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ORIGINAL RESEARCH

Acute Coronary Syndrome Subphenotypes Based on Repeated Biomarker Measurements in Relation to Long-Term Mortality Risk

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BACKGROUND: We aimed to identify patients with subphenotypes of postacute coronary syndrome (ACS) using repeated measurements of high-sensitivity cardiac troponin T, N-terminal pro-B-type natriuretic peptide, high-sensitivity C-reactive protein, and growth differentiation factor 15 in the year after the index admission, and to investigate their association with long-term mortality risk.

METHODS AND RESULTS: BIOMArCS (BIOMarker Study to Identify the Acute Risk of a Coronary Syndrome) was an observational study of patients with ACS, who underwent high-frequency blood sampling for 1 year. Biomarkers were measured in a median of 16 repeated samples per individual. Cluster analysis was performed to identify biomarker-based subphenotypes in 723 patients without a repeat ACS in the first year. Patients with a repeat ACS (N=36) were considered a separate cluster. Differences in all-cause death were evaluated using accelerated failure time models (median follow-up, 9.1 years; 141 deaths). Three biomarker-based clusters were identified: cluster 1 showed low and stable biomarker concentrations, cluster 2 had elevated concentrations that subsequently decreased, and cluster 3 showed persistently elevated concentrations. The temporal biomarker patterns of patients in cluster 3 were similar to those with a repeat ACS during the first year. Clusters 1 and 2 had a similar and favorable long-term mortality risk. Cluster 3 had the highest mortality risk. The adjusted survival time ratio was 0.64 (95% CI, 0.44–0.93; *P*=0.018) compared with cluster 1, and 0.71 (95% CI, 0.39–1.32; *P*=0.281) compared with patients with a repeat ACS.

CONCLUSIONS: Patients with subphenotypes of post-ACS with different all-cause mortality risks during long-term follow-up can be identified on the basis of repeatedly measured cardiovascular biomarkers. Patients with persistently elevated biomarkers have the worst outcomes, regardless of whether they experienced a repeat ACS in the first year.

Key Words: acute coronary syndrome ■ cardiovascular biomarkers ■ death ■ phenotypes ■ repeated measurements

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CLINICAL PERSPECTIVE

What Is New?

- We report a comprehensive cluster analysis of repeatedly measured high-sensitivity troponin T, N-terminal pro-B-type natriuretic peptide, high-sensitivity C-reactive protein, and growth differentiation factor 15 in the year following an acute coronary syndrome admission, and investigated their association with long-term mortality risk.
- In the low-risk patients who had no repeat acute coronary syndrome in the first year, 3 subphenotypes were identified, displaying significant differences in their longitudinal biomarker profile: cluster 1 showed relatively low and stable biomarker concentrations, cluster 2 had elevated concentrations that subsequently decreased, and cluster 3 showed persistently elevated concentrations.
- The low-risk patients with persistently elevated biomarker concentrations (cluster 3) had adverse prognoses similar to those who experienced a repeat acute coronary syndrome in the first year. Patients with relatively low biomarker concentrations at 1 year follow-up (clusters 1 and 2) showed the best prognosis.

What Are the Clinical Implications?

- Our findings highlight the importance of biomarkers for long-term acute coronary syndorme prognostication and personalized risk assessment.
- Incorporating both favorable and unfavorable biomarker profiles into clinical practice could potentially foster targeted care to individuals who may require different levels of monitoring and intervention.

Nonstandard Abbreviations and Acronyms

BIOMArCS	Biomarker Study to Identify the Acute Risk of a Coronary Syndrome
GDF-15	growth differentiation factor 15
hs-cTnT	high-sensitivity cardiac troponin T

In recent decades, circulating biomarkers such as cardiac troponin and NT-proBNP (N-terminal pro-B-type natriuretic peptide) have been shown to provide objective and accurate prognostic information in patients with established coronary artery disease.[1–5] These cardiac biomarkers could facilitate tailoring of appropriate treatment in individual patients.[6–9] Obviously, while pursuing personalized management, appropriate longitudinal risk assessment and stratification of patients are crucial. In this context, clustering patients with established coronary artery disease based on dynamic changes in their cardiovascular biomarker profile may be a powerful tool to provide information on individual patients' risk and to improve prognostication. Nonetheless, studies clustering patients with cardiac disease based on their biomarker profile are scarce, mostly performed in patient cohorts with heart failure,[10–12] and generally limited to biomarker measurements at 1 point in time.[13,14]

BIOMArCS (the Biomarker Study to Identify the Acute Risk of a Coronary Syndrome) was specifically designed to study the longitudinal biomarker patterns in patients admitted for an acute coronary syndrome (ACS).[15,16] BIOMArCS enrolled 844 patients, who had a median of 17 repeated blood samples taken in the first year after the index event. Concentrations of hs-cTnT (high-sensitivity cardiac troponin T), NT-proBNP, hs-CRP (high-sensitivity C-reactive protein), and GDF-15 (growth differentiation factor 15) were measured in these samples, and a comprehensive unsupervised clustering analysis was conducted to identify biomarker-based phenotypes. The clinical characteristics of the resulting clusters were then studied, along with their relationship to all-cause death during long-term follow-up. Of particular interest were the dynamic biomarker patterns of low-risk patients who did not experience a repeat ACS in the first year. Patients who did experience a repeat ACS are considered high-risk patients[17] and are therefore, for comparison, studied as a separate cluster.

