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This is the author's accepted manuscript version of the article: Bashir Alaour, Torbjørn Omland, Janniche Torsvik, Thomas E. Kaier, Marit S. Sylte, Heidi Strand, Jasmine Quraishi, Sam McGrath, Luke Williams, Steven Meex, Simon Redwood, Michael Marber and Kristin M. Aakre. (2021). Biological variation of cardiac myosin-binding protein C in healthy individuals. *Clinical Chemistry and Laboratory Medicine*. Accepted for publication on 10 June 2021.

### **Full title**

**Biological Variation of Cardiac Myosin-Binding Protein C in Healthy Individuals**

### **Short title**

Biological Variation of Cardiac Myosin-Binding Protein C

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Supplemental material included

**Abstract:****Background:**

Cardiac myosin-binding protein C (cMyC) is a novel biomarker of myocardial injury, with a promising role in the triage and risk stratification of patients presenting with acute cardiac disease. In this study, we assess the weekly biological variation of cMyC, to examine its potential in monitoring chronic myocardial injury, and to suggest analytical quality specification for routine use of the test in clinical practice.

**Methods:**

Thirty healthy volunteers were included. Non-fasting samples were obtained once a week for ten consecutive weeks. Samples were tested in duplicate on the Erenna® platform by EMD Millipore Corporation. Outlying measurements and subjects were identified and excluded systematically, and homogeneity of analytical and within-subject variances was achieved before calculating the biological variability ( $CV_I$  and  $CV_G$ ), reference change values (RCV) and index of individuality (II).

**Results:**

Mean age was 38 (range, 21-64) years, and 16 participants were women (53%).

The biological variation, RCV and II with 95% confidence interval (CI) were :  $CV_A$  (%) 19.5 (17.8 – 21.6),  $CV_I$  (%) 17.8 (14.8 – 21.0),  $CV_G$  (%) 66.9 (50.4 – 109.9), RCV (%) 106.7 (96.6 – 120.1)/ -51.6 (-54.6 – -49.1) and II 0.42 (0.29 – 0.56).

There was a trend for women to have lower  $CV_G$ . The calculated RCVs were comparable between genders.

**Conclusion:**

cMyC exhibits acceptable RCV and low II suggesting that it could be suitable for disease monitoring, risk stratification and prognostication if measured serially. Analytical quality specifications based on biological variation are similar to those for cardiac troponin and should be achievable at clinically relevant concentrations.

**Keywords:**

Cardiac myosin-binding protein C, biological variation, Reference Change Value, index of individuality.

**List of abbreviations:**

cMyC: Cardiac myosin-binding protein C

APACE: Advantageous Predictors of Acute Coronary Syndromes Evaluation

AMI: acute myocardial infarction

NSTEMI: non-ST elevation myocardial infarction

$CV_A$  : analytical variation

$CV_I$  : within-subject variation

$CV_G$  : between-subject variation

RCV: reference change value

II: index of individuality

hs-cTn: high-sensitive cardiac troponin

eGFR: estimated glomerular filtration rate

NT-ProBNP: N-terminal pro B-type natriuretic peptide

**Introduction:**

Cardiac myosin-binding protein C (cMyC) is a novel biomarker of myocardial injury that was first identified in cardiac venous effluent approximately 10 years ago (1). More recently, a quantitative sandwich immunoassay was developed by selecting a pair of high-affinity mouse monoclonal antibodies to the N-terminal domain of cMyC. This assay can detect small quantities of myocardial injury in blood, equivalent to approximately 0.07 mg of the intact human heart, and less than 1% of the volume of myocardial necrosis needed to exceed the 99th percentile upper reference limit (2). In the systemic circulation, cMyC concentrations rise more rapidly than hs-cTn after timed iatrogenic, as well as spontaneous, Type 1 acute myocardial infarction (Type 1 AMI) (3-5).

