Temporal evolvement of high-sensitivity cardiac troponin serum concentrations during 1 year after acute coronary syndrome admission

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ABSTRACT

Background: Serum levels of high-sensitivity troponin (hsTn) are elevated in patients admitted for acute coronary syndrome (ACS). Detailed insights in the biological variation of hsTn and its temporal pattern following ACS are currently not available.

Purpose: To describe the temporal evolvement of hsTnI and hsTnT after admission for ACS, and to determine their variation during the clinically stable phase up to one year thereafter.

Methods: BIOMArCS is a prospective, observational study with high frequency blood sampling during one year post-ACS. 1507 blood samples from 191 patients who remained free from major adverse cardiac events (MACE) during follow-up were used to analyze the temporal evolvement during follow-up with linear mixed models. Biological variation was studied using the samples collected in the timeframe of 6-12 months after the index ACS, when patients were considered to have stable coronary artery disease.

Results: On average, both hsTnI and hsTnT were clearly elevated during ACS and remained elevated for 43 (hsTnI) and 15 days (hsTnT). The intra-individual variation in the 6-12 months window was 14.0% and 18.1% for hsTnI and hsTnT respectively, while the inter-individual variation was 94.1% and 75.9%. Using the first two samples taken from one month after the index ACS onwards, we were able to compose a patient-specific reference value for over 80% of the patients.

Conclusions: HsTnI and hsTnT remain elevated for a prolonged period after ACS. Given the low intra-individual and the large inter-individual differences, we propose further investigation of the use of patient-specific reference values over population-derived ones. Such patient-specific reference values can be adequately determined in the majority of patients using just two consecutive samples.

INTRODUCTION

High-sensitivity Troponins (hsTn) are now commonly used in clinical practice. In patients presenting with ischemic chest pain, serial cardiac hsTn measurements with at least one value above the 99th percentile of a healthy control group, in combination with a typical rise and/or fall of the serial measurements, are key elements of the diagnosis of myocardial infarction.[1,2] In addition, hsTn levels have also been demonstrated as prognostic markers in acute coronary syndrome (ACS) patients and in patients with known coronary artery disease (CAD). We demonstrated that asymptomatic post-ACS patients with mildly elevated hsTns have a doubled incidence of repeat ACS within one year.[3] Similar results were described by Ang et al in a study among 326 consecutive ACS patients and by Koenig et al among 1050 patients with previous hospitalization for ACS or coronary artery bypass grafting, associating persistently elevated hsTnT at approximately 7 weeks after index event with increased risk of cardiovascular events during a long-term follow up.[4,5]

Both in the context of diagnosing future re-ACS as well as in the context of personalizing the prognostication for future re-ACS, it is critical to be able to separate dynamic troponin changes due to myocardial necrosis, from their analytical and biological fluctuations. To date, studies on the biological variation of cardiac troponins, measured with contemporary high-sensitivity assays, are scarce and their sample sizes have usually been small.[6-10] Particularly in patients with known CAD or post-ACS patients, little to no information is available. Interpreting troponin values after ACS, is further complicated due to the prolonged period that the troponin value can remain elevated above the 99th percentile of normal.[11-13]

Against this background, we utilized the 'BIOMarker study to identify the Acute risk of a Coronary Syndrome' (BIOMArCS) with highly frequent blood sampling, investigating stabilization patterns of hsTnI and hsTnT after ACS. In particular, we aimed to determine the time until their stabilization, and, once stabilized, their intra- and inter-individual variation.

METHODS

Patients

The study design of BIOMArCS has been published previously.[14] In short, BIOMArCS is a multi-centre, prospective, observational study that was conducted in 18 participating hospitals in the Netherlands during 2008-2015. The study was designed to obtain detailed data on biomarker patterns until one-year follow-up post-ACS. Patients above 40 years presenting with ACS and at least one additional cardiovascular (CV) risk factor

were eligible for enrolment. Exclusion criteria were ischemia precipitated by a condition other than atherosclerotic CAD, a left ventricular ejection fraction <30%, or end-stage congestive heart failure (NYHA class >= 3), severe chronic kidney disease, or a coexistent condition with life expectancy <1 year. All patients were treated according to prevailing guidelines and at the discretion of the treating physician. The study protocol was approved by the Institutional Review Board of the participating hospitals, and all study subjects gave written informed consent.

Blood sampling and storage

Blood samples were collected at admission, at the day of hospital discharge and subsequently every fortnight during the first six months after discharge. If logistic circumstances hindered inclusion during hospitalization, patients could be included on the first outpatient visit within 6 weeks after discharge. In a subset of approximately 8% of patients, additional blood samples were collected within 24, 48, 72 and 96 hours after admission and at the day of hospital discharge with the specific aim to study the early evolution and normalization of the biomarkers. Follow-up was terminated permanently after coronary artery bypass grafting, hospital admission for heart failure, or a deterioration of renal function leading to a glomerular filtration rate <30ml/min/1.73 m^2.

