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Temporal Pattern of Growth Differentiation Factor-15 Protein After Acute Coronary Syndrome (From the BIOMArCS Study)

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Growth differentiation factor-15 (GDF-15) has appeared as a promising biomarker with strong predictive abilities in acute coronary syndrome (ACS). However, studies are solely based on *single* measurements in the *acute* phase of an ACS event. The way GDF-15 patterns in post-ACS patients behave on the long term is largely unknown. We conducted a nested case-control study within our multicenter, prospective, observational biomarker study (BIOMArCS) of 844 ACS patients. Following an index ACS event, high-frequency blood sampling was performed during 1-year of follow-up. GDF-15 was determined batchwise by electrochemiluminescence immunoassays in 37 cases with a recurrent event during 1-year follow-up, and in 74 event-free controls. Cases and controls had a mean ± standard deviation age of 66.9 ± 11.3 years and 81% were men. From 30 days onwards, patients showed stable levels, which were on average 333 (95% confidence interval 68 to 647) pg/mL higher in cases than controls (1704 vs 1371 pg/mL; p value 0.013). Additionally, in the post 30-day period, GDF-15 showed low within-individual variability in both cases and controls. In conclusion, post-ACS patients experiencing a recurrent event had stable and systematically higher GDF-15 levels during 30-day to 1-year follow-up than their event-free counterparts with otherwise similar clinical characteristics. Thus, postdischarge blood sampling might be used throughout the course of 1 year to improve prognostication, whereas, in view of the low within-individual variation, the number of repeated sampling moments might be limited. © 2019 Elsevier Inc. All rights reserved. (Am J Cardiol 2019;124:8−13)

In recent years, circulating growth differentiation factor-15 (GDF-15), a stress-induced cytokine, has emerged as a biomarker of interest due to its potential prognostic value in patients with cardiovascular disease.[1] In particular, elevated levels of GDF-15 are associated with an impaired prognosis after acute coronary syndromes (ACS).[2-6] However, the prognostic value of GDF-15 in ACS patients thus far, has been mainly based on **single** measurements in the **early, acute** phase of an acute ischemic event. Therefore, the optimal time point in the stabilized post-ACS phase for GDF-15 blood sampling to make prognostic implications remains not fully elucidated yet. We used our 'BIOMarker study to identify the Acute risk of a Coronary Syndrome' (BIOMArCS) with high-frequency blood sampling in post-ACS patients as a platform to describe the temporal evolution of GDF-15 during 1-year follow-up, to evaluate differences between patients with and without a recurrent event, and to study the individual variability of GDF-15.

Methods

We performed a nested case-control analysis within the main BIOMArCS study that was approved by the medical ethics committees of all participating hospitals. The rationale and design of BIOMArCS are described in detail elsewhere.[7] In brief, BIOMArCS is a prospective, multicenter, observational study conducted in 18 participating hospitals in the Netherlands. A total of 844 patients, admitted for an ACS, including unstable angina pectoris, non−ST-elevation

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myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI) and with at least one additional cardiovascular risk factor, were enrolled between 2008 and 2015. Patients underwent regular blood sampling after the initial admission for ACS according to a strict schedule to describe the temporal evolution of blood biomarkers in the post-ACS phase, and to reveal deviations in temporal biomarker patterns before a recurrent coronary event. Venepuncture was performed at admission, at hospital discharge and subsequently every fortnight during the first half year, followed by monthly blood sample collection until 1 year. Follow-up blood sampling 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 of <30 mL/min/1.73 m^2, since these conditions influence circulating biomarker concentrations. Ultimately, patients had 17 (median) repeated blood samples within 1 year. The study was performed in accordance with the criteria described in the declaration of Helsinki and all patients provided written informed consent for their participation.