The kinetic profile of cMyC has been tested clinically and compared to cardiac troponins in a retrospective analysis of 7000 sera from approximately 2000 patients in the Advantageous Predictors of Acute Coronary Syndromes Evaluation (APACE) cohort presenting with suspected non ST-segment elevation myocardial infarction (NSTEMI) of whom 340 had an adjudicated AMI. Analysis, confined to the initial presentation blood sample, showed that cMyC is at least as good as cardiac troponin measured with the leading assays in predicting the diagnosis of AMI, mortality and future cardiovascular events (6). Additionally, cMyC signalled improved triage over hs-cTnT of pre-hospital patients having blood drawn in the ambulance just 70 minutes after symptom onset (7).

In summary, cMyC shows promise as a biomarker of acute myocardial injury.

Understanding the long-term biological variation of an analyte has several applications including suggesting analytical quality specifications for routine assays, determining the number and frequency of sampling required to establish homeostatic set points of an individual, calculating the index of individuality, and determining physiological variations in consecutive results. The latter is useful for prognostication and risk stratification, chronic cardiovascular

disease monitoring and detecting or predicting cardiac damage in the context of chronic non-cardiac conditions or long-term exposure to cardiotoxic agents. The EFLM suggest three different models for setting analytical quality specifications (8) of which biological variation seems the most applicable for novel markers, until a larger number of outcome studies or robust state of the art data become available (9) The purposes of the current study are to examine the biological variation of cMyC in healthy individuals to 1) better understand its potential as a marker of chronic myocardial injury, and 2) provide data as for which analytical quality specification for routine use of the assay may be suggested.

## **Materials, subjects and methods**

### *Ethics*

This study was carried out according to the principles of the Declaration of Helsinki. The protocol was approved by the respective regional ethics committee at each centre: South Central - Berkshire Research Ethics Committee (London), and the Regional Committee for Medical and Health Research Ethics in Bergen (Bergen and Oslo).

Unified informed consent from across centres was obtained from all volunteers.

### *Volunteers*

Thirty healthy volunteers were recruited from London (King's College London and Guys and St Thomas' Hospital), Bergen (Haukeland University Hospital) and Oslo (University of Oslo and Akershus University Hospital), 10 volunteers were recruited from each centre.

The opportunity to participate in the study was advertised locally via posters and circulated "Research Opportunities" emails amongst the staff of each of the participating centres.

### *Screening, inclusion and exclusion criteria:*

Potential participants were screened according to the following criteria:

Inclusion criteria: Healthy individuals of age between 18 and 75.

Exclusion criteria: any evident disease, current pregnancy, use of cardiac medications, previous history of acute or chronic cardiac illness, any chronic non-cardiac illness including cancer in remission during the past 5 years, or any of the following abnormalities on screening blood tests

- eGFR <60 mL/min/1.73m<sup>2</sup>
- NT-ProBNP > local reference limit
- Troponin T (hs-cTnT) > 99th percentile value (> 13 ng/L)

#### *Sample collection, processing and analysis*

To minimise pre-analytical variability, a unified Standard Operating Protocol (SOP) was used across all centres (see appendix). Venous blood sampling was performed weekly, on the same weekday +/-1 day, for 10 consecutive weeks from October to December 2018. Non-fasting blood samples were drawn between 08.00 and 10.00 am. Smoking, alcohol intake and exercise were reviewed and documented during each visit.

Participants rested for 15 minutes before blood was collected into 3.5 mL plastic serum-separation Vacutainer SST II Advance gel tubes (Becton Dickinson) using a 21 Gauge winged blood collection set with flexible tube needle (Becton Dickinson). Samples were allowed to clot for 30 minutes at room temperature and then centrifuged at 2200 x g for 10 minutes at 20°C. Separated acellular serum (0.9 mL) was then aliquoted into matching cryovial tubes (1.5 ml Mikroröhre PCR-PT, SARSTEDT AG & Co. KG) before being frozen at -80 °C within 2 hours after phlebotomy.

Samples were shipped simultaneously from all centres on dry ice for cMyC measurement.

### *Sample analysis*

All serum samples were tested in duplicate on the Erenna® platform by EMD Millipore Corporation, Hayward California. LoD 0.4 ng/L; LoQ (20% CV<sub>A</sub>) of 1.2 ng/L; intra-series precision (CV, 11 +/- 3%) and inter-series precision (CV, 13 +/- 3%) (3). There were three missing samples.

