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ORIGINAL SCIENTIFIC PAPER
Acute Coronary Syndromes

Serially measured high-sensitivity cardiac troponin T, N-terminal-pro-B-type natriuretic peptide, high-sensitivity C-reactive protein, and growth differentiation factor 15 for risk assessment after acute coronary syndrome: the BIOMArCS cohort

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Aims Evidence regarding the role of serial measurements of biomarkers for risk assessment in post-acute coronary syndrome (ACS) patients is limited. The aim was to explore the prognostic value of four, serially measured biomarkers in a large, real-world cohort of post-ACS patients.

Methods and results BIOMArCS is a prospective, multi-centre, observational study in 844 post-ACS patients in whom 12 218 blood samples (median 17 per patient) were obtained during 1-year follow-up. The longitudinal patterns of high-sensitivity cardiac troponin T (hs-cTnT), N-terminal-pro-B-type natriuretic peptide (NT-proBNP), high-sensitivity C-reactive protein (hs-CRP), and growth differentiation factor 15 (GDF-15) were analysed in relation to the primary endpoint (PE) of cardiovascular mortality and recurrent ACS using multivariable joint models. Median age was 63 years, 78% were men and the PE was reached by 45 patients. The average biomarker levels were systematically higher in PE compared with PE-free patients. After adjustment for 6-month post-discharge Global

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Registry of Acute Coronary Events score, 1 standard deviation increase in log[hs-cTnT] was associated with a 61% increased risk of the PE [hazard ratio (HR) 1.61, 95% confidence interval (CI) 1.02–2.44, $P = 0.045$], while for log[GDF-15] this was 81% (HR 1.81, 95% CI 1.28–2.70, $P = 0.001$). These associations remained significant after multivariable adjustment, while NT-proBNP and hs-CRP were not. Furthermore, GDF-15 level showed an increasing trend prior to the PE (*Structured Graphical Abstract*).

Conclusion Longitudinally measured hs-cTnT and GDF-15 concentrations provide prognostic value in the risk assessment of clinically stabilized patients post-ACS.

Clinical Trial Registration The Netherlands Trial Register. Currently available at URL https://trialsearch.who.int/; Unique Identifiers: NTR1698 and NTR1106.

Structured Graphical Abstract

Key question

Biomarkers reflect pathophysiologic processes and their evolution which could prove beneficial in risk assessment. What is the prognostic value of serially measured hs-cTnT, NT-proBNP, hs-CRP, and GDF-15 in a cohort of post-ACS patients?

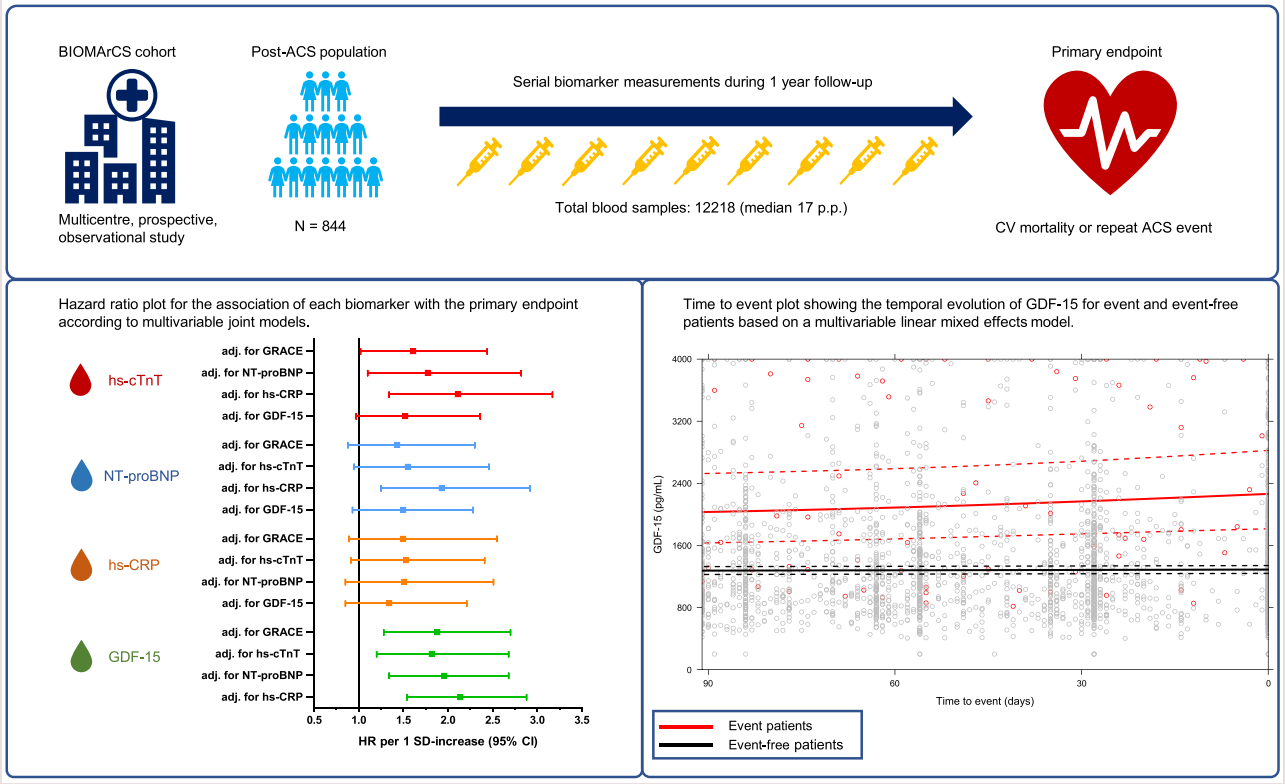
Key findings

Increase of GDF-15 level was associated with an 81% increased risk for a repeat event, while for hs-cTnT this was 61%, adjusted for GRACE score. GDF-15 associations remained significant adjusted for multiple biomarkers. NT-proBNP and hs-CRP were not significantly associated.

Take-home message

Serially measured GDF-15 was significantly and independently associated with the risk of recurrent cardiovascular event and concentrations rose towards the endpoint. Therefore, GDF-15 could improve risk assessment in the context of personalized medicine.

ACS, acute coronary syndrome; BIOMArCS, BIOMarker study to identify the acute risk of a coronary syndrome; CI, confidence interval; CV, cardiovascular; GDF-15, growth differentiation factor 15; GRACE, Global Registry of Acute Coronary Events risk score; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal-pro-B-type natriuretic peptide; pg/mL, picogram per millilitre; SD, standard deviation.