Blood samples were handled and securely stored on-site. After preparation, aliquots were frozen at –80 degrees Celsius within two hours after withdrawal. Samples were transported under controlled conditions to the department of Clinical Chemistry at the Erasmus MC for long-term storage.

Study patients and troponin analysis

For the analysis of the BIOMArCS study, hsTnI and HsTnT serum levels were measured in the samples of 187 patients. Of these 187 patients, 45 had a new ischemic event during the follow-up. For the current analysis, we removed the patients with a new ischemic event from the analysis set and enriched the set with 49 patients who had daily sampling during the first 4 days of the index ACS submission. Hence, our analysis set consisted of 191 endpoint-free patients. They contributed a median of 8 (25^{th}-75^{th} percentile 5-10) repeated samples per patient (altogether 1507 samples) that were used for the analysis of stabilization patterns.

Based on previous studies we presumed that hsTn levels would be biochemically stable at 6 months post-ACS.[15-17] Accordingly, the analysis of biological variation was based on 446 samples that were collected 6-12 months after the index ACS, and was limited to the 98 patients who had ≥3 measurements in that time window and who did not undergo a (staged) percutaneous coronary intervention (PCI) – thus iatrogenic distortion of the troponin levels caused by PCI was excluded.[18]

Troponin values were determined in a blinded fashion and in one batch using a hsTnI assay (Abbott) and a hsTnT assay (Roche). These assays have a lower limit of detection and upper reference limit (99th percentile) of 1.2 ng/L and 10 ng/L for hsTnI, and 5 ng/L and 14 ng/L for hsTnT, respectively.

Measures of biological variation

The coefficient of variation (CV) of a series of measurements is defined as 100% times the standard deviation (sd) of the measurements divided by their mean value ($\overline{X}$):

$$CV = 100\% * sd/\overline{X}$$

According to the methods by Fraser and Harris,[19] the total variation of a series of repeated measurements in individual subjects can be split in 3 components, which represent the variation due to the imprecision of the analytical process (CV_a), the intra-individual or within-subject variation (CV_i) and the inter-individual or between-subject variation (CV_g). CV_a can be determined by repeatedly measuring the same sample using different assays. However, since this procedure is expensive, time-consuming and resource draining, laboratories generally use the CV_a that is based on a reference sample. We used the lab-specific CV_a of 5.0% for hsTnI and 3.0% for hsTnT, respectively. Besides determining the different coefficients of variability, we also calculated the *Index of Individuality (II)* and the Reference Change Value (RCV) for both biomarkers. The II is the ratio of the combined within-subject and analytical variation relative to the between-subject variation. Previously it has been suggested that in case of an II <0.6, individual subjects should have their own reference values instead of a population based reference.[20] When the II >1.4, a population-based reference is preferred. The RCV reflects the limit of (relative) change in biomarker values in individual subjects that can be explained by the combined within-subject and analytical variation. A more detailed description of the parameters of variability and the formulas used to calculate them are included in the *supplementary files*.

Data analysis

Continuous variables are presented as mean (SD) or median (25th-75th percentile), depending on their distributions. Categorical variables are summarized as numbers and percentages. We used linear regression for investigating which factors were associated with the CV_i.

Stabilization

We used linear mixed models to describe the average troponin stabilization patterns over time. In these models, time was entered as the independent variable, and the log-

transformed troponin value as the dependent variable. A total of three cubic splines were placed in order to model the non-linearity of the association between time and troponin level. We used Akaike's information criterion and Bayesian information criteria for the optimal placing of these splines. Random slopes as well as random intercepts were included in the models to allow for individual variation. Using the mixed model, we calculated the average daily values of both hsTnI and hsTnT. These values were then used to determine the average time during which troponins were raised above the reference value after ACS, and the average time until stabilization. We defined stabilization (on group level) as a difference in (model-derived) average troponin level of less than one percent between two consecutive measurements.

The analyses of the post six-month blood samples revealed a low II for both hsTnI and hsTnT. Hence, individual based reference values are preferred in our ACS patients, which ideally, are to be known as early as possible. Since the average time until stabilization appeared less than a month for both troponins, we performed a post-hoc analysis, based on the samples taken after one month, to learn if patient-specific reference values for hsTnI and hsTnT can be determined in this early time window.

We calculated the average of the first two hsTn measurements and compared this with the first consecutive measurement. If the difference was less than 5 ng/L, we considered the average of the first two measurements to be the patient-specific reference. If a patient-specific reference value was observed, we verified this value by comparing it with the last available measurement of the same patient for differences larger than 5 ng/L and by using paired t-test. The 5 ng/L threshold is equal to the median patient-specific hsTnT level times the upper limit of the RCV.

All analyses were performed using R 3.1.1.