### *Statistics*

Data were analysed twice by two independent researchers: KMA and BA, using the following platforms: Excel 2016 and SPSS version 26.0 (KMA), and R version 3.6.1 (BA).

Baseline characteristics were described using percentage, means or medians (standard deviation and first quartile-third quartile where applicable).

Student's *t* test and Mann-Whitney U test were used for comparing groups as appropriate.

Shapiro-Wilk test was used to verify the normality of distribution.

1. *Analytical outliers* were identified as per Burnett's method (10). An outlier was defined as a result, which lies further than some multiple, *m* (*m* is a constant determined by the sample size) of standard deviations from the mean.
2. *Stability of subjects*: Subjects that expressed a *non-steady-state* were identified with simple linear regression. The trend was calculated as a percentage of change from the first result. Individual slopes (per participant) of linear regressions were derived. Unstable trends (significantly deviating from 0,  $p < 0.01$ ) were identified and respective subjects were excluded. Then homogeneity of the remaining slopes was tested using linear mixed effect models. ANOVA was used to test whether introducing the slope as

a random effect (allowing the slopes to vary) would improve the fit of the model. High ranked slopes were removed until homogeneity was achieved.

3. *Outliers in mean values of subjects* were defined according to Reed's criterion which rejects extreme values if the difference between them and the next highest (or lowest) exceeds one-third of the range of all values (11).
4. *The distribution of the residual data (means of duplicates)* was tested using Shapiro-Wilk test. As data did not conform to a Gaussian distribution, values were transformed into natural logarithms (12).
5. *Homogeneity of analytical and between-subject variances (ln transformed data)*  
Analytical (n= residual duplicates) and between-subject (n=residual subjects) variances were calculated and ranked. Homogeneity of variances was tested using Cochran's and Bartlett's methods, outlying values were excluded until homogeneity was achieved (13).
6. Calculations of  $\sigma_A$ ,  $\sigma_I$  and  $\sigma_G$  were done (ln transformed data) using nested ANOVA.

The  $\sigma$  was thereafter retransformed into  $CV_A$ ,  $CV_I$ , and  $CV_G$  using:

$$CV_{ln} = \sqrt{(\exp \sigma^2 - 1)} \times 100$$

in which  $\sigma$  is the estimated standard deviation for the ln-transformed data and  $CV_{ln}$  is the adjoining re-transformed CV.

The RCV values (with 95% confidence intervals) were calculated according to Fokkema et al (12). This method is applicable for skewed data as it will always return negative RCV data that are interpretable in clinical practice (not exceeding 100%):

$$RCV \text{ pos} = \left[ \exp \left( 1.96 \times 2^{\frac{1}{2}} \times (\sigma_A^2 + \sigma_I^2)^{\frac{1}{2}} \right) - 1 \right] \times 100$$

$$RCV \text{ neg} = \left[ \exp \left( -1.96 \times 2^{\frac{1}{2}} \times (\sigma_A^2 + \sigma_I^2)^{\frac{1}{2}} \right) - 1 \right] \times 100$$

in which  $\sigma_A$  is the analytic standard deviation and  $\sigma_I$  is the within-person standard deviation of the logarithmic data. Due to the  $CV_I$  exceeding 12%, we choose to also calculate the RCVs in the total cohort using the non-parametric method, as described by Røraas et al (14). This method is less precise compared to Fokkema, but fits all measurement distributions.

The index of individuality II was calculated using the retransformed data as follows:

$$II = \sqrt{CV_A^2 + CV_I^2} / CV_G$$

Separate calculations were performed in the total cohort, gender-stratified groups, using the methodology above for excluding the outliers and calculating biological variation, RCV and II.

## Results

None of the samples had undetectable cMyC concentrations (below LoD).