The primary study endpoint was a composite of cardiac death, nonfatal myocardial infarction, or unstable angina pectoris, requiring urgent coronary revascularization within 1-year follow-up. Study endpoints were adjudicated by a clinical event committee, blinded for any biomarker data, after the study was completed in 2015. In 2014, Roche Diagnostics GmbH offered the opportunity to determine GDF-15 with their precommercial assay in a limited number of BIOMArCS patients. Since no commercial GDF-15 assay would have been available within the foreseeable future, we decided to accept this one-time offer. We analyzed the blood samples of all patients with an investigator-reported endpoint event at that time, as well as 2 matched endpoint-free controls for each such event. Matching was based on admission hospital, age (± 5 year range), gender, diabetes mellitus, peripheral artery disease, and history of coronary artery disease (CAD). We kept the results unanalyzed until study completion and event-adjudication. After study completion, it appeared that 37 of the investigator-reported events were confirmed as study endpoint. In the current analysis, these events were included as cases, together with their corresponding 74 matched controls.

Blood samples were initially handled and securely stored on-site. Aliquots were frozen at −80°C within 2 hours after withdrawal. Long-term storage and batchwise GDF-15 analysis took place at the Department of Clinical Chemistry of the Erasmus MC, Rotterdam (the Netherlands). Laboratory personnel were blinded for any clinical data, including endpoint data. The plasma GDF-15 concentrations were measured using the quantitative sandwich electrochemiluminescence immunoassay "ECLIA" (Roche Diagnostics, Mannheim, Germany) on a Cobas e601 immunoassay analyzer. The lowest detection limit of GDF-15 analyte concentration was 400 pg/mL. No interference was found using in vitro tests to determine interference between 51 commonly used cardiovascular pharmaceuticals and the assay.

It is important to discern a fixed amount of individual biomarker variability from clinically relevant changes over time. Therefore certain parameters have been described to define individual variability, which are needed to interpret the relevance of changes of repeated measurements. 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,[8] 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 of GDF-15 in our laboratory appeared to be 1.75% and 1.88% for high and low concentrations, respectively. Subsequently, 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:

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

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

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

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

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

Since a high II (>1.4) indicates a relatively high within-subject variation and low between-subject variation, it is more likely that an unusual biomarker value will lie outside the borders of most overlapping values and therefore population-based reference intervals are sufficient. Conversely, when the II is low (<0.6), it is agreed that subjects should have their own reference values, based on previous samples. 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 skewed distribution a log-normal approach has been described, and the RCV limits can be determined as follows:

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

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

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

Since GDF-15 is known to be initially elevated post-ACS, all aforementioned variability parameters are based on >30 day blood samples. Although the exact pathophysiological substrate for an initial elevation of GDF-15 in the acute phase of ACS is unknown,[9] a possible "washout" effect due to an acute phase reaction is thereby hampered. After that period, biochemical as well as clinical

Table 1
Baseline clinical characteristics (n = 111)

Variable	Cases (n = 37)	Controls (n = 74)	p value
Age (years)	67.9 ± 11.7	66.3 ± 11.2	0.79
Men	30 (81%)	60 (81%)	0.95
ST-segment elevation myocardial infarction	13 (35%)	42 (57%)	<0.001
Non-ST-segment elevation myocardial infarction	18 (49%)	27 (37%)	0.17
Unstable angina pectoris	6 (16%)	5 (7%)	0.78
Smoker			
Current	13 (35%)	25 (34%)	0.91
Former	11 (30%)	23 (31%)	0.97
Never	13 (35%)	26 (35%)	0.95
Diabetes mellitus	12 (32%)	26 (35%)	0.52
Hypertension	19 (51%)	40 (54%)	0.77
Hypercholesterolemia	17 (46%)	30 (41%)	0.81
Prior myocardial infarction	12 (32%)	23 (30%)	0.93
Prior percutaneous coronary intervention	11 (30%)	20 (27%)	0.88
Prior coronary artery bypass grafting	9 (24%)	10 (14%)	0.64
Prior stroke	8 (22%)	7 (10%)	0.78
Prior peripheral vascular disease	10 (27%)	13 (18%)	0.73

Values are mean ± standard deviation or n (%). p values were obtained by the linear mixed model (continuous variable) or generalized linear mixed model (categorical variable), whichever was appropriate.

stabilization is expected to be reached for adequately determining GDF-15 variability. Thereafter, only patients with at least 3 available measurements in that time window are included, leaving 20 cases and 46 controls for variability analysis.