METHODS

Data Availability Statement

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

Study Population

BIOMArCS is an observational study that was conducted in 18 hospitals across the Netherlands from 2008 to 2015.[15,16] The study aimed to collect data on biomarker patterns in patients with ACS during a 1-year follow-up period. BIOMArCS enrolled patients aged ≥40 years who were admitted with an ACS and had at least 1 cardiovascular risk factor. Patients with severely impaired ejection fraction, severe chronic kidney disease, or a coexisting condition with a life expectancy of <1 year were excluded. Patients underwent regular blood sampling in the first year after the index ACS admission according to a strict schedule.[16] Ultimately, a total of 12 218 blood samples were obtained from 844 patients, with a median of 17 repeated blood samples

per patient. The patients received treatment according to prevailing guidelines and at the discretion of the treating physician. The current study included the 723 patients who were known to be alive 1 year after the index event, had at least 1 biomarker measurement available and did not experience a repeat ACS during the first year after the index event. Additionally, 36 patients who did experience a nonfatal repeat ACS were also included (see Figure S1 for details on patient selection).

BIOMArCS was conducted in compliance with the Declaration of Helsinki, and the study protocol was approved by the institutional review boards of the participating hospitals. All study subjects provided written informed consent. BIOMArCS is registered in The Netherlands Trial Register with the unique identifiers NTR1106 and NTR1698.

Sample Collection and Processing

To ensure the quality and reliability of the data, standardized protocols for sample collection, handling, and storage were applied. Blood samples were processed and stored at −80°C within a median of 82 minutes (25th to 75th percentile, 58–117) after collection.[15] The aliquots were then transported to the Department of Clinical Chemistry, Erasmus MC, Rotterdam, for long-term storage and batchwise central analysis. Storage duration was not correlated with the included biomarkers (Figure S2). To conduct the current investigation, specific assays were used to analyze the concentrations of hs-cTnT, NT-proBNP, GDF-15, and hs-CRP. The hs-cTnT, NT-proBNP, and GDF-15 concentrations were analyzed in a single batch using Elecsys quantitative sandwich electro-chemiluminescence immunoassays ECLIA on a cobas e 601 analyzer (Roche Diagnostics, Ltd., Rotkreuz, Switzerland). Meanwhile, hs-CRP concentrations were analyzed using a particle-enhanced immunoturbidimetric assay on a cobas c 501 analyzer (Roche Diagnostics). The study used 10 606 samples, with a median of 16 samples per patient, that were obtained in the period >30 days after the index ACS, during which patients were clinically stabilized. Samples collected within the 0- to 30-day window after a repeat ACS were excluded. Additional details on sample selection can be found in Figure S1.

Primary End Point

In the present study, the primary end point was all-cause death after the 1-year landmark point, which aligns with the end of the protocolized high-frequency blood sampling schedule of the BIOMArCS study. Vital status data were obtained from municipal registries, and follow-up on all-cause death was completed until January 2021 for 96.8% of patients. Patients with incomplete follow-up data were censored at the date they were last known to be alive.

Statistical Analysis

Biomarker measurements were studied as continuous variables, and values below the limit of blank (for hs-cTnT) or limit of detection (for NT-proBNP, hs-CRP, and GDF-15) were assigned a value at the limit of blank or limit of detection value divided by 2. A $\log_2$ transformation was applied to reduce skewness. Outliers in the data were defined as values with a distance >3 SDs from the mean and were excluded from analyses. Accordingly, 13 high-sensitivity troponin T measurements were excluded from analyses.

A cluster analysis was performed on the repeated biomarker data of the 723 patients who remained event free during the first year following the index ACS. The first step involved the application of linear mixed-effects modeling to estimate the concentrations of individual patients' biomarker concentrations at 30 days after the index ACS (ie, intercept coefficient), as well as the rate of change during the 30-day to 1-year post-ACS period (ie, slope coefficient). Specifically, both a random intercept and slope were modeled as random effects in the linear mixed-effects models (Data S1), using sampling time during follow-up as the time scale for slope. The intercept and slope coefficients were then subjected to 98% winsorization and standardization. Subsequently, K-means cluster analysis was performed on these model parameters, representing the complete trajectory of the biomarkers. K-means is an unsupervised clustering approach. The "optimal" number of clusters was determined using the NbClust R package,[18,19] which uses the majority rule of 30 separate indices. Cluster stability was verified by resampling using several schemes, including bootstrapping and noise replacement. Clusters with a Jaccard means >0.75 were considered valid and stable.[20,21] Following this methodology, the optimal number of biomarker-trajectory-based clusters in the patients who did not experience a repeat ACS turned out to be 3 (see Results section). Therefore, an unsupervised K-means cluster analysis was finally applied to partition these patients into 3 clusters.[22] The patients who experienced a repeat ACS in the first year after the index event were considered a separate fourth cluster.