Keywords Acute coronary syndrome • Biomarkers • Repeated measurements • Risk assessment • Prognosis

Introduction

A fundamental part of the management of coronary artery disease is to accurately identify patients at high risk for a (recurrent) cardiovascular (CV) event and treat with more intensive medical therapy or opt for an early revascularization. To this end, several risk assessment tools exist of which Global Registry of Acute Coronary Events (GRACE) is recommended for patients presenting with an acute coronary syndrome (ACS).[1–3] GRACE has been well validated and demonstrated high discriminatory accuracy.[4–6] Still, in the current era of personalized medicine, there is a need for additional methods to individualize and therefore more accurately determine risk and prognosis. One such method is circulating biomarkers reflecting the pathobiological mechanisms underlying the disease. In patients presenting with ACS, the cardio-specific biomarker troponin plays a central role in diagnosis and prognostication.[1–3] However, there are several components to the pathophysiology of ACS aside from cardiomyocyte injury or myocardial necrosis, like inflammation, haemodynamic stress, and ventricular dysfunction.[7,8] Each of these processes are represented by (a) different (set of) biomarkers, hence requiring more than only cardiac troponin to explain the entire pathophysiology.

Recently, there has been more attention for other potentially relevant biomarkers in ACS and high-sensitivity (hs-) assays.[7,9] Biomarkers like N-terminal pro-B-type natriuretic peptide (NT-proBNP),[10,11] C-reactive protein (CRP),[10,12] and growth differentiation factor 15 (GDF-15)[13] have been shown to be promising markers for prognostication in the ACS population. Most studies, however, have only based their findings on a single measurement at baseline, not taking into consideration the dynamics during follow-up and across the clinical spectrum of disease. Therefore, evidence regarding temporal biomarker evolution and the relationship with recurrent events is limited.

We hypothesized that the temporal pattern of these biomarkers is associated with prognosis in (clinically stabilized) patients post-ACS admission. Previously, we published the prognostic value of serially measured hs-Troponin, NT-proBNP, and hs-CRP in a subset of 187 patients from the BIOMarker study to identify the acute risk of a coronary syndrome (BIOMArCS) according to a case–cohort design.[14] In another preliminary report, we showed promising results with respect to the prognostic value of serially measured GDF-15 post-ACS patients.[15] To expand upon these findings, we now present an analysis of the full BIOMArCS cohort of 844 patients with serially measured hs-cardiac troponin T (hs-cTnT), NT-proBNP, hs-CRP, and GDF-15 in a total of 12 218 repeated samples (median 17 per patient) in relation to recurrent ACS events during 1-year follow-up.

Methods

Study design

The BIOMArCS study has been described in detail previously.[16,17] Briefly, patients aged >40 years who were hospitalized for ACS, including ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and unstable angina pectoris (UAP), with at least one additional CV risk factor were included. Excluded were patients with myocardial ischaemia precipitated by a condition other than atherosclerotic coronary artery disease, severely impaired left ventricular function [ejection fraction <30% or end-stage congestive heart failure (HF) Class III or IV], severe chronic kidney disease, or a co-existent condition with a life-expectancy less than 1 year. Patients underwent highly frequent blood sampling during 1 year after the index admission for ACS with a median of 17 samples per patient [inter-quartile range (IQR) 12–20]. The primary composite endpoint (PE) was reached by 45 patients, defined as the first event of CV mortality ($n = 8$) or recurrent non-fatal ACS event including myocardial infarction (MI) ($n = 29$) or UAP requiring urgent coronary revascularization ($n = 8$). These were adjudicated by a Clinical Event Committee who was blinded to biomarker data.

This study complied with the Declaration of Helsinki and was approved by the Institutional Review Boards of the Erasmus MC (MEC-2007-185) and of each of the participating centres. Informed consent was provided by all participants before the study procedures were carried out. The procedures followed were in accordance with institutional guidelines. BIOMArCS has been registered in the Netherlands Trial Register with the identifiers: NTR1106 and NTR1698.

Biomarker measurements

Blood samples were taken by means of venepuncture according to a fixed protocol: at admission, discharge, every 2 weeks during the first half year and monthly afterwards. Blood samples were first handled on-site before being stored at −80°C. The aliquots were then transported under controlled conditions to the Department of Clinical Chemistry, Erasmus MC, Rotterdam, for long-term storage and (batch-wise) central analysis after study completion. For the current analysis, hs-cTnT, NT-proBNP, and GDF-15 concentrations were (re-)analysed using their specific Elecsys® quantitative sandwich electro-chemiluminescence immunoassays on a cobas® e 601 analyser (Roche Diagnostics, Ltd., Rotkreuz, Switzerland). hs-CRP concentrations were analysed using a particle-enhanced immunoturbidimetric assay on a cobas® c 501 analyser (Roche Diagnostics, Ltd., Rotkreuz, Switzerland).

Analysts were blinded to patient characteristics and endpoint data.

Statistical analysis

Continuous variables are reported as mean and standard deviation (SD) or as median and IQR based on normality, which was assessed by visually exploring the histograms and Q–Q plots. Differences between continuous variables were evaluated using Student's *t*-test or Mann–Whitney *U* test. Categorical variables are presented as counts and percentages, and differences between categorical variables were evaluated using the χ^2 or Fisher's exact test.

The longitudinal biomarker analyses were performed on samples that were collected >30 days after the index ACS admission, a period in which the patients were clinically stabilized and acute biomarkers were largely normalized.[18] A total of 30 patients reached the PE during this period. In these patients, only the samples that were obtained prior to the PE were analysed.

Biomarker levels were log2-transformed and standardized by subtracting the mean and then dividing by the SD (i.e. the Z-scores were constructed), which facilitates the comparison of effect estimates between markers. Correlations between the four biomarkers were studied based on all samples using the Spearman method which we applied on the Z-scores. We ran linear mixed effects (LME) models to describe the average biomarker trajectories during the 30-day to 1-year period after the index ACS event. We studied these trajectories in relation to the GRACE risk score, using the 6-month post-discharge model,[19] and in relation to the PE. The association between the serially measured biomarkers and the PE were further analysed using multivariable joint models which combine an LME model (describing the patient-specific longitudinal biomarker evolution) with a time-to-event model.[20] Results are presented as hazard ratios (HR) per 1 SD difference in biomarker level (on the log2-scale) with corresponding 95% confidence intervals (CI), which represent the relative risk measure of the PE at any given timepoint during follow-up. The joint models were defined as follows:

(1) Model 1 was unadjusted and the LME sub-model was adjusted for clinical confounders; GRACE score (continuous), sex, diabetes mellitus, coronary artery bypass graft, valvular heart disease, stroke, and peripheral vascular disease.
(2) Model 2 was adjusted for GRACE score (continuous) and the LME sub-model was adjusted for the same clinical confounders.
(3) Model 3 (multi-marker) was adjusted for one additional biomarker and the LME sub-model was adjusted for the same clinical confounders.

