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Stabilization patterns and variability of hs-CRP, NT-proBNP and ST2 during 1 year after acute coronary syndrome admission: results of the BIOMArCS study

Abstract

Objectives: Details of the biological variability of high-sensitivity C-reactive protein (hs-CRP), N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and ST2 are currently lacking in patients with acute coronary syndrome (ACS) but are crucial knowledge when aiming to use these biomarkers for personalized risk prediction. In the current study, we report post-ACS kinetics and the variability of the hs-CRP, NT-proBNP and ST2.

Methods: BIOMArCS is a prospective, observational study with high frequency blood sampling during 1 year post-ACS. Using 1507 blood samples from 191 patients that remained free from adverse cardiac events, we investigated post-ACS kinetics of hs-CRP, NT-proBNP and ST2. Biological variability was studied using the samples collected between 6 and 12 months after the index ACS, when patients were considered to have stable coronary artery disease.

Results: On average, hs-CRP rose peaked at day 2 and rose well above the reference value. ST2 peaked immediately after the ACS but never rose above the reference value. NT-proBNP level rose on average during the first 2 days post-ACS and slowly declined afterwards. The within-subject variation and relative change value (RCV) of ST2 were relatively small (13.8%, RCV 39.7%), while hs-CRP (41.9%, lognormal RCV 206.1/-67.3%) and NT-proBNP (39.0%, lognormal RCV 185.2/-64.9%) showed a considerable variation.

Conclusions: Variability of hs-CRP and NT-proBNP within asymptomatic and clinically stable post-ACS patients is considerable. In contrast, within-patient variability of ST2 is low. Given the low within-subject variation, ST2 might be the most useful biomarker for personalizing risk prediction in stable post-ACS patients.

Keywords: acute coronary syndrome (ACS); C-reactive protein (CRP); myocardial infarction; N-terminal prohormone of brain natriuretic peptide (NTproBNP); ST2; variability.

Introduction

Elevated serum levels of high-sensitivity C-reactive protein (hs-CRP), N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and soluble ST2 (ST2) have been associated with adverse cardiovascular events in patients

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with coronary artery disease (CAD) and acute coronary syndrome (ACS), and have been proposed in prognostic models [1–8]. However, the differences in serum levels between the patients with and without cardiovascular events are often not large. For example, in a study by Zebrack et al. among 2554 patients undergoing coronary angiography, in the group without CAD and the lowest event rate during a mean follow-up of 2 years median CRP levels was from 1.15 mg/dL, compared median levels of 1.28 mg/dL in the group with the most severe CAD and highest event rate during follow-up [4].

While aiming for personalized risk prediction, appropriate stratification of patients is crucial. Thus, it is important to know if differences in biomarkers levels between subjects, and changes over time within a patient, truly reflect differences in health state, or if it is caused by analytical or by biological variability. Studies on the variability of hs-CRP, NT-proBNP and ST2 during stable health have mostly been performed in (small sets of) healthy subjects, or in heart failure (HF) patients [9–18]. Remarkably, data on their performance in stable post-ACS/CAD patients is scarce [19].

Against this background, we aimed to provide a detailed description of the influence of an ACS on hs-CRP, NT-proBNP and ST2 levels, and to investigate the within- and between-patient variability of these biomarkers in serial blood samples during stable health after ACS. Our analyses are embedded in the BIOMarker study to identify the Acute risk of a Coronary Syndrome (BIOMArCS), which was specifically designed to study longitudinal biomarker patterns in (post-)ACS patients [20].

Materials and methods

BIOMArCS is a multi-center, prospective, observational study that was conducted in 18 participating hospitals in the Netherlands during 2008–2015. The study was designed to obtain data on biomarker patterns in ACS patients during 1-year follow-up. Details of the BIOMArCS design and main findings have been published previously [20–22].

Briefly, patients above 40 years presenting with ACS and at least one additional cardiovascular risk factor were eligible. Preferably, patients were enrolled during hospital admission, but inclusion at the first outpatient visit post-discharge (usually 4–6 weeks later) was allowed. Blood samples were collected at admission, at the day of hospital discharge and subsequently every fortnight during the first 6 months after discharge. Additional blood samples were collected at 24, 48, 72 and 96 h after admission and at the day of hospital discharge in a subset of 8% of patients, with the specific aim to study the evolution and normalization of biomarkers in the early post-ACS phase. Follow-up was terminated permanently after coronary artery bypass grafting, hospital admission for HF, or a deterioration of renal function leading to a glomerular filtration rate <30 mL/min/1.73 m², as circulating biomarker concentrations may be significantly influenced by these conditions.