The clinical characteristics of the patients in the identified clusters were compared using ANOVA, Kruskal-Wallis tests, χ^2 tests, and Fisher's exact tests, depending on variable distributions. To study all-cause death after the 1-year landmark, the Kaplan–Meier method was used, and differences between clusters were evaluated by Fleming–Harrington statistics with $\rho=1$, $\gamma=1$, which is appropriate for crossing survival curves.[23] Additionally, due to the observed violation of the proportional hazard assumption, accelerated failure time models with a Weibull distribution of the error terms were fitted. The survival time ratio produced by accelerated failure time models can be interpreted as the average survival time

since the 1-year landmark of 1 cluster relative to a reference cluster. Unadjusted and adjusted accelerated failure time models were explored, where adjustment factors included age, sex, diabetes, hypertension, hypercholesterolemia, smoking status, body mass index, history of coronary artery disease, history of stroke, and estimated glomerular filtration rate.

Single imputation was used to account for missing values of the clinical data that were included in the survival models using the *mice* package.[24] All statistical analyses were conducted using R software version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as a 2-sided *P* value of <0.05.

RESULTS

Clustering Based on Repeated Biomarker Measurements

Three distinct clusters were identified on the basis of the longitudinal biomarker profile of patients who did not experience a repeat ACS during the first year (see Figure 1 and Table 1). In cluster 1272 (38%) patients had relatively low and stable biomarker concentrations during the 30-day to 1-year post-ACS period. In cluster 2210 (29%) patients showed slightly elevated concentrations of hs-cTnT and NT-proBNP 30 days after the index ACS, which subsequently decreased. One year after the index ACS, patients in cluster 2 reached similar concentrations as patients in cluster 1. In cluster 3241 (33%) patients had elevated concentrations of all 4 biomarkers 30 days after the index ACS, which gradually decreased during the months thereafter, but remained elevated compared with clusters 1 and 2. Patients in cluster 3 had temporal biomarker patterns similar to those with a repeat ACS during the first year after the index event. Notably, the clustering algorithm did not identify a cluster of patients characterized by increasing concentrations of hs-cTnT, NT-proBNP, or hs-CRP during follow-up.

Cluster Membership in Relation to Clinical Characteristics

Table 2 and Figure S3 present an overview and summary of the baseline characteristics of the patients in the separate clusters. The patients in cluster 3 had the poorest clinical profile, with higher age, greater prevalence of cardiovascular risk factors (particularly diabetes and hypertension) and cardiovascular disease, higher GRACE risk scores, and lower estimated glomerular filtration rate than the patients in clusters 1 and 2. Overall, the clinical characteristics of patients in cluster 3 were similar to those of patients with a repeat ACS, although patients in cluster 3 were slightly older, had lower systolic blood pressure, and were more frequently diagnosed with ST-segment–elevation myocardial infarction. Patients in clusters 1 and 2 had a similar prevalence of cardiovascular risk factors but differed in their cardiovascular history and diagnosis at presentation. Specifically, patients in cluster 1 more often had a history of myocardial infarction and percutaneous coronary intervention and were more likely to present with non–ST-segment–elevation myocardial infarction or unstable angina pectoris compared with cluster 2.

Cluster Membership in Relation to Long-Term Survival

Over a median follow-up of 9.1 (25th to 75th percentile, 7.3–10.4) years after the 1-year landmark, 141 (18.6%) patients reached the primary end point of all-cause death. Patients in cluster 3 had a significantly higher cumulative incidence of all-cause death (cumulative incidence, 39% at 10.4 years) compared with those in cluster 1 (14%) and cluster 2 (9%), who had a similar prognosis (Figure 2). The long-term cumulative incidence of all-cause death of patients in cluster 3 was numerically higher than those who experienced a repeat ACS in the first year after the index event (26%). These findings were largely confirmed by the accelerated failure time models (Table 3). After correcting for multiple cardiovascular risk factors, the survival time ratio for cluster 3 was 0.64 (95% CI, 0.44–0.93; *P*=0.018) relative to cluster 1 and 0.71 (95% CI, 0.39–1.32; *P*=0.281) relative to the patients with a repeat ACS, indicating a 36% and 29% lower expected survival time, respectively. The adjusted survival times of patients in clusters 1 and 2 were similar, as expressed by an adjusted survival time ratio of 1.04 (95% CI, 0.64–1.67; *P*=0.882) for cluster 2 relative to cluster 1.