The variables used are similar to the previous (case–cohort) analysis.[14] Missing data in baseline variables are shown in Supplementary material online, *Table S1*. There were no missing values in covariates used within the post-30 longitudinal analyses. No relevant differences were observed based on index event.

Table 1 Baseline characteristics of the overall BIOMArCS cohort ($n = 844$), patients without the primary endpoint ($n = 799$) and patients with the primary endpoint ($n = 45$)

Variable	Overall ($n = 844$)	PE-free ($n = 799$)	PE ($n = 45$)	*P*-value
Age (years)	62.5 (54.3–70.2)	62.4 (54.3–69.9)	67.4 (57.1–76.6)	**0.026**
Men	657 (78%)	621 (78%)	36 (80%)	0.732
White	792 (96%)	748 (95%)	44 (98%)	0.715
Admission diagnosis				0.079
STEMI	430 (52%)	414 (53%)	16 (36%)	
NSTEMI	314 (38%)	292 (37%)	22 (49%)	
UAP	88 (11%)	81 (10%)	7 (16%)	
Culprit coronary artery				
Right	277 (33%)	265 (34%)	12 (27%)	0.343
Left main	21 (3%)	20 (3%)	1 (2%)	1.00
Left anterior descending	267 (32%)	253 (32%)	14 (31%)	0.903
Left circumflex	138 (17%)	129 (16%)	9 (20%)	0.516
PCI performed at index event	676 (86%)	642 (86%)	34 (87%)	0.875
Cardiovascular risk factors				
Diabetes mellitus	196 (24%)	179 (23%)	17 (38%)	**0.020**
Hypertension	463 (56%)	441 (56%)	22 (49%)	0.358
Hypercholesterolaemia	411 (49%)	391 (50%)	20 (44%)	0.505
Family history of CAD	421 (60%)	401 (60%)	20 (56%)	0.616
Smoking status				0.655
Current smoker	337 (40%)	320 (41%)	17 (38%)	
Former smoker	250 (30%)	238 (30%)	12 (27%)	
Never smoker	246 (30%)	230 (29%)	16 (36%)	
GRACE risk score[a] (continuous)	96 (78–118)	94 (77–116)	121 (98–141)	**<0.001**
GRACE risk—low	397 (48%)	389 (49%)	8 (18%)	**<0.001**
GRACE risk—intermediate	260 (31%)	246 (31%)	14 (31%)	
GRACE risk—high	175 (21%)	152 (19%)	23 (51%)	
Prior cardiovascular disease				
Myocardial infarction	224 (27%)	391 (50%)	20 (44%)	0.505
Coronary artery bypass grafting	83 (10%)	72 (9%)	11 (24%)	**0.003**
Percutaneous coronary intervention	218 (26%)	204 (26%)	14 (31%)	0.438
Stroke	75 (9%)	66 (8%)	9 (20%)	**0.015**
Peripheral vascular disease	74 (9%)	64 (8%)	10 (22%)	**0.004**
Chronic heart failure	20 (2%)	16 (2%)	4 (9%)	**0.019**
Valvular heart disease	18 (2%)	14 (2%)	4 (9%)	**0.013**
Physical examination				
Body mass index (kg/m^2)	28.0 ± 5.9	28.0 ± 6.0	27.2 ± 3.7	0.180
Heart rate (b.p.m.)	75 ± 19	75 ± 19	75 ± 16	0.751
Systolic blood pressure (mmHg)	140 ± 27	139 ± 27	146 ± 24	0.106
Killip Class I	780 (94%)	743 (94%)	37 (82%)	**0.005**
Medication after 7 days post-ACS				
Aspirin	758 (95%)	720 (95%)	38 (90%)	0.462
P2Y12 inhibitor	758 (95%)	719 (95%)	39 (93%)	0.651
Vitamin K antagonist	55 (7%)	48 (6%)	7 (17%)	**0.020**
Statin	768 (96%)	730 (97%)	38 (90%)	0.326
Beta-blocker	718 (90%)	680 (90%)	38 (90%)	1.00
ACE-i/ARB	662 (83%)	626 (83%)	36 (86%)	0.638

Continued

Table 1 ***Continued***

Variable	Overall ($n = 844$)	PE-free ($n = 799$)	PE ($n = 45$)	*P*-value
Biomarkers at first sample[b]				
hs-cTnT (pg/mL)	28 (11–387)	29 (10–394)	27 (10–282)	0.512
NT-proBNP (pmol/L)	44 (18–106)	43 (18–101)	146 (33–176)	**0.006**
hs-CRP (mg/L)	2.9 (1.1–8.4)	2.9 (1.1–8.4)	3.3 (1.1–11.6)	0.567
GDF-15 (pg/mL)	1302 (940–1922)	1288 (937–1879)	1788 (1330–3738)	**<0.001**

ACE-i, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CAD, coronary artery disease; GDF-15, growth differentiation factor 15; GRACE, Global Registry of Acute Coronary Events risk score; hs-CRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; mg/L, milligram per litre; NSTEMI, non-ST-elevation myocardial infarction; NT-proBNP, N-terminal-pro-B-type natriuretic peptide; PCI, percutaneous coronary intervention; pg/mL, picogram per millilitre; pmol/L, picomole per litre; STEMI, ST-elevation myocardial infarction; UAP, unstable angina pectoris.

Values are in mean ± standard deviation, median [inter-quartile range (IQR) 25th–75th percentile] or *n* (%). 1 pg/mL = 1 ng/L. 1 pmol/L = 8.46 pg/mL. Significant *P*-values (<0.05) are in bold.

[a]GRACE risk score was calculated using the model containing age, pulse, systolic blood pressure, initial serum creatinine, initial positive cardiac enzyme, ST-segment depression, prior myocardial infarction, prior congestive heart failure, and percutaneous coronary intervention. Categorization into three categories (low—intermediate—high) was done according to the cut-off values provided in the risk table at https://www.outcomes-unmassmed,org/grace/grace_risk_table.aspx, additionally taking into account whether there was ST-elevation at the index event. Median (IQR) GRACE risk scores per category were 77 (65–100) for low, 108 (101–115) for intermediate, and 136 (126–149) for high category.

[b]First sample was taken at a median (IQR) of 14 (2–31) days.