Baseline characteristics of participants contributing to total and gender-stratified cohorts are shown in table 1. 16 participants were women (53%). Mean age was 38 (range, 21-64), there was no significant age difference between women and men (mean age, 41 and 35 respectively;  $p = 0.173$ ). Two participants were daily smokers. NT-proBNP concentrations were higher in women compared to men (61 +/- 36.5 vs 33 +/- 15.7 ng/L, respectively;  $p = 0.013$ ), however, none of the participants had NT-proBNP above the reference interval. Otherwise, both groups had similar baseline characteristics as listed in table 1.

The distribution of cMyC concentrations across participants is shown in figure 1.

*Total cohort:*

216 samples from 22 participants (11 women and 11 men) were included in the calculation of biological variability, after the exclusion of outliers, as described in the method section, figure 2 and table 1 supplemental data. None of the excluded subjects were smokers. The following results were obtained:

$CV_A$  19.5 % (17.8 – 21.6 %),  $CV_I$  17.8 % (14.8 – 21.0 %),  $CV_G$  66.9 % (50.4 – 109.9 %), RCV 106.7 % (96.6 – 120.1 %)/ -51.6 % (-54.6 – -49.1 %) and II 0.42 (0.29 – 0.56), (table 2).

When RCV was calculated using the non-parametric method, corresponding values were 100.1% and -50.5%, respectively.

*Gender- stratified subgroups:*

A total of 118 samples from 12 women and 116 samples from 12 men were included in the calculation of gender-specific biological variability. The number of included individuals and samples were different from the total cohort, as the whole procedure of outlier exclusion was repeated in each gender-stratified data set (table 1, supplemental data).

A significant difference in cMyC values between women and men was observed: median (Q1-Q3) 3.54 (2.47 – 5.25) vs 4.58 (3.25 – 6.58) ng/L; respectively;  $p = 0.007$ . The  $CV_I$  was comparable across both groups, 19.7 % (15.5 – 24.5 %) and 20.3 % (16.6 – 24.6 %) for women vs men, respectively. There was a trend for women to have higher  $CV_A$  20.2 % (17.9 – 23.3 %) vs 16.8 % (14.9 – 19.4 %) and lower  $CV_G$  55.7 % (37.9 – 110.8 %) vs 83.1 % (55.6 – 195.9 %).

Calculated RCVs were comparable in both groups, + 117 %/-54% vs +106 %/-51 % for women vs men, respectively, however, women had higher II at 0.53 (0.30 – 0.78 ) compared to men 0.35 (0.20 – 0.52) (table 2).

## Discussion

The main finding in this study is that the weekly biological variation, RCV of cMyC in healthy individuals, quantified with the Erenna® platform at EMD Millipore Corporation, is moderate and comparable to other cardiac ischemia markers (cardiac troponin). The II is low. No important gender differences were observed. These measures of variation are important to define the minimal magnitude of change in the concentration of cMyC beyond which pathological processes are likely to be present, and to help guide analytical performance criteria for the assay when implemented in the routine laboratory.

Our data demonstrate a within-subject  $CV_I$  and between-subject  $CV_G$  of 17.8 % (14.8 – 21.0 %), and 66.9 % (50.4 – 109.9 %), respectively. Both fall within the range of respective CV calculated in similar cTn long term biological variability studies (Table 3) (15-23). The derived index individuality II was also similar to that for cTn. The low II suggesting high individuality. This favours interpreting serial changes of cMyC concentration in the individual patient rather than using population-based reference intervals, since the later could increase the fraction of falsely interpreted results (24). Overall, the RCVs were 106.7 % (96.6 – 120.1 %) / -51.6 % (-54.6 – -49.1%), which also lie within the range of RCVs observed for cTn in similar long-term biological variability studies (Table 3). The moderate long-term biological variation and RCVs demonstrated in this study suggest that serial measurement of cMyC might have a value in monitoring chronic cardiac disease activity and the vulnerability of the heart to damage secondary to chronic non-cardiac pathology. Of note, the RCV value is dependent on the analytical variation. Laboratories with higher or lower  $CV_A$  will produce different RCVs compared to those we report. This could be adjusted for by including the local  $CV_A$  in the RCV calculations. The RCV is also reference-cohort and condition-dependent (25). Cohorts with different types of pathology are likely to modify the haemostatic set-point and the variation

around it. As a consequence, some advocate measuring biological variation and RCV in more “relevant” cohorts than healthy volunteers, i.e. measuring long-term RCV in patients with chronic but stable heart failure or renal disease, and short-term RCV in patients presenting to emergency department with non-cardiac chest pain (26). Such data are of interest and should be reported, preferable together with data from healthy subjects for comparison.