Clustering Based on Single 1-Year Biomarker Estimates

As described above, patients in clusters 1 and 2 showed similar biomarker concentrations 1 year after the index event, which were considerably lower than those of patients in cluster 3. No cluster was identified with increasing biomarker concentrations during 1-year follow-up. Therefore, we conducted patient clustering again using individual patients' linear mixed-effects – based estimates (see Methods section) of biomarker concentrations at 1 year as a single value for each biomarker (Table 1). As expected, this analysis resulted in 2 clusters of 427 and 296 patients with low and high estimated biomarker concentrations at 1 year, respectively. These low and high biomarker clusters largely, but not entirely, overlapped with the combined clusters 1 and 2, and cluster 3, respectively (see Figure 3). The estimated 1-year biomarker concentrations of patients in the high biomarker cluster were similar to those of patients with a repeat ACS, except for hs-CRP, which was

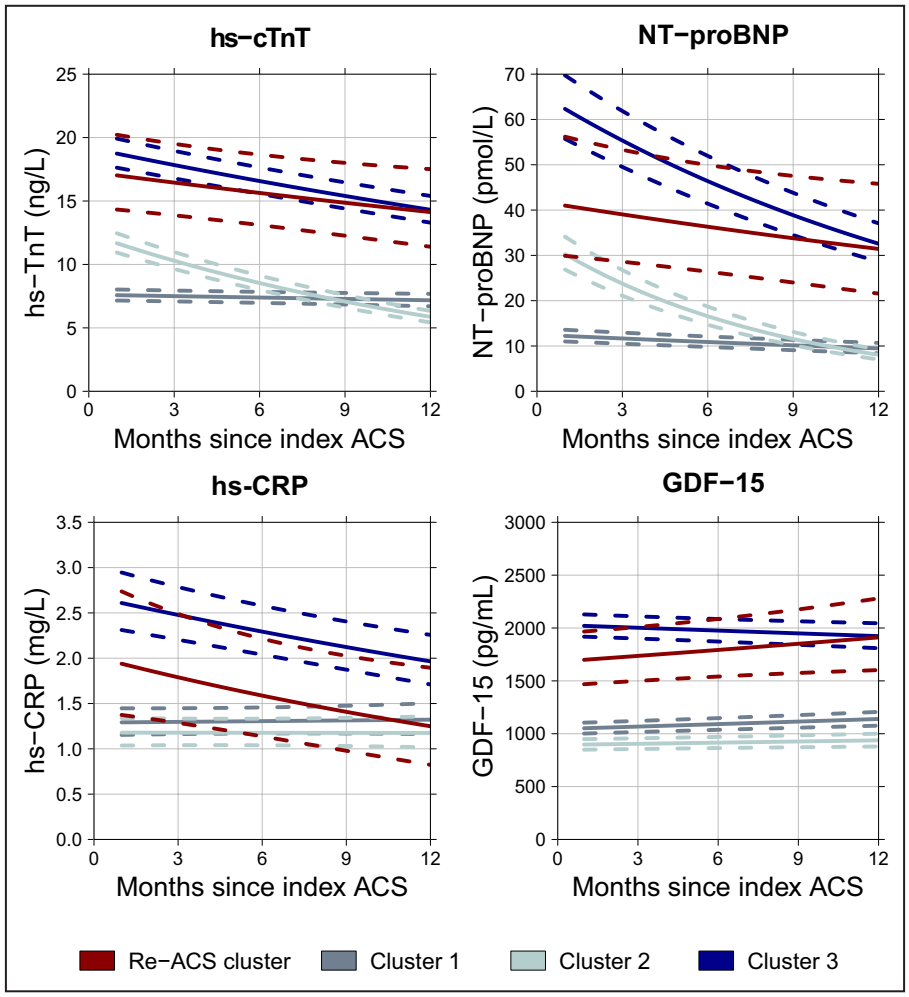

Figure 1. Clusters' biomarker trajectories during the first year following index ACS. Biomarker trajectories of repeated measurements-based clusters during the first year following the index ACS. The solid lines depict the average biomarker evolutions in each cluster separately. The dashed lines represent the 95% CI. Samples taken within 30 days after index ACS or repeat ACS are excluded from analyses. ACS indicates acute coronary syndrome; GDF-15, growth differentiation factor 15; hs-CRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

lower in the patients with repeat ACS (see Figure S4). Patients with high estimated biomarker concentrations had significantly worse death outcomes than patients with low biomarker concentrations. The cumulative incidence at 10.4 years was 37% versus 10% (P<0.001), respectively, and the adjusted survival time ratio for patients in the high biomarker cluster was 0.56 (95% CI, 0.39–0.82; P=0.003) (see Figure S5 and Table S1) relative to patients in the low biomarker cluster.

DISCUSSION

We conducted a comprehensive cluster analysis of repeatedly measured hs-cTnT, NT-proBNP, hs-CRP, and GDF-15 in the year following an ACS and studied their associations with clinical characteristics and long-term mortality risk after the 1-year landmark. Based on their temporal biomarker patterns, this study identified 3 patients with subphenotypes of low-risk post-ACS. These subphenotypes displayed significant differences in their longitudinal biomarker profile, clinical characteristics, and long-term mortality risk. Patients with persistently elevated biomarker concentrations had the worst prognosis, even compared with the apparent high-risk patients who experienced a repeat ACS in the first year.[17] Furthermore, no subphenotype characterized by increasing biomarker concentrations was identified.

