion of individuals where mean cardiac troponin ≤1.0 ng/L. [c]Concentrations expressed in median (range). [d]Refers to log-normal increase or decrease.

and median bidirectional relative change in cTnI concentration from pre- to post-dialysis was 16.9 and 17.3% respectively.

Discussion

We report the CV_I of cTn in healthy individuals and in patients with end-stage renal failure requiring haemodialysis or cardiomyopathy. The CV_I of cTnI is similar between patients with renal failure requiring haemodialysis and patients with cardiomyopathy, with a corresponding RCV of ~20–30% in the short-term and 50–60% in the long-term. However, the CV_I of cTnI was found to be greater in healthy individuals, with a corresponding short-term RCV of 46% and long-term RCV of 121%. The greater proportional RCV is likely secondary to the very low cTnI concentrations seen in this very healthy cohort of individuals and the greater assay imprecision at lower cTnI concentrations. Studies on CV_I in different cohorts advise the clinical interpretation of serial cTn results, guiding the magnitude of change required to define pathologic myocardial injury.

Table 3: Change in cardiac troponin I concentration from pre- to post-haemodialysis.

Participant	Number of sessions	Mean relative change, %	Median relative change, %, with range
1	5	20.0	19.3 (14.0–26.7)
2	7	11.7	9.6 (0.0–21.3)
3	7	13.0	13.3 (2.7–21.1)
4	5	23.2	23.2 (16.7–28.1)
5	5	19.6	20.1 (15.6–22.6)
6	7	21.6	13.3 (0.0–63.6)
7	7	18.0	19.5 (0.6–25.2)
8	0	N/A	N/A
9	7	8.4	6.8 (0.9–19.8)
All patients	50	16.9	17.3 (0.0–63.6)

Previous studies have yielded inconsistent CV_I results for cTnI. Using the Abbott Architect hs-cTnI assay, Apple et al. and Goldberg et al. found short-term CV_I to be 15.2 and 37.1% respectively [27, 28]. However, Aakre et al. and van der Linden et al., who also used the Abbott Architect assay in healthy individuals, found considerably lower short-term CV_I at 5.0% (mean cTnI concentration 3.9 ng/L) and 8.6% (median cTnI concentration 4.7 ng/L) respectively [16, 29]. Similar values for cTnI were found with the Singulex assay by Wu et al. and the Beckman Coulter assay by Vasile et al. [19, 30]. As a result of these differences, calculated RCVs have ranged from 30 to 70%, thus making it difficult to define an appropriate diagnostic cut-off percentage for a significant rise and/or fall in cTn concentration [16, 19, 27–30]. The disparity in results between studies may be due to factors such as differences in methodology, participant selection, definition of healthy status, time interval between serial sampling and the assay used [5]. The distribution of cTn can also affect results depending on which statistical method is used to estimate CV_I and RCVs [31]. For example, log-normal transformations may not necessarily be valid in cases where measurements are not log-distributed [31].

Few studies have assessed the hourly CV_I of cTnI or cTnT in patients with end-stage renal failure and patients with cardiac disease [16, 17, 32]. Some studies have found that the CV_I of cTn may be similar between healthy individuals and patients with renal failure requiring haemodialysis [16, 29, 33]. In addition, similar CV_I has also been demonstrated in studies in patients with cardiac disease [17, 34, 35]. The 2.6% short-term and 19.1% long-term cTnI CV_I for renal failure patients in our study is comparable to that of Aakre et al., who found values of 3.3% (mean cTnI concentration 41.5 ng/L) and 14.3% respectively (mean cTnI concentration 27.7 ng/L) [16]. Nordenskjöld et al. evaluated the CV_I of cTnI over 24 h using the Abbott Architect assay in a cohort of patients with coronary artery disease and found a CV_I of 13.5%, which is higher than our 4.2% short-term value [17]. Other studies in patients with cardiac disease have assessed CV_I over longer time periods, rather than over hours [36, 37]. However, as the diagnosis of acute myocardial injury typically relies on serial testing over hours, hourly short-term CV_I findings are particularly relevant.

According to current guidelines, a 50–60% relative change in cTn concentration may be suggestive of an acute myocardial injury when the initial cTn concentration is at or below the 99th percentile [1]. However, these are arbitrary thresholds for cTn concentration, which is a continuous variable. Although the majority of patients with renal failure and cardiomyopathy in our study had cTnI concentrations below the 99th percentile, the short-term RCV was found to be in the order of 20–30%, which is considerably lower than the suggested 50–60% change. Using the 50–60% change criteria may therefore result in improved diagnostic specificity but reduced diagnostic sensitivity. On the other hand, the short-term RCV for healthy individuals with very low baseline cTnI concentrations in our study is within the suggested change. However, these findings must be interpreted with caution, as relative changes in cTn concentration of 50% or more may reflect small absolute changes at very low baseline cTn concentrations (i. e. a change from 2 to 3 ng/L) despite the absence of significant myocardial injury. Such a change in cTn concentration may be statistically significant based on RCVs, but not necessarily clinically significant.