Data were analysed using R Statistical Software version 3.6.3 (Vienna, Austria) using the 'JMBayes' package for joint models.[20] Two-sided *P*-value <0.05 was considered statistically significant.

Results

Baseline characteristics

Majority of patients were men (78%). Overall median age was 63 (IQR 54–73) years, while patients with PE were older than those without (*Table 1*). The most common admission diagnosis overall was STEMI, but patients with PE more often had NSTEMI. Percutaneous coronary intervention at the index event was performed in 86% and to a similar degree in patients with PE and those without. Over a quarter of the patients had prior MI. Overall, cardiovascular disease burden was higher in patients with PE than in those without, and a lower proportion of PE patients was in Killip Class I. Moreover, guideline-recommended medication use was largely similar between both groups.

Serial measurements and longitudinal evolution of biomarkers

The mean level at first sample (during the acute phase) for each of the biomarkers is shown in *Table 1*. NT-proBNP and GDF-15 levels were significantly higher in patients who developed the PE than in those without ($P = 0.006$ and <0.001, respectively), while this was not the case for hs-cTnT and hs-CRP.

The number of repeated biomarker measurements available for analysis was on average 14.8 per patient. *Figure 1* shows the serial measurements for each of the biomarkers with corresponding level arranged according to the average biomarker level for each patient during follow-up. GDF-15 had the lowest overall variation suggesting a lower within-individual variation, whereas there was considerable spread in hs-CRP level within an individual patient. However, patients with the PE were mostly ranked higher (more towards the right side of the *x*-axis) with higher average biomarker levels following a larger spread pattern.

The average baseline GRACE score was higher in patients who experienced the PE than in those who did not (121 vs. 94, $P < 0.001$) and a larger proportion of them was in the highest risk category (51% vs. 19%, $P < 0.001$) (*Table 1*). Biomarker levels were significantly elevated in the highest compared with the lowest risk category with their longitudinal evolution visualized in *Figure 2*. The mean levels of the cardiac markers like hs-cTnT were dropping from an initially higher level during the acute phase, while for GDF-15 the mean levels were rather stable at the time of the index ACS admission. Moreover, biomarker levels were on average higher in patients who reached the PE compared with PE-free patients irrespective of the risk categories (*Table 2*).

Time-to-event biomarker pattern

The time-to-event pattern for each biomarker is shown in *Figure 3*. The average biomarker level was higher in patients with the PE compared with PE-free patients. There was no clear divergent pattern in the biomarker levels between the groups, except for GDF-15 which showed a significant, steady rise prior to the PE, while levels remained considerably stable in PE-free patients.

Association of serially measured biomarker level with prognosis

The geometric mean of the patient-specific means of the biomarkers were significantly higher in PE patients for all biomarkers except for hs-CRP (*Table 3*). Correlation between biomarkers was mostly present between hs-cTnT and NT-proBNP which was moderate (ρ=0.56, $P <$ 0.001). The univariable HRs for the PE of 1 SD increase in biomarker level (on the log-scale) were 2.09 (95% CI 1.43–2.90) for hs-cTnT, 2.04 (95% CI 1.35–2.08) for NT-proBNP, 1.72 (95% CI 1.04–2.79) for hs-CRP, and 2.24 (95% CI 1.62–3.04) for GDF-15 (*Table 3*). These associations remained significant for hs-cTnT (HR 1.61, 95% CI 1.02–2.44) and GDF-15 (HR 1.88, 95% CI 1.28–2.70) after adjustment for GRACE score, but not for NT-proBNP or hs-CRP, despite a trend towards increased risk. In the multi-marker models, hs-cTnT and GDF-15 remained strong independent prognostic factors, except when hs-cTnT was adjusted for GDF-15 ($P = 0.070$).

Discussion

In this analysis of clinically stabilized post-ACS patients undergoing high frequency blood sampling, elevated levels of longitudinally measured hs-cTnT and GDF-15 were associated with an increased risk for CV