RESULTS

Baseline characteristics

Baseline characteristics are presented in table 1. The mean age of the patients in the analysis set was 63.0 (11.1) years and 78% were men. More than half of the population had hypertension (52.1%) and a large proportion had hypercholesterolemia (47.5%) and/or a family history of CAD (53.5%). STEMI was the most common index event (46.2%), followed by NSTEMI (40.7%). No relevant differences in baseline characteristics could be identified when comparing the analysis set (n=191) and the 122 and 98 patients with more than three readily available Tn measurements after 1 and 6 months of follow-up.

Table 1. Baseline characteristics		
	Analysis set (n = 191)	Post 6 months (n=98)
Age. Y (SD)	62.4 (10.6)	62.8 (9.5)
Male gender (%)	148 (77.5)	77 (78.6)
Cardiovascular risk factors (%)		
Diabetes Mellitus	33 (17.3)	17 (17.3)
Hypertension	101 (52.9)	52 (53.1)
Hypercholesterolemia	92 (46.5)	54 (58.2)
Family history of CAD	87 (53.0)	47 (59.5)
Current smoker	80 (41.9)	41 (41.8)
History of cardiovascular disease (%)		
MI	50 (26.2)	30 (30.6)
CABG	14 (7.3)	6 (6.1)
PCI	44 (23.2)	28 (28.9)
Stroke	19 (9.9)	7 (7.1)
Admission diagnosis (%)		
STEMI	93 (49.0)	47 (48.0)
NSTEMI	74 (38.7)	37 (37.8)
UAP	24 (12.6)	14 (14.3)
Revascularisation during index admission		
PCI	147 (82.1)	79 (86.8)
CABG	5 (2.6)	1 (1.1)
Physical examination		
Body mass index (SD)	27.5 (3.6)	27.5 (3.6)
Killip class 1 (%)	177 (92.7)	94 (95.9)
Heart rate (IQR)	73 (62-84)	70 (61-81)
Systolic blood pressure (IQR)	137 (117-152)	136 (119-151)
eGFR, ml/min/1,73 m^2 (SD)	98 (30)	97 (28)
Discharge medication (%)		
Aspirin	183 (96.3)	95 (96.9)
BetaBlocker	167 (87.9)	83 (84.7)
ACEi	138 (72.6)	68 (69.4)
ARB	22 (11.6)	11 (11.2)
Statin	183 (96.3)	96 (98.0)

SD: Standard deviation; IQR: Interquartile range; Y: year; CAD: coronary artery disease; eGFR: estimated glomerular filtration rate; MI: Myocardial infarction; CABG: coronary artery bypass grafting; PCI: percutaneous coronary intervention; STEMI: ST-elevation myocardial infarction; NSTEMI: non ST-elevation myocardial infarction; UAP: unstable angina pectoris

Post 6 months: Analysis set minus (1.) an elective PCI more than 150 days after the index event and (2.) patients with less than 3 samples available after 6 months.

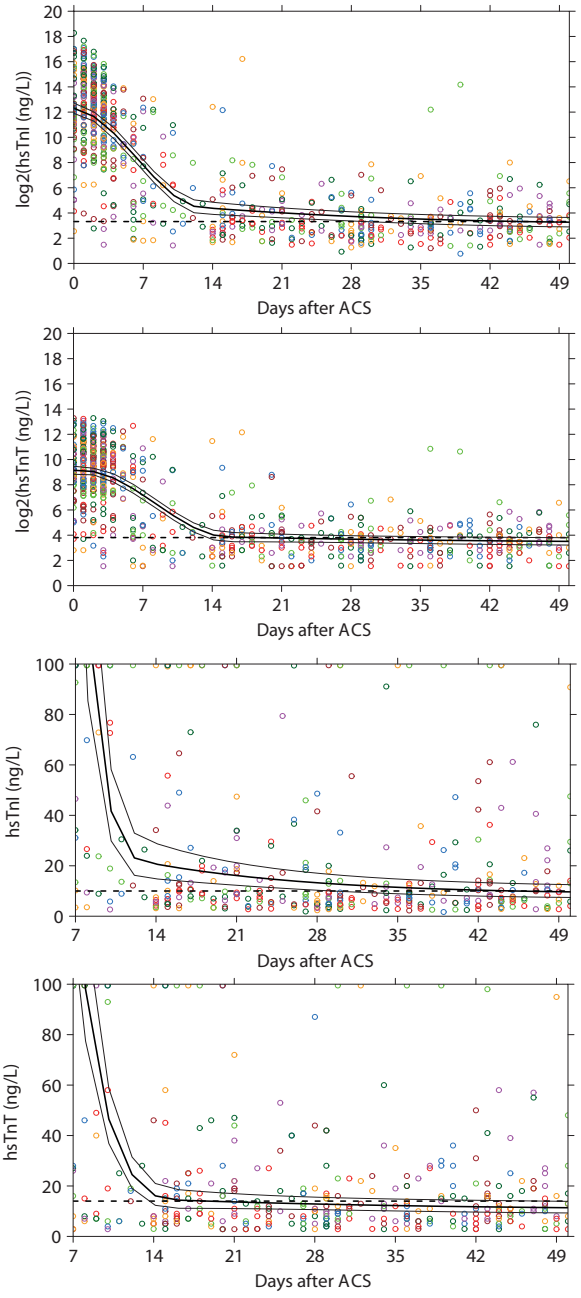

Figure 1. Average stabilization patterns of high-sensitivity troponins after ACS
The X-axes depict the number of days since the ACS. The Y-axes represent the troponin levels. The upper two plots are on the log scale with base number 2. A 1 point increase can thus be interpreted as a doubling of the value. The black lines depict the cohort average; the dashed lines the corresponding 95% confidence interval.