Categorical data are presented as numbers and percentages. Continuous variables are presented as mean ± standard deviation (SD) or as median and interquartile range (IQR), depending on their distribution. Normality of continuous variables was examined by visual inspection of the histogram and by normal Q-Q plots. The examined biomarker GDF-15 (outcome) showed a skewed distribution and was therefore ^{2}log-transformed for further analyses. GDF-15 biomarker trajectories were examined across different follow-up time intervals after the ACS index event during one year of follow-up. Within the first 7 days from admission, each patient's maximum biomarker value was determined. The median values of the patient-level maximum were compared between the cases and controls by linear mixed effect (LME) models. Then, the patient-average biomarker trajectories in 7 to 30 days from admission and from 30 days onwards were compared between cases and controls by LME models with nested random effects. Time from index ACS event until each blood sample measurement and a group variable (case/control) were entered as fixed effects in the model, paired individuals as random effects and serial GDF-15 measurements as the dependent variable (model 1). Subsequently, we fitted multivariable LME models with adjustment for age and gender (model 2), and with additional adjustment for admission diagnosis, diabetes mellitus, smoking, hypertension, hypercholesterolemia, BMI, history of revascularization, history of myocardial infarction and serum creatinine value (which was measured at each sampling moment) (model 3). Values were eventually backtransformed to present mean differences (95% confidence intervals [CI]) between cases and controls on the linear scale. All data were analyzed with SPSS (version 21) and R statistical software (version 3.5.1). All statistical tests were two-tailed and p values <0.050 were considered statistically significant.

Results

Baseline clinical characteristics are presented in Table 1. The matching procedure appeared successful, as there were no relevant differences between cases and controls, except for admission diagnosis of STEMI (p value <0.001). During the first 7 days after the index ACS, GDF-15 levels reached maximum values (median [IQR]) of 2436 [2286 to 4236] pg/mL in cases and 1804 [1207 to 3749] pg/mL in the controls (p value 0.22). These levels slightly decreased within the first 30 days, and the mean value within the 7 to 30 day period was 1908 pg/mL and 1590 pg/mL in cases and controls, respectively. This mean difference of 318 (95% CI ranging from −215 to 1058) pg/mL was statistically nonsignificant (p value 0.26). From 30 days after the index ACS onwards until 1-year follow-up, cases had systematically higher GDF-15 levels than controls (Table 2,

Table 2
Mean GDF-15 (pg/mL) values in cases and controls in the 30 days to 1 year period after ACS admission

	Cases	Controls	Mean difference (95% CI)	p value
Model 1*	1780	1414	366 (26, 788)	0.034
Model 2[†]	1744	1415	329 (2, 732)	0.049
Model 3[‡]	1704	1371	333 (68, 647)	0.013

CI = confidence interval.

* Unadjusted for patient characteristics.

[†] Adjusted for age and gender.