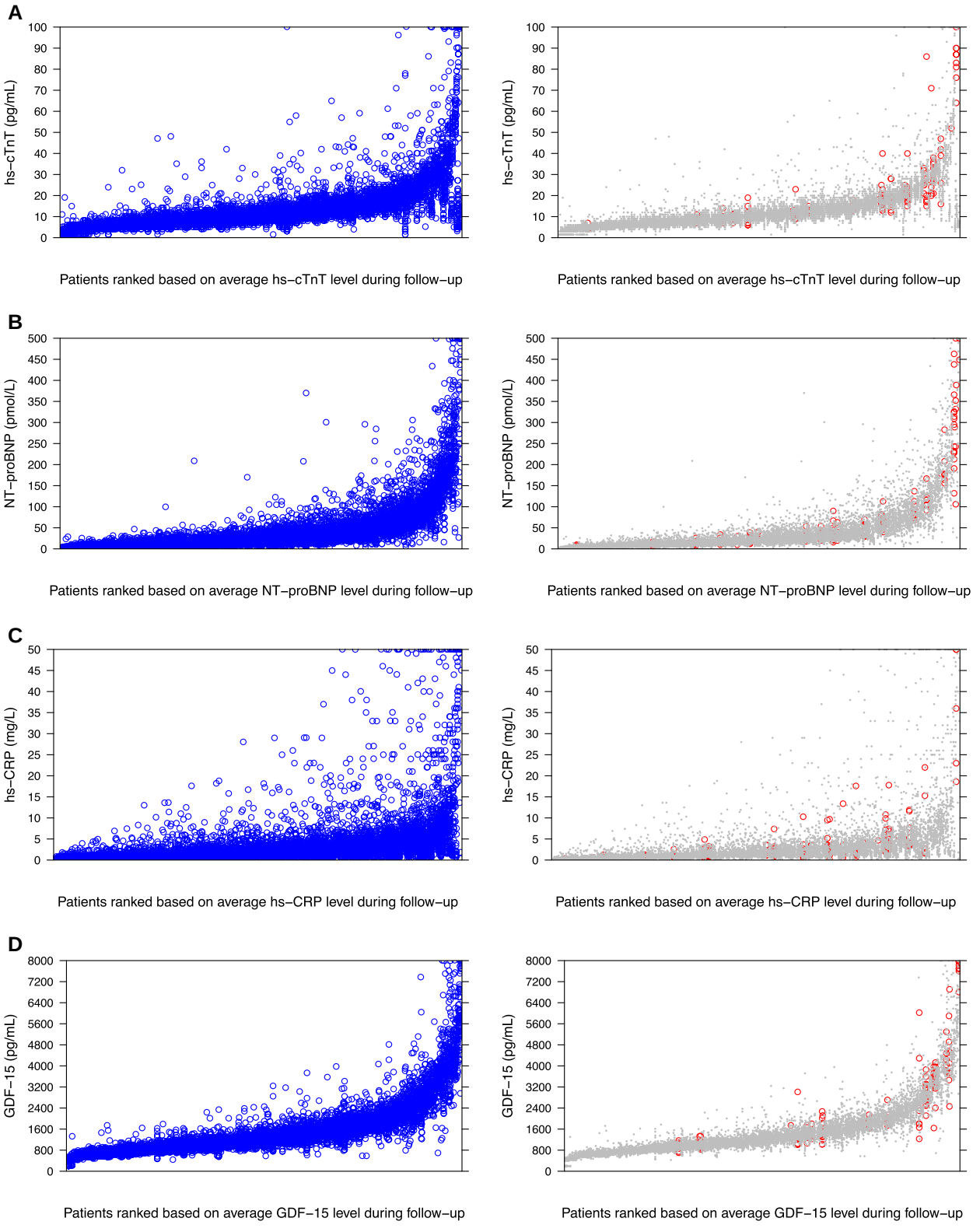


Figure 1 Biomarker level of each measurement per patient during follow-up for high-sensitivity cardiac troponin T *(A)*, N-terminal-pro-B-type natriuretic peptide *(B)*, high-sensitivity C-reactive protein *(C)*, growth differentiation factor 15 *(D)*. On the *x*-axis is the relative position of each individual patient ranked according to their average biomarker level across several repeated measurements during follow-up. The patient with the lowest average level is depicted on the far left of the *x*-axis, while the highest is depicted on the far right. Each of the measurements during follow-up belonging to an individual patient are shown vertically with the corresponding levels. All measurements are shown on the left side (blue circles) and the same measurements highlighted for patients who reached the primary endpoint (red circles) vs. those who did not (small grey dots) on the right side. mg/L, milligram per litre; pg/mL, picogram per millilitre; pmol/L, picomole per litre.

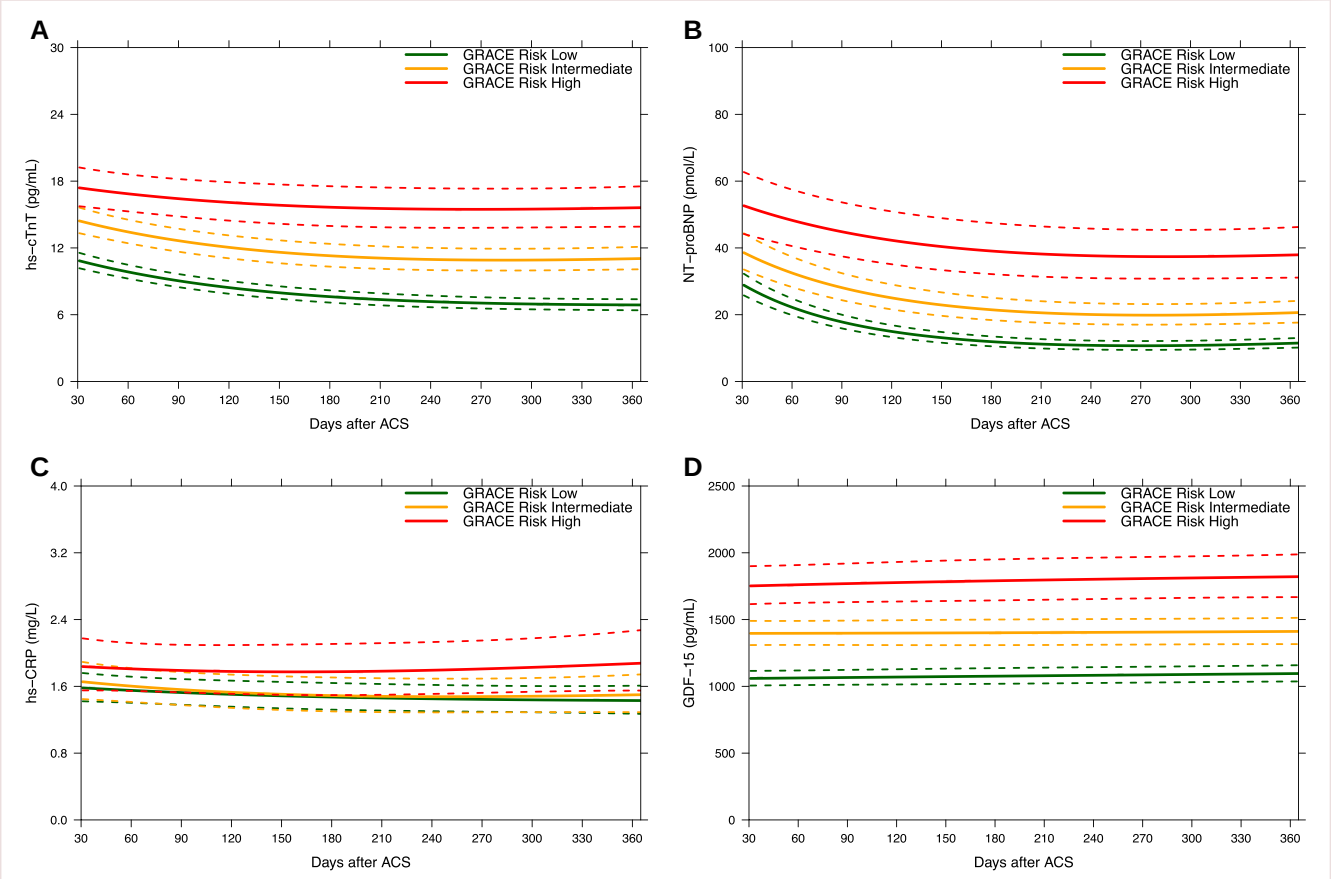


Figure 2 Longitudinal evolution of the average estimated biomarker levels (back-transformed to the original linear scale) of hs-cTnT *(A)*, N-terminal-pro-B-type natriuretic peptide *(B)*, high-sensitivity C-reactive protein *(C)*, growth differentiation factor 15 *(D)* according to linear mixed effects models of the biomarker on log2-scale as dependent variable adjusted for time-to-sample with GRACE risk score category interaction during follow-up post-acute coronary syndrome. Categories were defined according to the cut-off values provided in the online GRACE risk table, additionally taking into account whether there was ST-elevation at the index event. Solid green (low risk), orange (intermediate risk), and red (high risk) lines denote the mean values of each biomarker according to the appropriate category. Dashed lines denote the corresponding 95% confidence intervals. mg/L, milligram per litre; pg/mL, picogram per millilitre; pmol/L, picomole per litre.

mortality or recurrent non-fatal ACS during 1-year follow-up. There appeared to be no prominent divergence in average level of hs-cTnT, NT-proBNP, and hs-CRP prior to recurrent events and the latter two were not significantly associated with the PE. In contrast, GDF-15 steadily rose towards the endpoint (*Structured Graphical Abstract*). Hence, serial measurements seem most promising for GDF-15 to provide early insight into an upcoming event.