Patients with high concentrations of hs-cTnT, NT-proBNP, hs-CRP, and GDF-15 at baseline and during

Table 1. Biomarker Patterns of Clusters of Patients With ACS Based on Repeated Biomarker Measurements During 1-Year Follow-Up

	Cluster 1	Cluster 2	Cluster 3		Re-ACS cluster	
	n=272	n=210	n=241	*P* value*	n=31	*P* value†
First measurement >30 d after the index ACS‡						
hs-cTnT, ng/L	8.0 (6.0–11.0)	12.0 (9.0–17.0)	19.0 (15.0–28.0)	<0.001§	19.0 (12.0–25.5)	0.321
NT-proBNP, pmol/L	16.0 (9.0–28.0)	47.0 (27.0–87.5)	76.0 (42.0–153.0)	<0.001§	44.0 (22.5–109.5)	0.018§
hs-CRP, mg/L	1.3 (0.7–2.5)	1.2 (0.6–2.5)	2.7 (1.1–5.7)	<0.001§	1.3 (0.6–4.4)	0.056
GDF-15, pg/mL	1047.5 (801.5–1325.2)	896.5 (699.5–1139.0)	1875.0 (1435.0–2736.0)	<0.001§	1383.0 (1061.5–2288.5)	0.029§
Estimated concentration at 30 d after the index ACS‡						
hs-cTnT, ng/L	8.1 (6.0–10.6)	11.1 (8.9–14.7)	18.1 (14.1–23.4)	<0.001§	19.0 (12.1–22.5)	0.427
NT-proBNP, pmol/L	13.4 (7.7–22.8)	30.6 (17.7–49.2)	58.2 (35.2–120.1)	<0.001§	38.2 (21.5–99.8)	0.069
hs-CRP, mg/L	1.4 (0.8–2.3)	1.2 (0.7–2.0)	2.4 (1.2–4.6)	<0.001§	1.8 (1.0–3.3)	0.106
GDF-15, pg/mL	1040 (830–1314)	901 (718–1158)	1872 (1443–2573)	<0.001§	1536 (1083–2375)	0.120
Estimated rate of change 30-d to 1-y post-ACS‖						
hs-cTnT, % change /mo	−1.3 (1.9)	−5.0 (2.3)	−2.5 (2.2)	<0.001§	−2.2 (1.8)	0.452
NT-proBNP, % change/mo	−3.4 (3.3)	−9.8 (3.5)	−6.0 (4.1)	<0.001§	−4.4 (4.7)	0.048§
hs-CRP, % change/ mo	−0.4 (2.9)	−0.5 (3.0)	−1.5 (3.1)	<0.001§	−1.6 (2.7)	0.781
GDF-15, % change/ mo	0.6 (1.4)	0.3 (1.4)	−0.1 (1.4)	<0.001§	0.6 (1.7)	0.010§
Estimated concentration at 1 y after the index ACS‡						
hs-cTnT, ng/L	6.8 (5.2–9.4)	6.6 (4.8–8.7)	13.3 (10.0–19.2)	<0.001§	13.9 (8.9–17.6)	0.702
NT-proBNP, pmol/L	8.8 (5.1–15.5)	9.8 (5.3–18.1)	31.9 (16.1–60.0)	<0.001§	32.9 (9.8–58.0)	0.427
hs-CRP, mg/L	1.4 (0.7–2.2)	1.1 (0.7–2.0)	2.1 (1.0–3.8)	<0.001§	1.7 (0.8–2.9)	0.099
GDF-15, pg/mL	1061 (851–1470)	945 (719–1179)	1806 (1392–2680)	<0.001§	1668 (1149–2574)	0.383

ACS indicates acute coronary syndrome; GDF-15, growth differentiation factor 15; hs-CRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

*Cluster 1 vs cluster 2 vs cluster 3.

†Cluster 3 vs re-ACS cluster.

‡Biomarker concentrations are presented as median (25th to 75th percentile).

§$P<0.05$ is considered statistically significant.

‖Rate of change in biomarker concentration is presented as mean (SD).

follow-up showed the highest risk of death. This finding is consistent with our previous report on BIOMArCS,[5] as well as other studies that have linked elevated concentrations of these biomarkers to an increased risk of adverse cardiovascular outcomes in patients with ACS.[3,5,25–27] Patients who experienced a repeat ACS during the first year had similar biomarker patterns as patients in cluster 3. Hence, based on their longitudinal biomarker pattern during the first year after the index event, patients in cluster 3 had an increased risk of a repeat ACS but ultimately remained event free. Nowadays, it is still a considerable challenge to predict the exact timing of the event in high-risk individuals, despite the increase in knowledge on risk factors, including biomarkers, for the occurrence of an ACS. The uncertainty surrounding the timing of an ACS occurrence is disconcerting but may be inherent to the syndrome. Nevertheless, patients with a repeat ACS had better long-term prognosis than patients in cluster 3, which could be explained by the fact that patients with a repeat ACS were younger at the time of the index event and may have received intensified management following the repeat event.