Temporal evolvement after the index ACS

The average concentrations of the different biomarkers from the time of the ACS until day 50, are shown in Figure 1. Both hsTnI and T were clearly elevated at the onset of ACS and gradually returned to levels beneath the reference values. The moment at which biomarker levels did stabilize was 20 days for hsTnI and 16 days for hsTnT. However, hsTnI remained above the population reference value on average almost three times longer (43 days) than hsTnT did (15 days).

Biological variation

The distributions of the hsTn measurements after 6 months are shown for each patient in figure 2. None of the samples had a hsTnI level below the detection limit; 14.0% had a hsTnT level below the detection limit (but none were below the limit of blank). In total 12.6% of the values were above the population reference value for hsTnI and 17.3% for hsTnT.

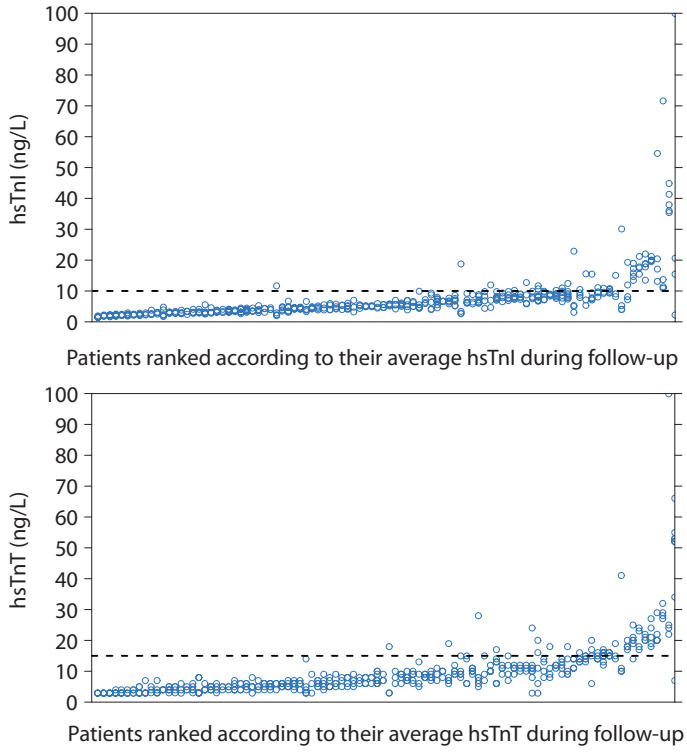

Figure 2. Distribution of the high-sensitivity troponins after six months
On the horizontal axes are the individual patients ranked based on their average troponin values. The vertical axes depict the troponin levels resulting from the repeated measurements. The dotted lines show the reference value of the troponin

The CV_i was slightly higher for hsTnT. We could not identify any baseline characteristics that were significantly associated with the observed CV_i (supplementary table 1). In contrast to the small CV_is, the CV_gs were large, reflecting relatively large differences in average troponin levels between patients. Consequently, both biomarkers had II's <0.6, The RCV limits ranged between −33.6% and 50.5% for hsTnI and −39.6% and 65.5% for hsTnT, respectively. In practice, this means that for a patient with a steady state hsTnI level of 5 ng/l, the maximum hsTnI rise based on analytical and within-subject variation is 3 ng/l. A larger increase would thus most likely be caused by pathological processes. An overview of the different parameters for the biological variation is presented in table 2.

Table 2. Overview of parameters of biological variation

							Log-normal	
	Mean patient level	CVa	CVi	CVg	II	RCV(%)	RCV low (%)	RCV up (%)
HsTropI	5.3 (3.7-8.3)	5.0	14.0	94.1	0.16	38.7	−33.6	50.5
HsTropT	7.8 (5.1-11.1)	3.0	18.1	75.9	0.24	50.1	−39.6	65.5

HsTropI: high-sensitivity troponin I; HsTropT: high-sensitivity troponin T; CVa: analytical coefficient of variation; CVg: interindividual coefficient of variation; CVi: intraindividual coefficient of variation; II: index of individuality; RCV: reference change value

Patient-specific reference

In a total of 122 patients, with 3 or more samples post 30 days, a patient-specific reference values could be determined in 85.2% (hsTnI) and 83.6% (hsTnT) using the first two post-30 day measurements. The median (IQR) reference value was 7.1 ng/L (4.4-10.6) for hsTnI and 8.5 ng/L (6.5-12.9) for hsTnT. In addition, we compared the observed patient-specific references values with the last available measurement from the same patient. The difference between the patient-specific reference value and their last available measurement was less than 5 ng/L in more than 81.7% (hsTnI) and 77.5% (hsTnT) of the patients. A paired t-test confirmed that there were no significant differences between the two values for both hsTnI (mean 10.0 vs 10.4 ng/L, p = 0.80) and hsTnT (mean 10.5 vs 10.4 ng/L, p = 0.91).