On gender-stratified analysis, slightly higher RCVs were reported in women than in men, driven by higher analytical variability calculated in women, a rather expected result considering that the significantly lower median cMyC concentrations reported in women should return a higher  $CV_A$ . Lower levels of cTn in women compared to men have also been reported in healthy individuals in similar studies. Furthermore, a lower  $CV_G$  was found in women compared to men, 55.7% vs 83.1%, respectively, resulting in an overall higher II in women, 0.53 vs 0.35. Both IIs remained less than 0.6, suggesting high individuality in both groups. The overlapping confidence intervals shown for these values indicate that no certain gender difference is evident. The majority of cTn biological variation studies did not report gender-stratified biological variation or RCVs. However, studies are encouraged to do so considering that gender-specific 99<sup>th</sup> percentile value of biomarkers are increasingly reported. Until more data from outcome studies investigating the biomarker in different clinical situations become available analytical performance specifications might be based on biological variation. Our data suggest that the  $CV_A$  for the cMyC assay at concentrations used for routine diagnosis should be below 9% (half of  $CV_I$ ) (27), which is very similar to current recommendations for cTn. Our calculated  $CV_A$  was 19.5% (17.8 – 21.6%), which is higher than  $CV_A$  reported in the majority of long term cTn biological variation studies (Table 3). However, our  $CV_A$  % was obtained from duplicates with median cMyC of 4.38 (2.75 – 5.97) ng/L, which is considerably lower than median cMyC found in patient with adjudicated diagnosis of acute coronary syndrome 237 (71 – 876) ng/L in the APACE cohort (6), and only

~ 5% of the 99<sup>th</sup> percentile (derived from patients without coronary artery disease) (28). Lower  $CV_A$  should be expected at higher (more clinically relevant) concentrations, and we predict lower  $CV_A$  with future automated assays of cMyC. Further, a higher ratio of mean cTn to respective assay-specific 99<sup>th</sup> percentile was reported in similar long-term cTns biological variation studies (16).

Finally, our data indicate that the desirable analytical bias (i.e. calculated as  $1/4(\sqrt{CV_I^2 + CV_G^2})$ ) should be 17% or lower. This is similar to what is commonly seen for lot variations for immunoassays. The allowable total error (precision and bias merged) should be below 28%.

This study has several strengths: 1) it is multi-centre, with unified protocol and standard operating procedure to minimise pre-analytical variability; 2) it included a relatively large number of participants, of which, 53 % were women; 3) “healthy status” was clearly defined, 4) exclusion of outliers was performed systematically and is described in the manuscript; 5) gender-stratified variability, RCV and II were measured. The RCVs were calculated using two different models, ln transformed data according to Fokkema and the more robust but less precise non-parametric method suggested by Røraas, the results were similar. The statistical analysis was performed by two independent researchers using two different software platforms. The study also has limitations – the participant mean age was lower than in patients with chronic primary or secondary cardiac disease so the reported data may not be valid for cohorts with other characteristic. Samples were analysed 18 months after collection, however, these were continuously stored at -80 °C and thawed once for the analysis.

## **Conclusion**

cMyC exhibits acceptable biological variation, RCV and low II suggesting that it could be suitable for disease monitoring, risk stratification and prognostication if measured serially.

Analytical quality specifications based on biological variation data are similar to those for cTn and should be achievable at clinically relevant concentrations. However, testing the RCV in cohorts with chronic cardiac disease and reported/measured outcomes is necessary to testify its ability to monitor disease activity and predict outcomes. However, future use of the biomarker will determine if specification should be based on clinical outcomes or biological variation.