All patients were treated to prevailing guidelines and at the discretion of the investigator. The study was approved by the medical Ethics Committees and conducted in accordance with the Declaration of Helsinki. All patients signed informed consent for their participation in the study.

Blood sampling and storage

Blood samples were handled and securely stored on-site within 4 h after venipuncture. After preparation, aliquots were frozen at –80 °C within 2 h after withdrawal. Samples were transported under controlled conditions to the Department of Clinical Chemistry at the Erasmus MC for long-term storage. After all material was collected and follow-up was completed, batch-wise analysis of blood samples was performed in a central laboratory. Laboratory personnel were blinded for patient characteristics.

Biomarker measurements were performed in the serum EDTA plasma after a median average storage time of 4.9 (25th–75th percentile 3.8–6.2) years. Hs-CRP was determined using the Coulter 5800 series (Beckman Coulter, Brea, CA, USA), lower limits of detection (LLOD) 0.2 mg/L, and population reference value 5 mg/L. ST2 was determined with the Presage ST2 assay (Critical diagnostics, San Diego, CA, USA), LLOD 1.31 ng/mL, and reference value 49.3 ng/mL (male) or 33.5 ng/mL (female). NT-proBNP was measured with a custom-built ELISA method using an antibody against HRP-conjugated MAB mouse anti-human N-terminal proBNP (Hytest, 13G12 [4NT1C]), which shows very good agreement with other commercially available assays. The intra-assay CV was 4%, LLOD 6.25 pmol/L, and reference value 30 pmol/L.

Analysis of the biomarker stabilization patterns

For the analysis of the BIOMArCS study, hsTnI and HsTnT serum levels were measured in the samples of 187 patients [21]. 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 (25th–75th percentile 5–10) repeated samples per patient (altogether 1507 samples) that were used for the analysis of stabilization patterns. We used linear mixed effect (LME) models to describe biomarker stabilization patterns over time. A maximum of two cubic splines were placed to model a possible non-linear evolvement. Mean values of hs-CRP, NT-proBNP and ST2 at each post-ACS day were then determined using the fitted LME models. The biomarker was considered stabilized when the difference in mean level between two consecutive days was less than 1%.

Measures of biological variability

A coefficient of variability (CV) of a series of measurements is defined as 100% times the standard deviation (SD) of the measurements divided by their mean value ($\bar{X}$):

$$CV = 100\% * SD / \bar{X}$$

According to the methods by Fraser and Harris [23], the total variability of a series of repeated measurements in individual subjects can be split in three components, which represent the variability due to the imprecision of the analytical process (CV_a), the intra-individual or within-subject variability (CV_i) and the inter-individual or between-subject variability (CV_g). Besides these measures of variability, we also determined the index of individuality (II) and the reference change value (RCV). 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 while the II is calculated for investigating if population-based reference values are adequate. A more detailed description of the different measures of variability and the formulas used to calculate them can be found in the Supplementary Files.

Based on previous studies investigating cardiac remodeling and biomarker levels post-ACS, we presumed that ACS patients would be biochemically stable after 6 months [1, 24, 25]. Hence, for the analysis of biological variability, those patients that had ≥3 measurements in the 6–12 months post-ACS time window were selected. This resulted in a total of 446 samples and was limited to 98 patients.

We performed sensitivity analyses, investigating if the biological variation was influenced by the New York Heart Association (NYHA)-classification and Canadian Cardiovascular Society (CCS) grading. NYHA class and CCS grade were determined at all sampling moments. In our sensitivity analyses, we calculated the measures of biological variation while excluding patients who reported an elevated NYHA-class (NYHA ≥1) and/or elevated CCS grading (CCS ≥1) at any sampling moment.

All statistical analyses were performed with R 3.3.1. p-Values below 0.05 (2-sided) were considered statistically significant.

Table 1: Baseline characteristics.