DISCUSSION

In BIOMArCS we observed that after the hsTn peak and plateau of the index ACS, hsTnT reached values below the clinically used reference value faster than hsTnI. The individual variation of hsTnI and hsTnT was low, while the differences between patients were large. This combination of characteristics led to a low II (<0.6) for both troponins, which stresses the need for a patient-specific instead of a population-based reference hsTn value[20] in patients with known stable CAD after having previously endured an ACS. We

demonstrated that in the majority of the patients two samples taken after at least one month sufficed to find such a reference value.

The parameters of variation are comparable to earlier reports in healthy populations. A study by Wu et al. of 17 healthy subjects also found a long-term individual variation of 14% for hsTnI.[9] The CV_g in their report was low (63%) in comparison to our study which indicates that there is a larger variation in troponin levels in stable ACS patients than in healthy individuals. The larger between-subject variation in a diseased population compared to a healthy one, is also confirmed by a study of Meijers et al. comparing biological variation in 83 patients with heart failure to 28 healthy subjects.[21] They reported a CV_g for hsTnT of 96.6% and 51.2% respectively. The CV_is however, were similar in both populations and comparable to our cohort.

Our data, as well as earlier research, all showed a low II for both hsTnI and hsTnI which means that a patient specific reference value is preferred over a population based reference value. We are the first to demonstrate that such a value could be retrieved in the majority of patients based on a limited number of consecutive measurements. These reference values showed good agreement with samples taken later during the year of follow-up. In a real life setting, the method could even be further optimized. If a treating physician identifies a patient with a prolonged stabilization the following measurement could be postponed. Also outliers could be neglected. Although it would take some extra visits of the patient for blood sampling to the clinic, determining the patient specific reference value could provide clinical benefits by leading to a more precise diagnosis of ACS or better rule-out. Furthermore, using our method, we can identify patients with a patient specific reference value above the population based reference value. In BIOMArCS we observed that in clinically asymptomatic patients that had endured an ACS more than 6 months ago, 12.6% of the hsTnI and 17.3% of the hsTnT values were above the population reference value, i.e. 99th percentile of a healthy reference population. This is exactly in line with our previous observation in the ATHEROREMO study in which hsTnT was above the 14 ng/L cut-off in 19.5% of 212 stable CAD patients.[22]

A recent study conducted at the emergency department of the Karolinska University Hospital also identified that approximaly one fifth, i.e. 4052 out of 21189 chest pain patients without ACS, present with an initial hsTnT concentration above 14 ng/L, once again confirming that an initial hsTnT elevation outside the setting of an ACS is a common finding in the emergency department.[23]

Similarly, the fact that a large percentage of patients with known stable CAD has chronic Tn elevation above the 99th percentile has to be accounted for when evaluating these patients in the emergency room. A patient specific reference value could aid the diagnostic process in such cases. Finally, the patient specific reference is also useful in determining the prognosis of the patient since there is a strong and graded association between hsTn and adverse outcome.[3,15,23-26]

At this point, there is no clear definition of the transition point at which post-ACS patients are considered to be stable CAD patients. From a biomarker perspective, our data show that all ACS-patients reach a point after which the Troponins remain within a constant, patient-specific range.

Due to the highly-frequent blood sampling, the BIOMArCS provides a unique platform to study longitudinal biomarker patterns in a real-world post-ACS population. However, a limitation is that the study protocol did not specify the time of sampling during the day and that we have no information of the patients activity prior to sampling. HsTns are known to be influenced by (heavy) physical activity[27] and to have a circadian rhythm.[28] We have investigated the variation of the time of sampling and found that all measurements were taken between 8 o'clock in the morning and 4 o'clock in the afternoon. Moreover, we found that, although not specified in the protocol, the vast majority of the patients always came in at the same hour for their blood sampling. Hence, the within-patient variation in biomarker levels found in this study cannot be explained by changes in sampling time. A second limitation of our study protocol is that we excluded all patients with recurrent events for determining the parameters of variability. Although correct, as we do not want to take into account possible distortion from an imminent ischemic event, this could potentially compromise generalizability of our parameters. However, in a sensitivity analysis also comprising the patients with ischemic events, the parameters only changed marginally (data not shown). A final limitation is that using our data, we cannot confirm that using a patient-specific reference value enhances the diagnostics for future ACS. This should be the focus of future research.