[‡] Adjusted for age, gender, admission diagnosis, diabetes mellitus, smoking, hypertension, hypercholesterolemia, BMI, history of revascularization, history of myocardial infarction and serum creatinine value (measured at each time-point).
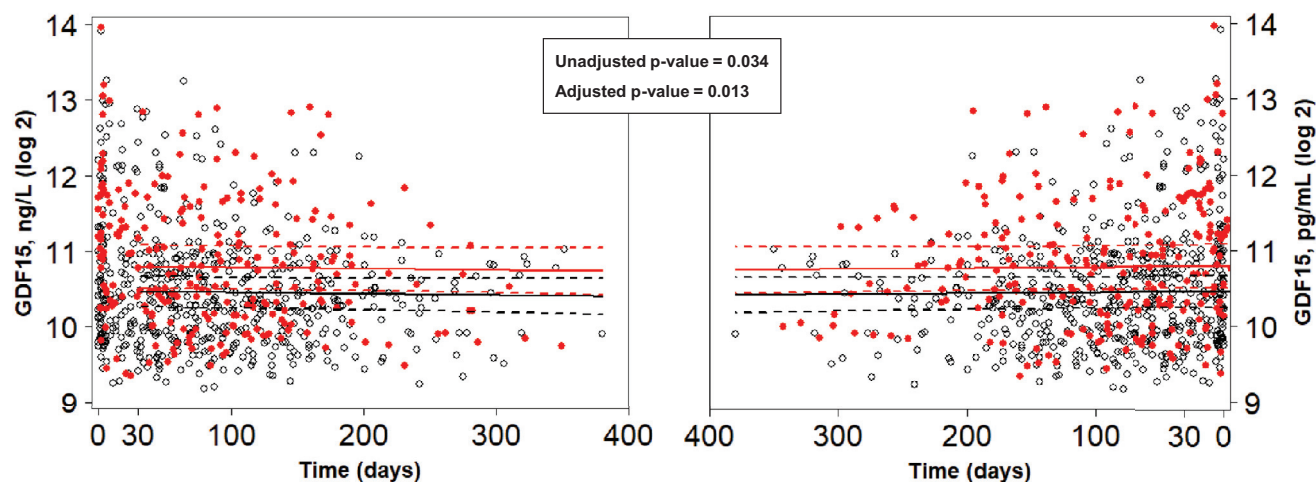
Figure 1. Serial measurements and temporal evolvement of GDF-15 (pg/mL) in cases (red) and controls (black). (Color version of figure is available online.) The left graph shows the evolvement of GDF-15 since the index event (t = 0) until 1-year follow-up. The right graph shows the evolvement of GDF-15 before the study endpoint (t = 0 in cases), or until the last blood sample moment (t = 0 in controls). The points represent measurements in individual patients. The lines represent the group average values *(bold lines)* and the 95% confidence intervals *(dashed lines)*, using linear mixed models with nested random effects.

Figure 1). This difference remained significant after correction for age, gender and multiple cardiovascular risk factors (p value 0.013). These findings are confirmed in strata according to gender, diabetes mellitus, smoking, serum creatinine value, and admission diagnosis (Supplemental Tables 4−8). No differences were observed in GDF-15 levels across the various subgroups (all p values for heterogeneity were >0.05).

An overview of the different variability parameters, calculated for a selected amount of cases and controls, is presented in Table 3. With a CV_a of 2%, both groups displayed limited within-subject variability (CV_i of 16.3 for the cases and 11.5 for the controls), whereas the between-subject variability showed larger variation (CV_g of 73.1 for the cases and 62.0 for the controls). This is also shown by a plot (Figure 2), which illustrates low within-subject variability ($CV_i/[CV_i + CV_g] = 16\%$ to 18%) and large between-subject variability ($CV_g/(CV_i + CV_g)$ 82% to 84%) with a minimum of 579 pg/mL and a maximum of 9748 pg/mL. As could be expected from low within-subject variability and high between-subject variability in both

groups, the II was low (below the threshold value of 0.6), and thus individual reference values are preferred. Thereby we found that the limits of change between subsequent measurements (RCV) are allowed to range from −36% to 57% in cases and from −28% to 38% in controls.

Discussion

This is the first study to describe GDF-15 patterns in post-ACS patients in great detail, utilizing a high-frequency blood sampling design during 1 year. Four key lessons were learned from our analysis. First, in individual patients, after reaching a peak value in the first week after admission, GDF-15 concentrations leveled off to levels that remain stable throughout 1-year follow-up. Second, importantly, there was no steady or sudden change in GDF-15 level before a recurrent event. Thus, no significant changes in GDF-15 values occurred after the initial post-ACS phase. Third, patients who experienced a recurrent event had on average 26% higher GDF-15 levels than those who remained event-free. Although the prognostic value of GDF-15 has already

Table 3
Parameters describing the biological variability of GDF-15 serial measurements 30 days after the acute coronary syndrome index event in both cases and controls

	Cases (n = 20)	Controls (n = 46)
Average biomarker level (pg/mL), median [IQR]	1423 [1122−2594]	1317 [966−1705]
Analytical coefficient of variation (CV_a)	2%	2%
Intra-individual coefficient of variation (CV_i)	16.3	11.5
Inter-individual coefficient of variation (CV_g)	73.1	62.0
Index of individuality (II)	0.2	0.2
Reference change value (RCV)	45%	32%
Reference change value, upper limit	57%	38%
Reference change value, lower limit	−36%	−28%

Parameters describing the biological variability of GDF-15, as calculated by formulas presented in the method section.