	Stabilization pattern set (n=191)	Biological variation set (n=98)
Age, year (SD)	62.4 (10.6)	62.8 (9.5)
Male gender, n (%)	148 (77.5)	77 (78.6)
Cardiovascular risk factors, n (%)		
Diabetes mellitus	33 (17.3)	17 (17.3)
Hypertension	101 (52.9)	52 (53.1)
Hypercholesterolemia	91 (47.6)	53 (54.1)
Family history of CAD	87 (53.0)	47 (59.5)
Current smoker	80 (41.9)	41 (41.8)
History of cardiovascular disease, n (%)		
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, n (%)		
STEMI	94 (49.2)	48 (49.0)
NSTEMI	72 (37.7)	36 (36.7)
UAP	25 (13.1)	14 (14.3)
Physical examination		
Body mass index (SD)	27.5 (3.6)	27.5 (3.6)
Killip class 1, n (%)	177 (89.4)	94 (95.9)
Heart rate (IQR)	73 (62–84)	70 (61–81)
Systolic blood pressure (IQR)	137 (117–152)	136 (119–151)

CABG, coronary artery bypass grafting; CAD, coronary artery disease; IQR, interquartile range; MI, myocardial infarction; n, number; PCI, percutaneous coronary intervention; SD, standard deviation; STEMI, ST-elevation myocardial infarction; UAP, unstable angina pectoris.

Results

Patient characteristics

The mean age (standard deviation) of the patients was 62.4 (10.6) years and 78% were men (Table 1). A substantial percentage of patients had hypertension (53%), hypercholesterolemia (48%) and a family history of premature CAD (53%). ST-elevation myocardial infarction (STEMI) was the most common index event (49%). No relevant differences in baseline characteristics were identified between the two analysis sets.

Stabilization patterns

The average stabilization patterns of the three biomarkers of interest in the post-ACS period are shown in Figure 1. Hs-CRP increased until day 2, and reached on average a maximum level of 14.9 mg/L. Thereafter, hs-CRP steadily declined. The population reference value was reached at day 15, and the marker had stabilized at day 30.

NT-proBNP also increased until day 2, where it reached an average maximum level of 94 pmol/L. NT-proBNP only slowly declined. The marker stabilized at day 15, but levels remained on average above the population reference value during follow-up. ST2 showed on average a maximum levels of 44.3 ng/mL at the day of the index ACS, which was well below the population reference value. Although still slowly declining, serum levels stabilized at day 5.