Studies on serial measurements of biomarkers in combination with GRACE score are limited. Moreover, the available studies differ in patient population, frequency of measurements, type of assay, and the definition of the endpoint along with the statistical methods used to describe the association. Therefore, it is challenging to properly compare our findings with previous literature. Nevertheless, our current findings on the complete BIOMArCS cohort utilizing 12 218 samples of 844 patients largely confirms our previous results from the case–cohort design of 187 patients[14] for hs-cTnT, NT-proBNP, and hs-CRP, further emphasizing the validity of the cost-efficient case–cohort design in the analysis of biomarker samples. However, contrary to our current analysis, Oemrawsingh *et al.* demonstrated a large and significant association of NT-proBNP with the endpoint even after adjustment. This is due to the use of an in-house custom-built enzyme-linked immunosorbent assay to measure NT-proBNP which explains the discrepancy in level and significance compared with the current automated Roche NT-proBNP assay. Although, their standardized values (Z-scores) that were ultimately used in longitudinal analysis, were still highly correlated (ρ=0.83, $P < 0.001$). Furthermore, the addition of GDF-15 assays to the risk assessment arsenal of patients with post-ACS showed promising results in preliminary analysis[15] and is confirmed based on our current study.

hs-cTnT reflects cardiomyocyte injury or necrosis, and based on expert consensus a combination of criteria is necessary to make the diagnosis of an acute MI. This includes at least a (preferably high-sensitivity) troponin value above the diagnostic threshold that is the 99th percentile of the upper reference limit, measured with an assay with a coefficient of variation < 10%.[1–3] Utilizing the Roche assay, this comes down to a threshold of ≥14 ng/L (14 pg/mL) based on a healthy reference population. In our study, patients who reached the study endpoint had an elevated hs-cTnT level with respect to this threshold compared with endpoint-free patients during the stable phase after index ACS admission. The guidelines also mention repeat sampling of hs-cTnT within a few hours after onset of symptoms based on the appropriate algorithm to determine if the myocardial injury is evolving or resolving, but nothing is stated about long-term repeated sampling during follow-

Table 2 Biomarker levels across Global Registry of Acute Coronary Events risk score categories and in patients with the primary endpoint vs. those without

Biomarker	GRACE category	Overall mean[a]	*P*-value	PE-free mean[b]	PE mean[b]
hs-cTnT (pg/mL)	Low	8.3	Reference	8.2	13
	Intermediate	12	**<0.001**	12	13
	High	16	**<0.001**	16	25
NT-proBNP (pmol/L)	Low	15	Reference	15	40
	Intermediate	25	**<0.001**	25	41
	High	42	**<0.001**	41	62
hs-CRP (mg/L)	Low	1.5	Reference	1.5	2.2
	Intermediate	1.5	0.765	1.5	2.1
	High	1.8	**0.047**	1.8	2.5
GDF-15 (pg/mL)	Low	1074	Reference	1071	1363
	Intermediate	1401	**<0.001**	1389	1753
	High	1777	**<0.001**	1711	2777

GDF-15, growth differentiation factor 15; GRACE, Global Registry of Acute Coronary Events risk score; hs-CRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; mg/L, milligram per litre; NT-proBNP, N-terminal-pro-B-type natriuretic peptide; pg/mL, picogram per millilitre; pmol/L, picomole per litre. Significant P-values (<0.05) are in bold.

[a]Average estimated biomarker level during follow-up based on LME model with biomarker on log2-scale as dependent variable and GRACE risk score category as independent variable. The estimate was back-transformed to the original (linear) scale and signifies the geometric mean biomarker level for the entire post-30 study population.

[b]Average estimated biomarker level during follow-up based on LME model with biomarker on log2-scale as dependent variable and GRACE risk score category as independent variable with PE status interaction. Geometric mean biomarker level for PE vs. PE-free patients.

up post-ACS for purposes of prognostication. A few studies have investigated the prognostic value of temporal changes in cardiac troponin based on two measurements.[21,22] Eggers *et al.*[22] showed that hs-cTnI has only a moderate prognostic value for CV events, while deFilippi *et al.*[21] demonstrated a large prognostic value of hs-cTnT for CV mortality even after adjustment for demographics and other biomarkers such as NT-proBNP and CRP. Important to note is that these studies were performed in community-dwelling older adults as opposed to our study which was performed in a post-ACS population with a more frequent sampling strategy. The current analysis again[23] confirms that measurement of hs-cTnT for prognostication (or patient reassurance) at outpatient visits after ACS admission should be considered and that two measurements would be sufficient during follow-up.

GDF-15 is an emerging biomarker that is part of the transforming growth factor-beta cytokine superfamily and plays a role in oxidative stress, inflammation, and cardiac remodelling.[24,25] Literature has suggested a pro-inflammatory role of GDF-15 in atherosclerosis released by activated macrophage cells and injured endothelial cells.[26] A 2019 meta-analysis of 43 547 ACS patients from 13 studies demonstrated a significant association between high baseline GDF-15 level and increased risk of mortality or recurrent MI.[27] The largest study in this meta-analysis was the study by Hagström *et al.*[28] which accounted for 39% of the participants. Most (older) studies in this meta-analysis had a higher quartile cut-off of >1800 ng/L (pg/mL), while the more recent ones including Hagström *et al.*[28] had > 2000 or even >2200. A recent individual patient meta-analysis of eight studies encompassing 53 486 patients showed that a single, baseline GDF-15 measurement >1800 ng/L was independently and prognostically associated with CV death and future MI in stabilized patients after recent ACS using Cox regression analysis.[29] Another study of GDF-15 showed incremental prognostic value and improved model fit with a cut-off of >1800 ng/L being independently associated with all-cause mortality and major adverse cardiac events (MACE).[30] In contrast, Walter *et al.*[31] demonstrated that the cut-off level of 1560 ng/L GDF-15 outperformed even hs-cTnT and GRACE for predicting 2-year mortality in acute MI patients. In our study, patients in the higher GRACE score category or who reached the endpoint also had on average systematically higher GDF-15 levels, even at the index ACS admission, possibly due to chronic low-grade inflammation of the coronary arteries.[32] Our study additionally demonstrated the strong prognostic value for outcomes in the context of serial measurements, as opposed to most previous studies that describe only a single timepoint analysis, and beyond traditional risk factors like GRACE score and other biomarkers. Moreover, while preliminary analysis of GDF-15 in a subset of BIOMARCS[15] did not demonstrate a sudden rise in level towards the endpoint, we now observe a steady rise in patients who experienced the study endpoint. This further denotes the utility of serial measurement of GDF-15 as a reflection of ongoing inflammatory processes leading to cardiac remodelling. Although hs-cTnT has a high positive predictive value for the diagnosis of ACS, levels of GDF-15 might be more informative on the long-term prognosis post-ACS. A recent serially measured multi-marker analysis of the Translational Initiative on Unique and Novel Strategies for Management of Patients With Heart Failure (TRIUMPH) data set regarding 496 patients with acute HF showed a significant association of GDF-15 with the endpoint of all-cause mortality and HF hospitalization and a prominent divergence in level nearing the event compared with event-free patients.[33] In our current analysis in a post-ACS population, GDF-15 was also a strong prognostic marker; however, this divergence was much less prominent. This is most likely due to theunderlying aetiology and inherent to the more acute trajectory of ACS.