Conclusion

In conclusion, hsTnT levels stabilize on average after 16 days and TnI levels after 20 days after ACS, although hsTnI levels remain above the 99th percentile reference value longer (43 days) than hsTnT levels (15 days). Once the troponins have stabilized, within-patient variation is small, and comparable to healthy populations. Between-patient variation, however, is much higher in post-ACS patients than in population controls. Finally, with two samples taken at least one month after ACS, a patient-specific reference value can be determined in the vast majority of the patients. This reference value could aid in future diagnostics and could help provide an indication of the prognosis of the patient.

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REFERENCES

1. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, Bax JJ, Borger MA, Brotons C, Chew DP, Gencer B, Hasenfuss G, Kjeldsen K, Lancellotti P, Landmesser U, Mehilli J, Mukherjee D, Storey RF, Windecker S, Baumgartner H, Gaemperli O, Achenbach S, Agewall S, Badimon L, Baigent C, Bueno H, Bugiardini R, Carerj S, Casselman F, Cuisset T, Erol C, Fitzsimons D, Halle M, Hamm C, Hildick-Smith D, Huber K, Iliodromitis E, James S, Lewis BS, Lip GY, Piepoli MF, Richter D, Rosemann T, Sechtem U, Steg PG, Vrints C, Luis Zamorano J, Management of Acute Coronary Syndromes in Patients Presenting without Persistent STSEotESoC. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J 2016;**37**(3):267-315.
2. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD. Third universal definition of myocardial infarction. Circulation 2012;**126**(16):2020-2035.
3. Oemrawsingh RM, Akkerhuis KM, de Mulder M, Umans VA, Kietselaer B, Schotborgh C, Ronner E, Lenderink T, Liem A, Haitsma D, van der Harst P, Asselbergs FW, Maas A, Oude Ophuis AJ, Ilmer B, Dijkgraaf R, de Winter RJ, Kie The SH, Wardeh AJ, Hermans W, Cramer E, van Schaik RH, Hoefer IE, Doevendans PA, Simoons ML, Boersma E, Investigators BI. High-Frequency Biomarker Measurements of Troponin, NT-proBNP, and C-Reactive Protein for Prediction of New Coronary Events After Acute Coronary Syndrome. Circulation 2019;**139**(1):134-136.
4. Ang DSC, Kao MPC, Dow E, Lang C, Struthers A. The prognostic value of high sensitivity troponin T 7 weeks after an acute coronary syndrome. Heart 2012;**98**(15):1160-1165.
5. Koenig W, Breitling LP, Hahmann H, Wüsten B, Brenner H, Rothenbacher D. Cardiac troponin T measured by a high-sensitivity assay predicts recurrent cardiovascular events in stable coronary heart disease patients with 8-year follow-up. Clinical chemistry 2012:clinchem. 2012.183319.
6. Frankenstein L, Wu AH, Hallermayer K, Wians FH, Jr., Giannitsis E, Katus HA. Biological variation and reference change value of high-sensitivity troponin T in healthy individuals during short and intermediate follow-up periods. Clin Chem 2011;**57**(7):1068-71.
7. Vasile VC, Saenger AK, Kroning JM, Jaffe AS. Biological and analytical variability of a novel high-sensitivity cardiac troponin T assay. Clin Chem 2010;**56**(7):1086-90.
8. Vasile VC, Saenger AK, Kroning JM, Klee GG, Jaffe AS. Biologic variation of a novel cardiac troponin I assay. Clin Chem 2011;**57**(7):1080-1.
9. Wu AH, Lu QA, Todd J, Moecks J, Wians F. Short- and long-term biological variation in cardiac troponin I measured with a high-sensitivity assay: implications for clinical practice. Clin Chem 2009;**55**(1):52-8.
10. Nordenskjold AM, Ahlstrom H, Eggers KM, Frobert O, Jaffe AS, Venge P, Lindahl B. Short- and long-term individual variation in cardiac troponin in patients with stable coronary artery disease. Clin Chem 2013;**59**(2):401-9.
11. Katus HA, Remppis A, Scheffold T, Diederich KW, Kuebler W. Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction. The American journal of cardiology 1991;**67**(16):1360-1367.
12. Mair J, Thome-Kromer B, Wagner I, Lechleitner P, Dienstl F, Puschendorf B, Michel G. Concentration time courses of troponin and myosin subunits after acute myocardial infarction. Coronary artery disease 1994;**5**(10):865-872.