Biological variation

The median patient average serum levels in the 6–12 months post-ACS period are 2.4 mg/L (interquartile range [IQR] 1.2–3.1) for hs-CRP, 54.4 pmol/L (IQR 29.1–97.8) for NT-proBNP and 30.2 ng/mL (IQR 25.2–35.0) for ST2. The distribution of the hs-CRP, NT-proBNP and ST2 measurements in the 6–12 months post-ACS period are shown for each patient in Figure 2. All hs-CRP and ST2 measurements were above the LLOD. NT-proBNP was below the LLOD in 7.2% of the samples. Hs-CRP values were above
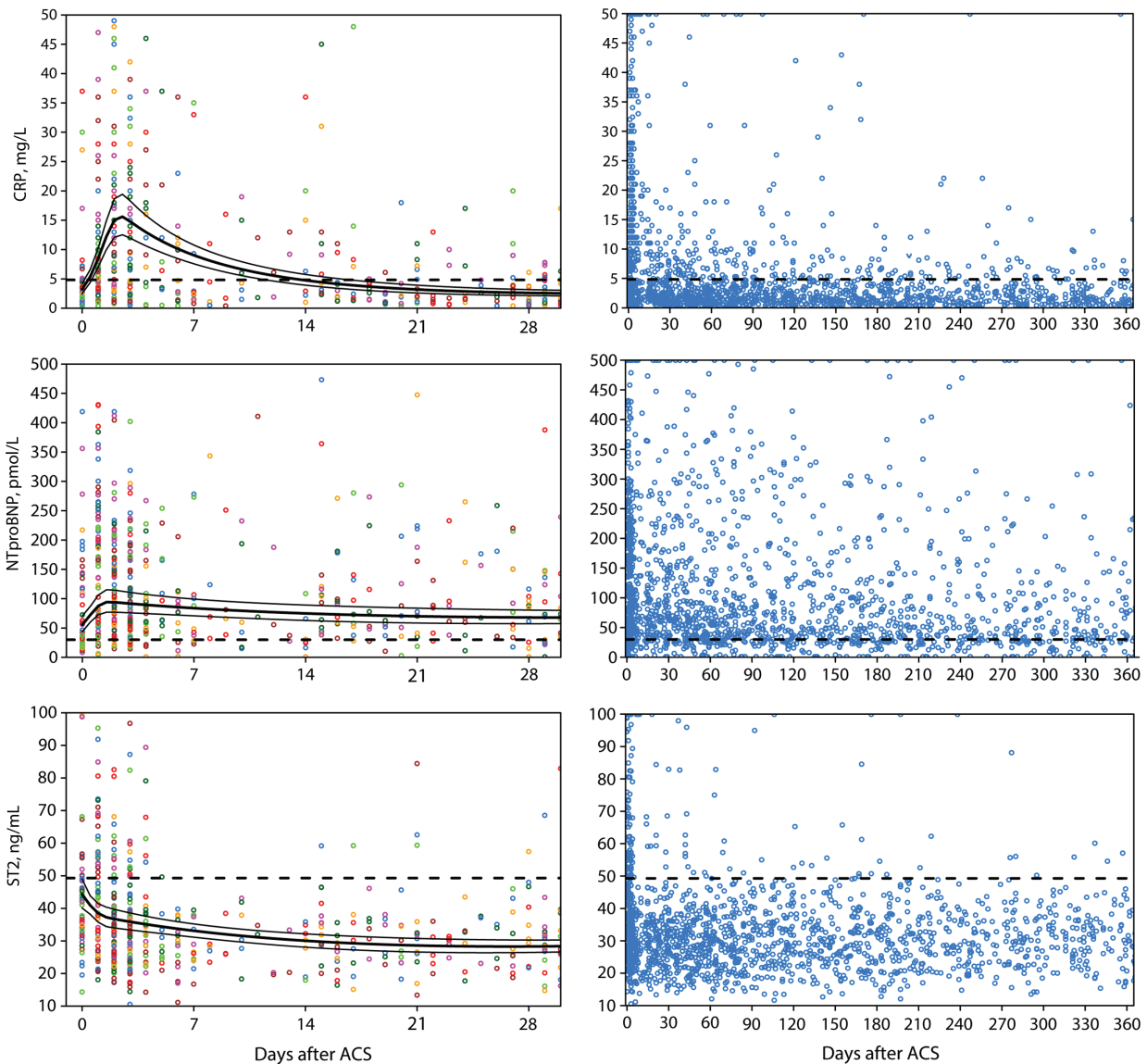
Figure 1: Temporal patterns of hs-CRP, NT-proBNP and ST2 after ACS.
Left pictures depict the washout pattern after the ACS, the right pictures show all measurements during the year of follow-up. hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; ST2, soluble suppression of tumorigenicity-2.

the population reference in 15.5% of the samples, NT-proBNP in 24.1%, and for ST2 in 3.5%.

Hs-CRP (CV_i 41.9%, lognormal RCV 206/-67%) and NT-proBNP (CV_i 39.0%, lognormal RCV 185/-65%) displayed a considerable within-individual variation and correspondingly wide RCVs, while the plasma concentrations of ST2 within a patient were rather stable (CV_i of 13.8%, RCV 40%). The within-subject variability of hs-CRP (Kruskal-Wallis, p = 0.36) and ST2 (p = 0.17) was not influenced by the patients average serum levels. In contrast, the within-subject variation of NT-proBNP (Kruskal-Wallis, p = 0.003) was much larger in patients with low serum concentrations (Figure 3). All three studies biomarkers had an II

below 0.6, indicating that a patient-based reference value, based on previous samples of the individual patient is preferred. A detailed overview of the parameters of variation is shown in Table 2.