Although the temporal biomarker pattern of patients in cluster 2 differed from that of cluster 1, both clusters had similar long-term prognoses. Specifically, hs-cTnT and NT-proBNP concentrations were elevated at baseline in cluster 2 compared with cluster 1 and showed a steeper negative slope in the months thereafter until they reached similar concentrations at 1 year. This prolonged elevation of biomarker concentrations observed in cluster 2 might reflect a greater degree of myocardial damage and dysfunction after the index ACS, which is consistent with the fact that cluster 2 composed mostly of patients with ST-segment–elevation myocardial infarction.[28,29] Nonetheless, it is important to highlight that these findings also suggest that once the 1-year landmark is reached, the patient's prognosis primarily hinges on the continued elevation of biomarkers, while the trajectory leading up to that point appears to be of lesser significance. Consequently, our results also

Table 2. Characteristics of Clusters of Patients With ACS Based on Repeated Biomarker Measurements During 1-Year Follow-Up

	Cluster 1	Cluster 2	Cluster 3		Re-ACS cluster	
	n=272	n=210	n=241	*P* value*	n=36	*P* value†
Age, y, mean (SD)	59.7 (9.6)	56.8 (9.0)	68.5 (9.6)	<0.001‡	64.1 (12.4)	0.013‡
Male sex, n (%)	214 (78.7)	172 (81.9)	183 (75.9)	0.303	27 (75.0)	1.000
White race, n (%)	256 (95.5)	200 (95.7)	223 (93.7)	0.547	36 (100.0)	0.248
Cardiovascular risk factors						
Diabetes, n (%)	51 (18.8)	28 (13.3)	86 (35.7)	<0.001‡	12 (33.3)	0.930
Hypertension, n (%)	147 (54.0)	99 (47.1)	157 (65.1)	<0.001‡	18 (50.0)	0.116
Hypercholesterolemia, n (%)	137 (50.4)	99 (47.1)	122 (50.6)	0.715	17 (47.2)	0.840
Current smoker, n (%)	114 (41.9)	104 (49.5)	74 (30.7)	<0.001‡	15 (41.7)	0.262
Body mass index, kg/m^2, mean (SD)	28.0 (4.5)	27.0 (3.6)	28.2 (5.2)	0.017‡	27.2 (3.6)	0.267
History of cardiovascular disease						
Myocardial infarction, n (%)	85 (31.2)	32 (15.2)	77 (32.1)	<0.001‡	11 (30.6)	1.000
CABG, n (%)	26 (9.6)	5 (2.4)	37 (15.4)	<0.001‡	9 (25.0)	0.226
PCI, n (%)	97 (35.7)	34 (16.3)	64 (26.6)	<0.001‡	11 (30.6)	0.762
Stroke, n (%)	15 (5.5)	12 (5.7)	34 (14.1)	0.001‡	8 (22.2)	0.309
Peripheral arterial disease, n (%)	15 (5.5)	7 (3.3)	32 (13.3)	<0.001‡	8 (22.2)	0.242
Chronic heart failure, n (%)	4 (1.5)	2 (1.0)	8 (3.3)	0.149	2 (5.6)	0.848
Presentation on admission						
GRACE risk score at discharge, mean (SD)	94.7 (27.2)	83.7 (23.0)	112.8 (30.8)	<0.001‡	119.9 (42.4)	0.227
Systolic blood pressure, mmHg, mean (SD)	139.3 (27.3)	137.4 (24.7)	140.5 (28.0)	0.479	150.8 (22.9)	0.037‡
eGFR, mL/min per 1.73m^2, mean (SD)	83.4 (19.7)	85.3 (19.8)	72.0 (22.6)	<0.001‡	75.1 (17.2)	0.438
Diagnosis						
STEMI, n (%)	110 (40.4)	144 (68.6)	125 (51.9)	<0.001‡	11 (30.6)	0.027‡
NSTEMI, n (%)	122 (44.9)	55 (26.2)	91 (37.8)	<0.001‡	20 (55.6)	0.064
UAP, n (%)	40 (14.7)	11 (5.2)	25 (10.4)	0.004‡	5 (13.9)	0.730
CAG performed, n (%)	267 (98.2)	206 (98.1)	223 (92.5)	0.001‡	33 (91.7)	1.000
PCI performed, n (%)	222 (84.1)	187 (93.5)	189 (85.5)	0.007‡	28 (87.5)	0.977
Discharge medication						
Aspirin, n (%)	267 (98.2)	207 (98.6)	229 (95.0)	0.036‡	32 (91.4)	0.633
P2Y12 inhibitor, n (%)	265 (97.4)	204 (97.1)	227 (94.2)	0.113	33 (91.7)	0.829
Vitamin K antagonist, n (%)	9 (3.3)	8 (3.8)	23 (9.5)	0.004‡	6 (16.7)	0.312
Statin, n (%)	264 (97.1)	208 (99.0)	229 (95.0)	0.045‡	32 (91.4)	0.633
Beta blocker, n (%)	244 (89.7)	193 (91.9)	215 (89.2)	0.598	31 (88.6)	1.000
ACE inhibitor or ARB, n (%)	217 (79.8)	178 (84.8)	204 (84.6)	0.235	32 (91.4)	0.419