13. Morrow DA, Cannon CP, Jesse RL, Newby LK, Ravkilde J, Storrow AB, Wu AHB, Christenson RH, Apple FS, Francis G. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: clinical characteristics and utilization of biochemical markers in acute coronary syndromes. Clinical chemistry 2007;**53**(4):552-574.
14. Oemrawsingh RM, Akkerhuis KM, Umans VA, Kietselaer B, Schotborgh C, Ronner E, Lenderink T, Liem A, Haitsma D, van der Harst P, Asselbergs FW, Maas A, Oude Ophuis AJ, Ilmer B, Dijkgraaf R, de Winter RJ, The SH, Wardeh AJ, Hermans W, Cramer E, van Schaik RH, Hoefer IE, Doevendans PA, Simoons ML, Boersma E. Cohort profile of BIOMArCS: the BIOMarker study to identify the Acute risk of a Coronary Syndrome-a prospective multicentre biomarker study conducted in the Netherlands. BMJ Open 2016;**6**(12):e012929.
15. Eggers KM, Lagerqvist B, Venge P, Wallentin L, Lindahl B. Persistent cardiac troponin I elevation in stabilized patients after an episode of acute coronary syndrome predicts long-term mortality. Circulation 2007;**116**(17):1907-14.
16. Funaro S, La Torre G, Madonna M, Galiuto L, Scara A, Labbadia A, Canali E, Mattatelli A, Fedele F, Alessandrini F, Crea F, Agati L, Investigators A. Incidence, determinants, and prognostic value of reverse left ventricular remodelling after primary percutaneous coronary intervention: results of the Acute Myocardial Infarction Contrast Imaging (AMICI) multicenter study. Eur Heart J 2009; **30**(5):566-75.
17. Galiuto L, Garramone B, Scara A, Rebuzzi AG, Crea F, La Torre G, Funaro S, Madonna M, Fedele F, Agati L, Investigators A. The extent of microvascular damage during myocardial contrast echocardiography is superior to other known indexes of post-infarct reperfusion in predicting left ventricular remodeling: results of the multicenter AMICI study. J Am Coll Cardiol 2008;**51**(5): 552-9.
18. Ndrepepa G, Colleran R, Braun S, Cassese S, Hieber J, Fusaro M, Kufner S, Ott I, Byrne RA, Husser O. High-sensitivity troponin T and mortality after elective percutaneous coronary intervention. Journal of the American College of Cardiology 2016;**68**(21):2259-2268.
19. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;**27**(5):409-37.
20. Petersen PH, Fraser CG, Sandberg S, Goldschmidt H. The index of individuality is often a misinterpreted quantity characteristic. Clin Chem Lab Med 1999;**37**(6):655-61.
21. Meijers WC, van der Velde AR, Muller Kobold AC, Dijck-Brouwer J, Wu AH, Jaffe A, de Boer RA. Variability of biomarkers in patients with chronic heart failure and healthy controls. Eur J Heart Fail 2016.
22. Oemrawsingh RM, Cheng JM, Garcia-Garcia HM, Kardys I, van Schaik RH, Regar E, van Geuns RJ, Serruys PW, Boersma E, Akkerhuis KM. High-sensitivity Troponin T in relation to coronary plaque characteristics in patients with stable coronary artery disease; results of the ATHEROREMO-IVUS study. Atherosclerosis 2016;**247**:135-41.
23. Roos A, Bandstein N, Lundback M, Hammarsten O, Ljung R, Holzmann MJ. Stable High-Sensitivity Cardiac Troponin T Levels and Outcomes in Patients With Chest Pain. J Am Coll Cardiol 2017; **70**(18):2226-2236.
24. Everett BM, Brooks MM, Vlachos HE, Chaitman BR, Frye RL, Bhatt DL, Group BDS. Troponin and Cardiac Events in Stable Ischemic Heart Disease and Diabetes. N Engl J Med 2015;**373**(7):610-20.
25. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, Tjora S, Domanski MJ, Gersh BJ, Rouleau JL, Pfeffer MA, Braunwald E, Prevention of Events with Angiotensin Converting Enzyme Inhibition Trial I. A sensitive cardiac troponin T assay in stable coronary artery disease. N Engl J Med 2009;**361**(26):2538-47.

26. Omland T, Pfeffer MA, Solomon SD, de Lemos JA, Rosjo H, Saltyte Benth J, Maggioni A, Domanski MJ, Rouleau JL, Sabatine MS, Braunwald E, Investigators P. Prognostic value of cardiac troponin I measured with a highly sensitive assay in patients with stable coronary artery disease. J Am Coll Cardiol 2013;**61**(12):1240-9.
27. Shave R, Baggish A, George K, Wood M, Scharhag J, Whyte G, Gaze D, Thompson PD. Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. Journal of the American College of Cardiology 2010;**56**(3):169-176.
28. Klinkenberg LJJ, van Dijk J-W, Tan FES, van Loon LJC, van Dieijen-Visser MP, Meex SJR. Circulating cardiac troponin T exhibits a diurnal rhythm. Journal of the American College of Cardiology 2014; **63**(17):1788-1795.