Sensitivity analyses

An NYHA class ≥1 was reported at 29 sampling moments (6%) in 15 different patients, while a CCS ≥1 was reported at 49 (11%) sampling moments in 27 different patients. In the majority of the cases, this concerned NYHA class and CCS class one (44.8% and 75.5%, respectively). The CVs
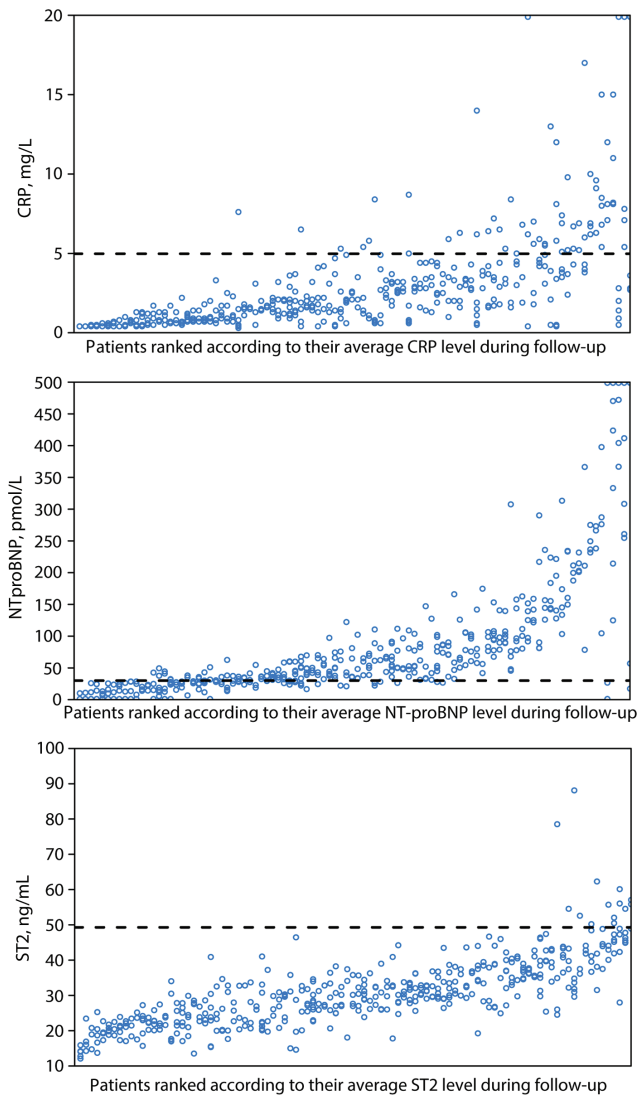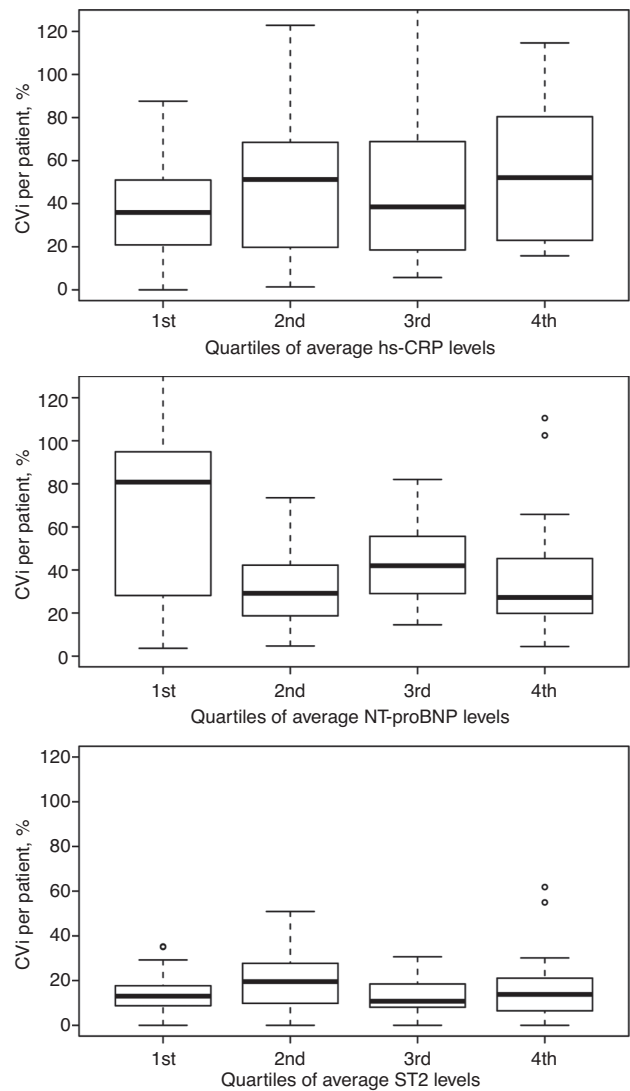
Figure 2: Distribution of measurements per patient.
Horizontal: patients ranked according to their average biomarker value. Vertical: Spread of biomarker measurement per patient.
hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; ST2, soluble suppression of tumorigenicity-2.