Proportion missing values was <0.3% except for ethnicity (1.1%), body mass index (0.5%), eGFR (2.0%), and PCI performed (5.5%). ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CABG, coronary artery bypass graft; CAG, coronary angiogram; eGFR, estimate glomerular filtration rate; GRACE, Global Registry of Acute Coronary Events; NSTEMI, non–ST-segment–elevation myocardial infarction; PCI, percutaneous coronary intervention; STEMI, ST-segment–elevation myocardial infarction; and UAP, unstable angina pectoris.

*Cluster 1 vs cluster 2 vs cluster 3.

†Cluster 3 vs cluster re-ACS.

‡$P<0.05$ is considered statistically significant.

indicate that highly frequent blood sampling during the first year of follow-up may not provide additional incremental prognostic information above a single measurement in clinical practice. Whether this also holds for cardiovascular adverse clinical outcomes requires further research.

In a previous report, we showed that BIOMArCS patients with a repeat ACS were charaterized by

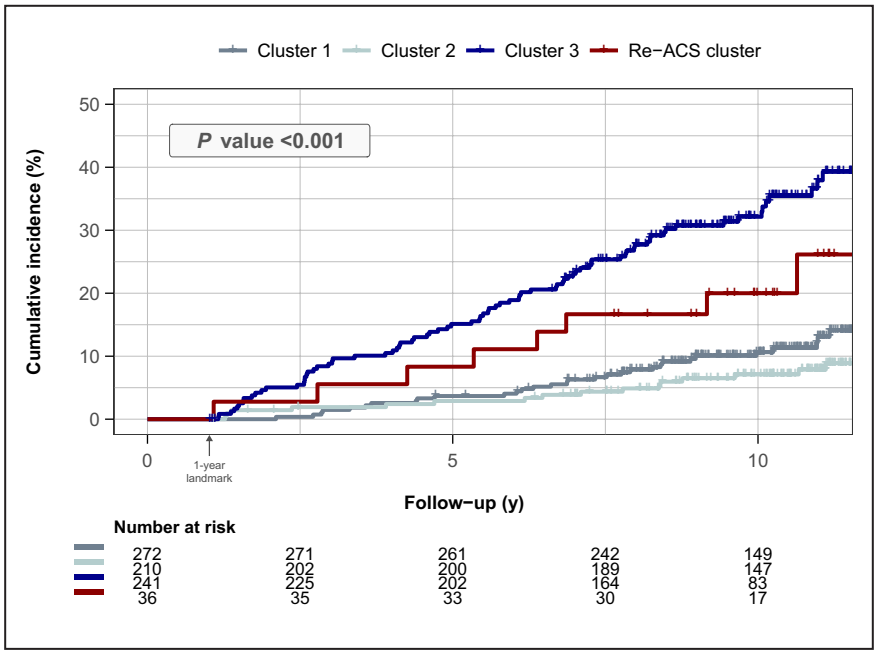

Figure 2. Association between clusters based on repeated biomarker measurements and long-term all-cause death.
ACS indicates acute coronary syndrome.

systematically elevated biomarker concentrations during follow-up and not by increasing concentrations.[5] The current analysis also did not reveal a cluster of patients with increasing biomarker concentrations. In fact, only 3% of our patients showed a relative hs-cTnT increase of >25% during the 30-day to 1-year study period (based on the linear mixed-effects model; data not shown). The findings in BIOMArCS contrast with previous reports by White et al,[30] Everett et al,[31] Cavender et al,[32] and Patel et al,[33] who reported a meaningful increase in cardiac troponin in up to 23% of patients with coronary artery disease and increasing concentrations were associated with a worse prognosis in all 4 studies. Indeed, increasing cardiac troponin concentrations are disconcerting, as these are potentially related to ongoing myocyte necrosis, chronic processes related to left ventricular hypertrophy,[34,35] increasing renal dysfunction,[36] or poor diabetes control.[37] We cannot entirely explain the absence of this phenotype in BIOMArCS, and the significant disparity in prevalence cannot be attributed solely to variations in sample size. Possibly, individuals choosing to participate in a highly frequent blood sampling study, such as BIOMArCS, might represent a selected subset of healthier patients with ACS

Table 3. Associations Between Clusters of Patients With ACS Based on Repeated Biomarker Measurements and All-Cause Death