NT-proBNP reflects volume overload and cardiac stress which is extensively used in heart failure (HF) but can also be elevated in ACS due to myocardial wall tension.[7] The guidelines state that measuring NT-proBNP can provide additional prognostic information.[2] In patients with non-ST-elevation ACS, an elevated BNP > 80 pg/mL (or 9.5 pmol/L) at presentation was associated with a higher risk of death and chronic HF with incremental information to cTnI.[11] Baseline NT-proBNP was an independent predictor for cardiac events in 215 patients with ACS.[10] This study also showed that patients with the combination of baseline hs-CRP level >3.5 mg/L and baseline NT-proBNP level > 500 pg/mL (or 59 pmol/L) had an 11-fold higher risk for cardiac events than patients with levels below these thresholds.

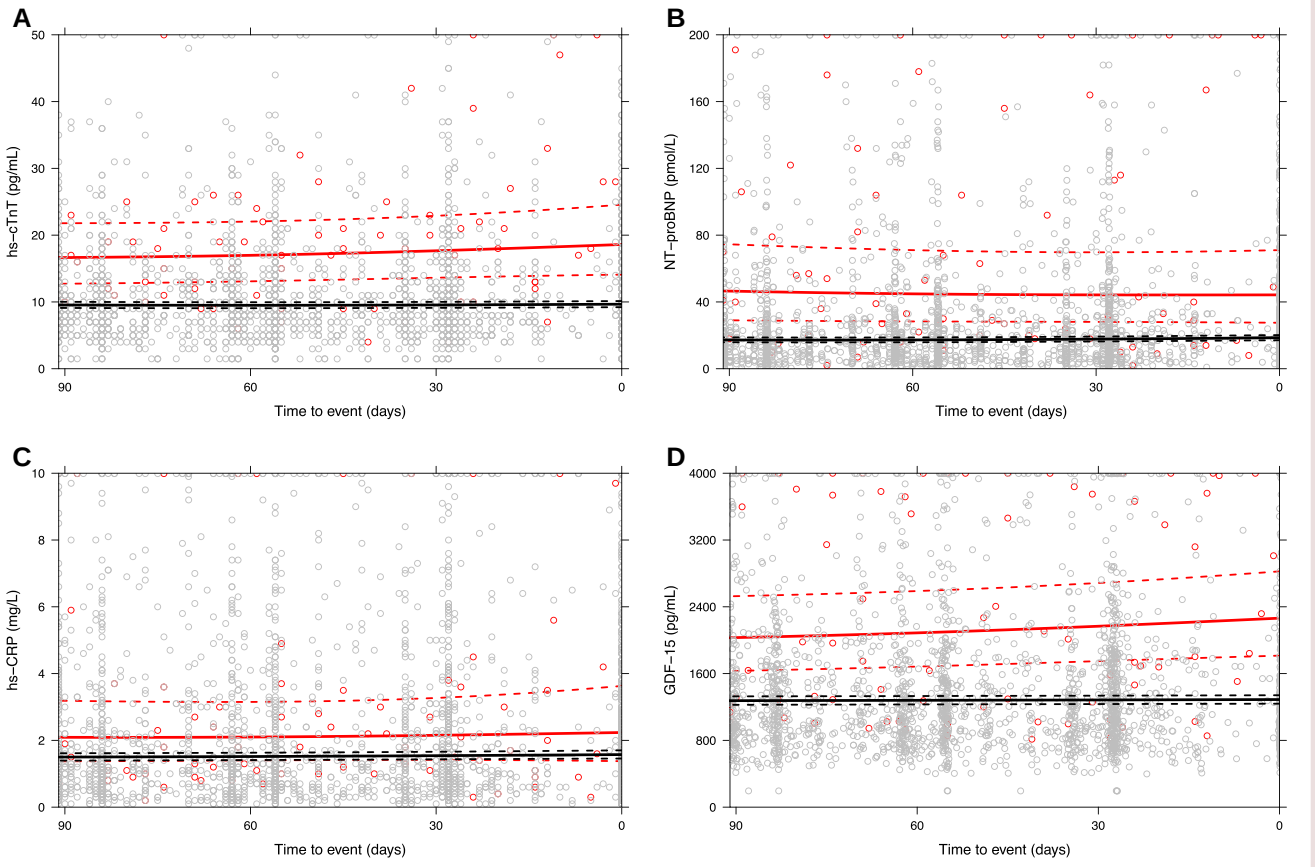


Figure 3 Longitudinal evolution of the average estimated biomarker level (back-transformed to the original linear scale) of hs-cTnT *(A)*, N-terminal-pro-B-type natriuretic peptide *(B)*, high-sensitivity C-reactive protein *(C)*, growth differentiation factor 15 *(D)* leading up to the event in patients who reached the primary endpoint or end-of-follow-up in patients who did not, according to linear mixed effects models of the biomarker on log2-scale as dependent variable adjusted for time-to-event with primary endpoint status interaction. The *x*-axis is reversed. Solid red (primary endpoint patients) and black (primary endpoint-free patients) lines denote the mean biomarker values, while the dashed lines denote the 95% confidence intervals. Dots represent the individual measurements. mg/L, milligram per litre; pg/mL, picogram per millilitre; pmol/L, picomole per litre.