Figure 3: Intra-individual variability in quartiles based on average biomarker level.
Boxplots of individual CV_is in the different biomarker quartiles.
hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; ST2, soluble suppression of tumorigenicity-2. Median [range] of quartiles: Hs-CRP 1st: 0.8 [0.4, 1.2] 2nd: 1.8 [1.3, 2.4], 3rd: 3.0 [2.6, 3.5], 4th: 5.1 [3.6, 21.3]. NT-proBNP 1st: 20 [3, 29] 2nd: 39 [29, 54], 3rd: 66 [55, 91], 4th: 174 [100, 783]. ST2: 1st: 22.2 [13.8, 25.2] 2nd: 27.3 [25.3, 30.2], 3rd: 31.4 [30.2, 34.9], 4th: 41.2 [35.0, 54.0].

calculated in the dataset excluding these patients, showed similar results as the full cohort (Supplementary Table 1).

Discussion

Levels of hs-CRP, NT-proBNP and ST2 appeared differently affected by an ACS. Both Hs-CRP and NT-proBNP reached maximum values at day 2, however, hereafter hs-CRP declined to levels below the population reference within 2 weeks, while the NT-proBNP only slowly declined and remained above the population reference value throughout the follow-up. ST2 was elevated at the time of the index ACS, but values remain below the population reference. Hs-CRP and NT-proBNP showed substantial within-subject variability and thus wide RCV, while the within-subject variability of ST2 measurement was low. The between-subject variability was much larger than the within-subject variability for all three biomarkers.

Hs-CRP is one of the most used biochemical marker of inflammation in medicine and is known to rise

Table 2: Overview of parameters of biological variation in serial measurements 6 months after ACS.

	Average patient level	CVa	CVi	CVg	II	RCV, %	Log-normal	
							RCV up, %	RCV low, %
CRP	2.4 (1.2–3.1)	3	42.0	99.1	0.42	116.3	206.1	−67.3
NT-proBNP	54.4 (29.1–97.8)	4	39.0	127.6	0.31	108.1	185.2	−64.9
ST2	30.2 (25.2–35.0)	5	13.4	25.4	0.56	39.7	48.4	−32.6

ACS, acute coronary syndrome; CVa, analytical coefficient of variation; CVg, interindividual coefficient of variation; CVi, intra-individual coefficient of variation; hs-CRP, high-sensitivity c-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; ST2, soluble suppression of tumorigenicity-2; II, index of individuality; RCV, reference change value.

after ACS due to inflammation of the ischemic areas of the heart. In agreement with our findings, Orn et al. described a delayed rise of CRP and a relatively fast near-normalization hereafter in 42 STEMI patients [26]. Similarly, among 962 patients with an episode of unstable CAD (NSTEMI and UAP), a peak in CRP serum concentration at 48 h after the start of symptoms was described. In this same population the CRP levels at 6 months was on average still elevated when compared to healthy controls, although not above the reference value [27]. We now show that although the CRP levels are quickly within the "normal" range, it can take much longer before the levels actually stabilize.

Details of the parameters of variability of hs-CRP had not yet been described in a post-ACS population. However, in healthy volunteers, the within-subject variability is known to be considerable while the between-subject variability is even larger [9–11]. In our post-ACS patients, we found comparable within-subject variability and RCVs as reported in healthy populations, but the variation between post-ACS patients appeared much larger. Given the high within-patient variability, it would take numerous numbers of samples to determine the habitual value needed to use CRP in personalized risk prediction in clinically stable CAD patients. This makes sense, as hs-CRP is not a specific cardiac marker and can be influenced by many other factors.