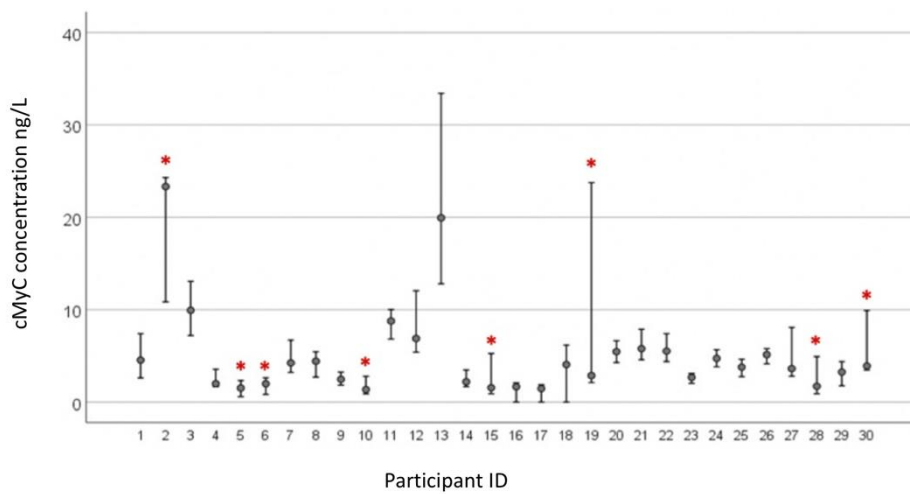
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## Figures and tables



\* Outliers identified and removed from the “total cohort”

Figure 1.



<b>Sample analysis</b>	Participants (n=30) Samples (n=297 *)	
<b>Analytical outliers according to Burnett</b>		Three analytical outliers were identified and excluded ID 2 weeks 4 and 9 ID 13 week 1
	Participants (n=30) Samples (n=297)	
<b>Steady state of subjects and homogeneity of slopes **</b>		One participant (ID 5) had a non-steady state and was excluded One participant (ID 19) was identified and excluded to achieved homogeneity of the slopes **
	Participants (n=28) Samples (n=277)	
<b>Between-subject outliers according to Reed</b>		No participant excluded
	Participants (n=28) Samples (n=277)	
<b>Homogeneity of within-subject and analytical variances according to Bartlett or Cochran's test</b>		Six participants, ID (2, 6, 10, 15, 28 and 30) were excluded due to within-subject non-homogeneity None was excluded due to analytical non-homogeneity
<b>Included in the calculation of RCV, II and biological variability</b>	Participants (n=22) Samples (n=216)	

\* Missing samples (n=3). \*\* slopes of linear regressions of temporal percentage of changes from first readings.

Figure 2.

Table 1. Baseline characteristics. Values displayed as mean (SD) unless stated otherwise

<b>Table 1. Baseline characteristics</b>				
	Total n=30	Women n=16	Men n=14	p value ( women vs men)
Age, mean (range)	38 (21-64)	41 (21-64)	35 (21-44)	0.173
BMI, kg/m <sup>2</sup>	22.8 (2.6)	22.35 (3)	23.38 (2.2)	0.303
Glucose, mmol/L	5.1 (0.6)	4.9 (0.5)	5.2 (0.6)	0.172
eGFR(CKD-EPIcreat), ml/min/1.73m <sup>2</sup>	97.7 (14.7)	95 (13.7)	100.6 (15.9)	0.308
Troponin T, ng/L <sup>a</sup>	3.3 (2)	3.3 (2.2)	3.3 (1.9)	0.917
NT-ProBNP, ng/L	47.9 (31.5)	61 (36.5)	33 (15.7)	0.013
Regular medications (%) <sup>b</sup>	3.3	6.25	0	0.359
<sup>a</sup> values below LoD were reported as 50% of the local lower limit of reportable result, 2 ng/L or 1.5 ng/L, respectively				
<sup>b</sup> non-cardiac drug				