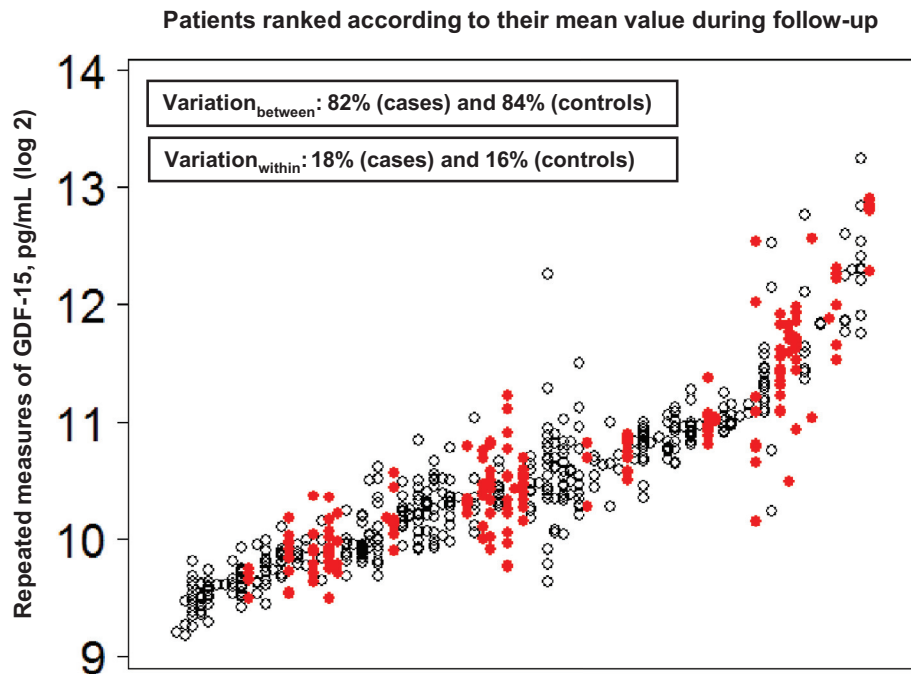**Patients ranked according to their mean value during follow-up**

Figure 2. Graphical illustration of GDF-15 variability by displaying the distribution of GDF-15 measurements per patient 30 days after the ACS index event. (Color version of figure is available online.)
The data points represent measurements in individual patients (cases in red; controls in black), ranked according to their mean value during post 30 days follow-up in order to display within- and between-individual variation.

been demonstrated by previous studies with one baseline measurement, we additionally proved that repeated postdischarge blood sampling of GDF-15 during 1 year might help improve accurate prognostication. Fourth, within-patient variability was much smaller than between-patient variability, meaning that the number of repeated blood samples to obtain a patient-specific stable GDF-15 level can be limited.

Considering the natural course of GDF-15 post-ACS in our analysis, peak values are present in the first 7 days after an ACS, whereafter it seems that GDF-15 subtly reaches a stabilized phase without significant changes, especially before a recurrent event. By the use of frequent serial measurements, the stability of the marker in individual patients on the long term was established. This finding is supported by previous data in post-ACS patients, demonstrating that GDF-15 concentrations show small alterations through the first 72 hours of hospitalization and potentially several months thereafter.[3,4] The fact that GDF-15 concentration levels remain significantly higher in patients who experience a recurrent event than in event-free patients over the course of a year in our study without any level changes around the event, suggests that GDF-15 is not merely a reflection of extent of myocardial damage or infarct size, but rather reflects severity of (chronic) atherosclerotic disease burden at any time point. This proposition is further supported by findings with cardiovascular magnetic resonance, demonstrating that GDF-15 is unrelated to infarct size and myocardial area at risk 2 to 4 days after the index event.[9] Furthermore, GDF-15 concentrations on admission seemed to be similar between NSTEMI and STEMI

patients, of whom more severe myocardial damage can be expected.[2,3] Thus, in support of our hypothesis, previous studies do not indicate that GDF-15 solely mirrors tissue damage.