SUPPLEMENTARY FILES

Methods

The CV_i was defined as the median value of the CVs of the repeated measurements in individual subjects ($CV_{subject}$), adjusted for the analytical variation:

$$CV_i = \sqrt{\text{median}(CV_{subject}^2) - CV_a^2}$$

Finally, CV_g was determined as 100% times the standard deviation ($sd_{\bar{x}_{subject}}$) of the mean values of the repeated measurements in individual subjects ($\bar{X}_{subject}$) by the (unweighted) mean of these means ($\bar{X}_{group}$):

$$CV_g = 100\% * sd_{\bar{x}_{subject}} / \bar{X}_{group}$$

The *Index of Individuality (II)* is the ratio of the combined within-subject and analytical variation relative to the between-subject variation:

$$II = \sqrt{CV_i^2 + CV_a^2/CV_g}$$

When the II <0.6, it is agreed that subjects should have their own reference values, based on previous samples.[17] When the II >1.4, a population-based reference is preferred.

The *Reference Change Value (RCV)* reflects the limit of (relative) change in biomarker values in individual subjects that can be explained by the combined within-subject and analytical variation. For biomarkers with a normal distribution, the RCV can be calculated as follows:

$$RCV = Z_{\alpha/2} * \sqrt{2(CV_i^2 + CV_a^2)}$$

where $Z_{\alpha/2}$ represents the critical value of the normal distribution for 100% * $(1-\alpha)/2$ confidence. For biomarkers with a skewed distribution a log-normal approach has been described,[18] and the RCV limits can be determined as follows:

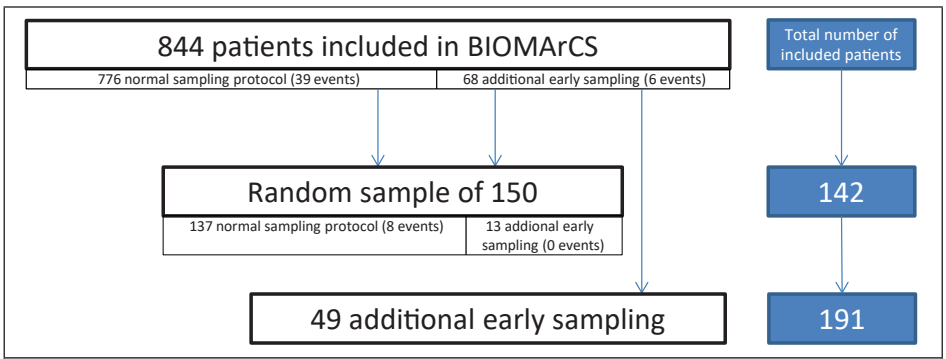
$$RCV_{downward} = e^{-Z_{\alpha/2}*\sqrt{2\ln(CV_w^2+CV_a^2+1)}} - 1$$

$$RCV_{upward} = e^{Z_{\alpha/2}*\sqrt{2\ln(CV_w^2+CV_a^2+1)}} - 1$$

We used $\alpha = 0.05$ (for 95% confidence), thus $Z_{0.025} = 1.96$.

Supplementary table 1.

	hsTnI		hsTnT	
	estimate (95%CI)	P-value	estimate (95%CI)	P-value
Male gender	0.002 (−0.686, 0.691)	0.994	−0.064 (−0.669, 0.541)	0.834
Age	−0.01 (−0.04, 0.02)	0.503	−0.022 (−0.048, 0.003)	0.088
Current Smoking	0.072 (−0.501, 0.645)	0.804	−0.15 (−0.652, 0.352)	0.555
Diabetes	−0.53 (−1.269, 0.209)	0.158	−0.213 (−0.867, 0.441)	0.519
Hypertension	−0.01 (−0.576, 0.556)	0.972	0.192 (−0.303, 0.688)	0.443
Hypercholesterolemia	0.094 (−0.474, 0.662)	0.744	−0.17 (−0.668, 0.328)	0.5
Family history of CAD	−0.061 (−0.633, 0.511)	0.832	0.193 (−0.379, 0.765)	0.503
BMI	−0.029 (−0.108, 0.05)	0.467	−0.032 (−0.101, 0.038)	0.368
Heart Rate	0.004 (−0.013, 0.021)	0.637	−0.005 (−0.02, 0.009)	0.485
Systolic blood pressure	−0.001 (−0.012, 0.009)	0.842	−0.002 (−0.011, 0.007)	0.702
Killip-class	0.782 (−0.859, −2.544)	0.947	0.52 (−0.92, −2.254)	0.717
Aspirin	0.516 (−1.122, 2.153)	0.533	0.556 (−0.881, 1.992)	0.445
BetaBlocker	−0.726 (−1.497, 0.045)	0.065	0.088 (−0.601, 0.777)	0.8
ACE inhibitor	0.059 (−0.555, 0.672)	0.85	−0.04 (−0.579, 0.498)	0.882
ARB	0.294 (−0.599, 1.188)	0.515	0.088 (−0.698, 0.874)	0.824
Statin	−1.864 (−3.827, 0.099)	0.062	−0.326 (−2.081, 1.428)	0.713



Supplementary figure 1.