NT-proBNP showed an initial rise and maximum value at day 2 followed by a slow decline thereafter, with the levels remaining above the population reference value. The early rise can be explained by the initial myocardial ischemia [28], while the slow decline is most likely caused by progressive remodeling combined with a degree of myocardial dysfunction post-ACS [29]. However, as repeated cardiac imaging was not part of our study protocol, we cannot confirm this. The post-ACS kinetics of NT-proBNP have previously been described by Taiwar et al. and Lidahl et al. in, respectively, 60 patients and 1216 myocardial infarction patients. Similar to our study,

they described a peak of the biomarker serum levels in the first 48 h after the index event and a slow decline hereafter [30, 31]. Other investigations – using a few samples taken weeks/months apart from each other and not specifically focusing on post-ACS kinetics –, also showed that the biomarker had a peak early after ACS, and only slowly declined between blood samples hereafter [1, 2]. Our study distinguishes itself from previous studies by three key elements: our study is conducted in the contemporary PCI-era; we systematically obtained a median of 4 (IQR 4–5) samples per patient at regular time points during 1-year follow-up; we applied state-of-the-art statistical methods, including LME models, in order to account for intra-patient correlation of consecutive measurements.

The biological variability of NT-proBNP in patients with CAD has been described earlier by Nordenksjold et al. in a total of 24 patients [19]. Using two samples taken a median of 23 (IQR 4–58) days apart, they found a CVi of 20.4 with a log-normal RCV of +76/−43%. We obtained a larger sample of patients and applied a higher blood sampling frequency. Also, we enrolled a homogeneous series of patients who were admitted for ACS, whereas Nordenksjold et al. studied patients undergoing coronary angiography, of whom only 50% patients ultimately underwent revascularization. These differences in study design could easily explain the differences in variability, and the corresponding RCVs found. The variability of NT-proBNP has also been investigated in healthy subjects and HF patients. Similar to our study results, in all studies previously performed, NT-proBNP serum levels consistently show considerable within-patient variability and large corresponding RCVs [12–15]. Because of this variability, a single NT-proBNP measurement does not suffice for determining the habitual value, and thus, also not for an adequate personalized risk prediction. Notably, the between-subject variability that we found was comparable to the HF patients (CV$_g$ of 116.3%) and larger than the healthy subjects (54.0%) that were described by Meijers et al. [15]. This can probably be explained by the larger

heterogeneity in health status among patient populations when compared to healthy populations.

The early post-ACS evolvement of serum ST2 has been described based on 403 NSTE-ACS patients who participated in GUSTO-IV, using blood samples at 24, 48 and 72 h after inclusion [5]. Similar as in our study, ST2 reached its maximum during the first sample and quickly declined hereafter. Our results add to this that, once stabilized, ST2 is a very stable marker with little variation over time in post-ACS patients. This is in line with previous studies investigating the biological variability of ST2, that all showed little within-patient variation and thus relatively small RCVs. Both Wu et al. and Dieplinger et al. report a CV_i of approximately 10% in small sets of healthy subjects [17, 18], which was similar to the CV_i in series of chronic HF patients [15, 16]. Interestingly, in the study by Meijers et al. the between-subject variability of ST2 in HF patients did not differ much from healthy controls (36.9% vs. 30.4%) [15]. Given the promising results of ST2 as a prognostic marker in patients with ACS and/or CAD [5–8], and the low within-patient variability of serum ST2 levels in post-ACS, a single, or a few, measurements would most likely improve personalized risk prediction.

Limitations

The BIOMArCS study provides us with a unique platform to investigate the effect of ACS on the different blood biomarker and to investigate their parameters of variability in clinically stable post-ACS patients. However, a few limitations of our work need discussion. Blood sampling in BIOMArCS was protocolized, but the exact sample moment on the day was not. Consequently, differences in physical activities and diet, as well as potential circadian variation could have influenced the measures of biological variation [32–34]. Still, importantly, all samples were taken between 8 am and 4 pm, whereas the vast majority of patients had their blood sampling at the same time, which, apparently best fitted in their private schedule. Secondly, as we used one central laboratory for the analysis of the blood samples, we could not investigate variability between different laboratories.

Conclusions

In conclusion, the within-patient variability of hs-CRP and NT-proBNP within asymptomatic and clinically stable post-ACS patients is substantial. This leads to clinically significant differences between serial measurements in the same patients. If used for personalized risk prediction, this would compromise the calibration and multiple samples would be needed in order to correctly classify the patients in the right risk category. In contrast, within-patient variability of ST2 is low. Given the low within-subject variation, ST2 might be the most useful biomarker for personalized risk prediction in stable post-ACS patients.

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