Elevated baseline CRP, an acute phase protein which is elevated due to inflammation, provided independent prognostic information for composite of death, MI, or congestive HF in 450 patients with ACS.[12] In a recent retrospective cohort study of suspected ACS by Kaura *et al.*,[34] hs-CRP elevation up to 15 mg/L was associated with mortality independent of troponin. In sharp contrast, Riedel *et al.*[35] showed hs-CRP was not an independent predictor of future events in patients post-ACS receiving optimal medical therapy for secondary prevention.

In our current analysis, serially measured NT-proBNP and hs-CRP did not have independent prognostic value despite a trend towards increased risk. Levels were not elevated above the aforementioned thresholds during the stable phase, and for hs-CRP the geometric mean was also not significantly higher in patients who reached the study endpoint. This could be due to the use of serial measurements during the stable phase post-ACS and due to more extensive adjustment including GRACE score in the models we applied.

Considering the pathophysiology, a multi-marker approach might be beneficial. Sabatine *et al.*[12] described a near doubling of mortality risk for each additional biomarker that was elevated at presentation including cTnI, BNP, and CRP, each providing unique prognostic information. Our analysis showed that GDF-15 remained significant even after adjustment for other biomarkers and could possibly be used in conjunction with GRACE score and hs-cTnT. Notably, some attenuation of effect in the multi-marker model containing hs-cTnT and NT-proBNP was observed due to correlation.

Biomarker measurements along with clinical risk scores should be considered a step in the right direction towards a more personalized medicine. The clinical implications of these results especially regarding GDF-15 need to be further investigated. Elevated GDF-15 might indicate an increased risk for a recurrent ACS event and an overall increased atherosclerotic disease burden. Its systemic not to mention pleiotropic nature,[36] however, along with the lack of insight into the exact proatherogenic mechanism, makes it difficult to directly implement in clinical practice. Instead, a lower value could aid clinicians in ruling out an impending ACS event and reassure patients. In order to further elucidate this, a prospective study should be conducted investigating the association between serially measured GDF-15 (and other biomarkers) plus GRACE score with the extent and complexity of disease.[37,38]

Limitations

We analysed the prognostic value of several biomarkers, measured in subsequent blood samples, for recurrent ACS events in a large real-world cohort of post-ACS patients using advanced statistical

Table 3 Association of serially measured biomarker levels with the primary endpoint

Biomarker	Geometric mean[a] PE vs. PE-free	Model[b]	HR[c] (95% CI)	*P*-value
hs-cTnT	18 vs. 10 pg/mL	1—Unadjusted	2.09 (1.43–2.90)	**<0.001**
	***P* < 0.001**	2—GRACE risk	1.61 (1.02–2.44)	**0.045**
		3—NT-proBNP	1.77 (1.10–2.82)	**0.012**
		3—hs-CRP	2.11 (1.34–3.17)	**<0.001**
		3—GDF-15	1.52 (0.97–2.36)	0.070
NT-proBNP	50 vs. 20 pmol/L	1—Unadjusted	2.04 (1.35–2.08)	**<0.001**
	***P* < 0.001**	2—GRACE Risk	1.43 (0.88–2.30)	0.153
		3—hs-cTnT	1.55 (0.95–2.46)	0.082
		3—hs-CRP	1.93 (1.25–2.92)	**0.004**
		3—GDF-15	1.50 (0.93–2.28)	0.092
hs-CRP	2.2 vs. 1.5 mg/L	1—Unadjusted	1.72 (1.04–2.79)	**0.039**
	P = 0.069	2—GRACE Risk	1.50 (0.89–2.55)	0.130
		3—hs-cTnT	1.53 (0.91–2.41)	0.102
		3—NT-proBNP	1.51 (0.85–2.51)	0.136
		3—GDF-15	1.34 (0.85–2.21)	0.224
GDF-15	2103 vs. 1269 pg/mL	1—Unadjusted	2.24 (1.62–3.04)	**<0.001**
	***P* < 0.001**	2—GRACE risk	1.88 (1.28–2.70)	**0.001**
		3—hs-cTnT	1.82 (1.20–2.68)	**0.004**
		3—NT-proBNP	1.96 (1.34–2.68)	**<0.001**
		3—hs-CRP	2.14 (1.54–2.88)	**<0.001**

GDF-15, growth differentiation factor 15; GRACE, Global Registry of Acute Coronary Events risk score; hs-CRP, high-sensitivity C-reactive protein; HR, hazard ratio; hs-cTnT, high-sensitivity cardiac troponin T; mg/L, milligram per litre; NT-proBNP, N-terminal-pro-B-type natriuretic peptide; pg/mL, picogram per millilitre; pmol/L, picomole per litre. Significant P-values (<0.05) are in bold.

[a]Average estimated biomarker level for PE vs. PE-free patients, based on LME model with biomarker on the log2-scale as dependent variable with PE status as independent variable. The estimate was back-transformed to the original (linear) scale.

[b]Joint models with the biomarker of interest (Z-score) as dependent variable and adjusted according to the following models: Model 1—unadjusted with LME sub-model adjusted for clinical confounders: GRACE risk score (continuous), sex, diabetes mellitus, coronary artery bypass graft, valvular heart disease, stroke, and peripheral vascular disease; Model 2—adjusted for GRACE risk score (continuous) with the LME sub-model adjusted for the same clinical confounders; Model 3—(multi-marker) additionally adjusted for one other biomarker (Z-score) with the LME sub-model adjusted for the same clinical confounders.

[c]Hazard ratio represents the instantaneous risk of the composite PE (CV mortality and recurrent non-fatal ACS) associated with a 1-SD difference in biomarker level (on the log2-scale) at any given timepoint during follow-up.

methods. However, some limitations should be acknowledged. First, the number of study endpoints was limited, although this was largely offset by the highly frequent measurement of biomarkers allowing us to observe alterations right before a recurrent ACS event. Furthermore, we might not have accounted for all potentially relevant confounders (such as medication use) despite our extensive adjustments in the models.

Conclusion

Our analysis of the complete BIOMArCS cohort shows that longitudinal hs-cTnT and GDF-15 are strong independent prognostic factors of CV mortality and recurrent non-fatal ACS during 1-year follow-up in clinically stabilized patients post-ACS. GDF-15 showed a steady rise before an ACS event and therefore would be especially suitable for a frequent sampling strategy. Adding serial measurements of GDF-15 to the existing armamentarium may refine risk assessment.

Supplementary material

Supplementary material is available at *European Heart Journal: Acute Cardiovascular Care* online.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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