Accelerated protocols for the diagnosis of non-ST-segment elevation myocardial infarction have been validated for the Abbott hs-cTnI assay, where cTn can be measured at 0 and 1 h after presentation [18]. The guideline-recommended cut-off levels for such protocols are assay-specific and the protocols need to be used in conjunction with the clinical context [18]. For the Abbott hs-cTnI assay, myocardial infarction may be ruled-out if the initial cTn concentration is less 5 ng/L and the increase within 1 h is less than 2 ng/L [18]. In the context of our study, hs-cTnI concentrations were less than 5 ng/L for all healthy individuals and the calculated RCV of 46% would result in an absolute change less than 2 ng/L. Alternatively, myocardial infarction may be ruled-in if the initial cTn concentration is greater than or equal to 52 ng/L, or if the increase within 1 h is greater than or equal to 6 ng/L [18]. The 20 and 30% short-term RCV for the renal failure and cardiomyopathy patients respectively would not result in an absolute change of more than 6 ng/L in our study. However, at low concentrations of cTn, using absolute change criteria rather than relative changes may have greater utility in the diagnosis of acute myocardial injury according to studies in the emergency department [38–40]. It currently remains debatable whether absolute or relative change should be used. Combining absolute change criteria at lower cTn concentrations with relative change criteria at higher concentrations has also been proposed, but the specificity and sensitivity of such algorithms would need to be assessed in future studies [5, 41].

Consistent with other studies, we found that the long-term CV_I of cTn is greater than that of short-term [16, 17, 19, 28]. In addition, the large CV_G of cTnI resulted in an II of less than 0.6 in the majority of our analyses. Therefore, a change in cTnI tests results in an individual is more sensitive than a population-based threshold for diagnostic purposes [11].

A secondary aim of our study was to examine the influence of haemodialysis on cTnI concentration. Some studies have demonstrated that cTnI decreases after haemodialysis, whereas cTnT increases [21, 23]. However, this was not replicated in other studies [22, 24, 42]. The mean pre-haemodialysis cTnI concentration was lower than the post-haemodialysis concentration in the current study. The mean relative change of 16.9% (median 17.3%) we observed is of a similar magnitude to that of the 17.6% short-term RCV for the renal failure cohort. However, Skadberg et al. demonstrated that cTnI concentrations reduced by a lesser extent of 7.6% (median), whereas Tarapan et al. demonstrated that cTnI concentrations reduced by a greater extent of 36% (median) after haemodialysis [43, 44]. Both studies measured cTnI concentrations using the Abbott assay. Interestingly, a study by Chen et al. demonstrated that cTnT decreases during haemodialysis and then rises after haemodialysis, before returning to baseline levels approximately 11 h after haemodialysis was completed [42].

A strength of our study was the recruitment of distinct and well-characterised cohorts, with a greater than 90% follow-up for both short- and long-term plasma collection.

Healthy participants were comprehensively screened via history, electrocardiogram and echocardiography, whilst the patients with chronic diseases met strict inclusion and exclusion criteria to ensure stable disease at recruitment, and to maximise the likelihood of follow-up. The strict screening process for healthy participants ensures that CV_I estimates are more reliable, as variations in cTn concentration are less likely to be due to disease processes. In addition, we employed measures in the laboratory to minimise pre-analytical and CV_A [8].

However, perhaps because the "healthy" individuals were so healthy and relatively young, the cTnI concentrations were significantly lower than anticipated with the majority below the limit of detection. This resulted in our analysis being performed after the exclusion of a large number of individuals with mean cTnI concentrations less than the limit of blank instead, which is an important limitation. A further limitation of our study is the lower number of patients with end-stage renal failure, a condition which is associated with high morbidity. Due to strict inclusion and exclusion criteria, many haemodialysis patients at our hospital's dialysis unit were ineligible for participation. However, such strict selection criteria are required in order to conduct a rigorous CV_I study, as cTn concentration may vary due to numerous cardiac or non-cardiac pathology [1]. For these reasons, our long-term CV_I data in these cohorts were based on calculations on a smaller number of individuals. As the cTnI concentrations were similar between the individuals analysed, this led to a lower than expected value for CV_G and an II that was greater than 1.4. In addition, samples were not collected by the same phlebotomist, which can result in inter-phlebotomist variability. However, it may be argued that this aspect of pre-analytical variation actually reflects real-world practices. Our study had an over-representation of men in the cardiomyopathy cohort and women in the renal failure cohort. We did not evaluate whether there was diurnal variation in cTnI concentration, which has been demonstrated mostly for cTnT [16, 45, 46]. Lastly, we did not measure albumin to correct for haemoconcentration, or a comparative molecule such as beta-2 microglobulin, to further investigate cTnI changes from pre- to post-haemodialysis.

In conclusion, we report the CV_I of cTnI using a hs-cTnI assay on the recently released Abbott Alinity analyser, thus providing additional information to inform clinical decision making and assist in the development of future recommendations for the diagnosis of acute myocardial injury. A relative change of greater than 20–30% may be clinically important in the short-term for patients with stable end-stage renal failure or cardiomyopathy with initial cTnI concentrations below the 99% percentile. We believe that much larger studies are required to definitely evaluate CV_I in very well-selected healthy individuals with very low cTnI concentrations. Whilst calculating RCVs is important, it is only by comparing statistically significant change criteria to outcome data that pathological significance will be determined. Therefore, further work should examine how CV_I studies can best be used in routine clinical practice to optimise the diagnosis of acute myocardial injury in health and disease states.

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