Table 2. Analytical and biological variation, RCV and II of cMyC

	Total	Women	Men
Number of participants	30	16	14
Number of participants <sup>a</sup>	22	12	12
Numbers of samples <sup>a</sup>	216	118	116
cMyC concentration, ng/L, median (Q1-Q3)	4.38 (2.75-5.97)	3.54 (2.47-5.25)	4.58 (3.25-6.58)
CV <sub>A</sub> , mean (95% CI), %	19.5 (17.8 – 21.6)	20.2 (17.9 – 23.3)	16.8 (14.9 – 19.4)
CV <sub>I</sub> , mean (95% CI), %	17.8 (14.8 – 21.0)	19.7 (15.5 – 24.5)	20.3 (16.6 – 24.6)
CV <sub>B</sub> , mean (95% CI), %	66.9 (50.4 – 109.9)	55.7 (37.9 – 110.8)	83.1 (55.6 – 195.9)
Positive RCV , mean (95% CI), %	106.7 (96.6 – 120.1)	117.2 (102.3 – 139.1)	106.2 (92.3 – 126.4)
Negative RCV, mean (95% CI), %	-51.6 (-54.6 – -49.1)	-54.0 (-58.2 – -50.6)	-51.5 (-55.8 – -48.0)
Index of individuality II	0.42 (0.29 – 0.56)	0.53 (0.30 – 0.78)	0.35 (0.20 – 0.52)
<sup>a</sup> after excluding the outliers			

Table 3. Cardiac troponins' long-term biological variation, RCV and II as reported in recent studies.

Author	Year <sup>a</sup>	n <sup>b</sup>	Frequency	Period	Age <sup>c</sup>	Assay	RCV (log-normal)	CVA (%)	CVI (%)	CVB (%)	II (%)
<b>cTnI</b>											
Lan et al.(13)	2020	20	weekly	7 weeks	40 (22-70)	hs-TnI Abbott Alinity ci-series	+269.9/-73	14	47.9	25.8	1.69
Ceriotti et al.(14)	2020	89	weekly	10 weeks	20-60	hs-TnI Singulex Clarity	+59.7/-37.4	11.6	16.6	F 40.3 M 65.3	F 0.44 M 0.23
		91				hs-TnI Siemens Atellica	+50.1/-33.4	10.7	13.9	F 36.3 M 36.5	F 0.40 M 0.40
Schindler et al.(15)	2016	10	<= twice a week	3 weeks	50.9 (51-64)	hs-TnI Abbott Architect	+53/-34	4.8	14.5	44	0.3
Aakre et al.(16)	2014	20	weekly	10 weeks	61 (46-68)	hs-TnI Abbott Architect	+77/-44	13.8	15.6	25.9	0.8
Vasile et al.(17)	2011	20	fortnightly	8 weeks	39 (25-56)	hs-TnI Beckman Coulter	+14/-10.6	2.7	2.6	41	0.1
Wu et al.(18)	2009	17	fortnightly	8 weeks	19-58	hs-TnI Singulex	+81/-45	15	14	63	0.39
<b>cTnT</b>											
Meijers et al.(19)	2017	28	monthly	4 months	43 (13)	hs-TnT Roche Modular	+83.4/-27.0	1.5	16	51.2	0.3
Corte et al.(20)	2015	11	weekly	5 weeks	21-50	S-TnT Roche Cobas e411	+35/-26	5.1	5.9	30.4	0.3
Aakre et al.(16)	2014	20	weekly	10 weeks	61 (46-68)	hs-TnT Roche Modular	+42/-30	9.7	8.3	26.8	0.48
Frankenstein et al.(21)	2011	17	weekly	5 weeks	32 (22-59)	hs-TnT E 170 assay	+138/-58	7.8	31	na	na
						hs-TnT Elecsys 2010 assay	+135/-58	9.7	30	na	na
Vasile et al.(17)	2010	20	fortnightly	8 weeks	39 (25-56)	hs-TnT Roche Modular	+315	94	92	94	1.4

<sup>a</sup> Year published. <sup>b</sup> n = number of subjects. <sup>c</sup> expressed in mean ( range) or range only, F = females, M = males