With regard to prognostication, GDF-15 has been thoroughly investigated in clinical studies and shown to be an independent prognostic marker of mortality and cardiovascular events in both healthy individuals and CAD patients, which is in accordance with our results.[4−6,9−11] Specifically, a recent meta-analysis focused on ACS patients, including 8 studies and 8,903 participants, showed a significant hazard ratio (95% confidence interval) of 1.66 (1.47 to 1.87) on the association between GDF-15 and mortality or recurrent MI.[6] However, most studies performed blood sampling only on admission at the onset of an ACS or at discharge during the recovery phase of an ACS. As we have demonstrated, initial GDF-15 peak values were largely present in the first 7 days after the index ACS, which is likely the expression of an acute phase reaction. Therefore, single blood samples timed in the early phase during the course of an ACS event may represent a peak level, which does not clarify its prognostic implications on long-term post-ACS. To our knowledge, only 2 clinical studies have performed a limited number of serial GDF-15 measurements in post−NSTEMI patients.[3,4] Wollert et al[3] collected blood samples on admission and at 24, 48, and 72 hours in a subgroup of 399 patients, whereas Eggers et al[4] measured GDF-15 at baseline and after clinical stabilization at 6 weeks, 3 months, 6 months in 950 patients. Both studies found significant associations with respectively 1-year and 5-year mortality at each time point. Along

with our data with highly frequent blood sampling, we have additionally demonstrated that obtained blood samples within a course of 1 year post-ACS will provide comparable prognostic information.

The biological variability of GDF-15 in ACS patients has not been described so far. We found low within-subject variability and high between-subject variability, which corresponds with findings from a study on the biovariability of GDF-15 conducted in 41 patients with stable chronic systolic dysfunction.[12] In this study, GDF-15 was measured at 4 blood sampling time points up until 3 months and showed very little biological (within-)variation, whereas there was an elevated between-individual variation (reflected by a low II). Altogether, describing biomarker variability is warranted to provide insight into the significance and interpretation of a biomarker in clinical practice. Our results indicate that changes in serial measurements of GDF-15 in an individual who experienced an ACS, independently of disease status (case or control), might be more useful than population derived reference values.

The unique design and character of this study enabled us to provide novel data on the temporal evolution and variability of GDF-15 post-ACS. Nevertheless, some limitations warrant to be acknowledged. Due to the study design and its observational character, this substudy is unable to demonstrate causal inference. Whether GDF-15 merely reflects CAD pathways, or directly contributes to coronary pathophysiology remains unknown. Also, as opposed to previous studies with large cohorts, we could not demonstrate significant differences in GDF-15 levels between cases and controls within the first 30 days. This is probably due to a lack of power with a limited number of measurements <30 days within a relatively small cohort. In line with this, we are aware of the fact that our study comprises a relatively small number of study patients and events. Further, by acknowledging previous studies that investigated the prognostic value of GDF-15 in large study populations, our study encompassing an exceptional blood sampling frequency method should rather be seen as hypothesis-testing with an extension to existing knowledge.

In conclusion, with detailed analysis of the longitudinal GDF-15 pattern post-ACS, we have demonstrated that GDF-15 concentrations remain stable during follow-up with limited within-individual variation. In patients who eventually experience a recurrent event, GDF-15 is systematically elevated, independently of clinical risk factors and serum creatinine. Thus, to enable risk stratification with GDF-15 in post-ACS patients, blood sampling might be used throughout the course of 1 year for prognostication, whereas the number of repeated sampling moments might be limited. Further exploration of the exact role of GDF-15 in risk stratifying post-ACS patients and deciding on clear cut off points is warranted in future studies in order to make accurate prognostications.

Disclosures

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2019.03.049.

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