	Model 1: unadjusted		Model 2: adjusted for age		Model 3: adjusted for age and sex		Model 4: adjusted for age, sex, and CVD risk factors†	
	STR (95% CI)*	*P* value	STR (95% CI)*	*P* value	STR (95% CI)*	*P* value	STR (95% CI)*	*P* value
Cluster 1	Ref		Ref		Ref		Ref	
Cluster 2	1.31 (0.80–2.13)	0.282	1.11 (0.69–1.77)	0.668	1.10 (0.69–1.75)	0.696	1.04 (0.64–1.67)	0.882
Cluster 3	0.34 (0.23–0.50)	<0.001‡	0.60 (0.42–0.87)	0.007‡	0.60 (0.42–0.87)	0.007‡	0.64 (0.44–0.93)	0.018‡
Re-ACS cluster	0.50 (0.26–0.95)	0.034‡	0.71 (0.38–1.32)	0.279	0.72 (0.39–1.34)	0.307	0.80 (0.43–1.50)	0.495
Re-ACS cluster	Ref		Ref		Ref		Ref	
Cluster 3	0.67 (0.35–1.27)	0.219	0.81 (0.43–1.51)	0.504	0.80 (0.43–1.49)	0.474	0.71 (0.39–1.32)	0.281

ACS indicates acute coronary syndrome; CVD, cardiovascular disease; and STR, survival time ratio.
*STR produced by accelerated failure time models can be interpreted as the average survival time since the 1-year landmark of a cluster relative to a reference cluster.
†Diabetes, hypertension, hypercholesterolemia, smoking status, body mass index, diagnosis of index ACS, history of coronary artery disease, history of stroke, and estimated glomerular filtration rate.
‡$P<0.05$ is considered statistically significant.

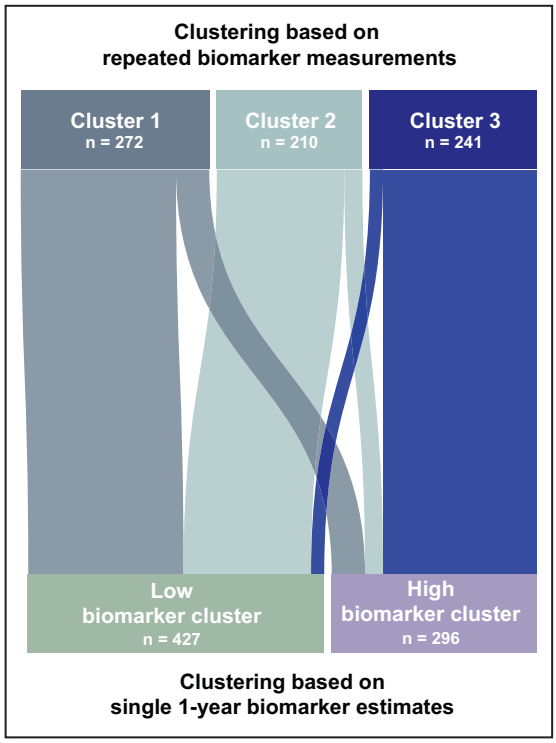

Figure 3. Reclassification diagram.
The low biomarker cluster based on single 1-year biomarker estimates coincides mostly with the combined clusters 1 and 2 on the basis of repeated biomarker measurements. The high biomarker cluster based on single 1-year biomarker coincides mostly with cluster 3 on the basis of repeated biomarker measurements.

with relatively high treatment compliance that could positively influence biomarker concentrations. On the other hand, we consider the regression to the mean phenomenon a serious limitation of previous studies that investigated change based on 2 measurements only.[30–33]

The current study shows that subphenotypes with differences in prognosis could be identified on the basis of repeated measurements of a set of biomarkers previously associated with cardiovascular disease. These findings are indicative of the potential utility of cardiovascular biomarkers in future clinical applications, such as personalized prognostication and patient management. For instance, patients with persistent high cardiovascular biomarker concentrations following ACS may benefit from more intensive monitoring, lower thresholds for diagnostic testing, and earlier intervention. In contrast, patients with a favorable biomarker profile can be reassured and potentially be followed-up less intensively. While clinical guidelines acknowledge the additional prognostic value of individual and combined biomarkers, their routine use for prognostication and therapeutic decision making has not yet been recommended.[1,38,39] Ideally, cardiovascular biomarkers would be used as a continuous, longitudinal measure in a dynamic cardiovascular risk prediction tool that incorporates sex, age, and clinical features. Further research is needed to investigate the incremental value of more extensive biomarker assays, such as multimarker assays currently used in heart failure populations,[10] and to determine whether these carry the potential to enhance ACS prognostication and personalized risk assessment. Furthermore, since noncardiovascular deaths will most likely not be preventable with cardiovascular secondary prevention strategies, further research is warranted to investigate whether repeated biomarker measurements hold promise in distinguishing patients with differing long-term prognoses for cardiovascular adverse events.

Our study has several strengths. First, this is the first study that investigated subphenotypes of patients following ACS on the basis of the temporal evolution of multiple cardiovascular biomarkers that were repeatedly measured at high frequency. Most biomarker studies classifying patients following ACS have explored 1 cross-sectional measurement only, or studied changes using just 2 repeated measurements several months apart.[30–33,40] Our data in combination with the statistical techniques (ie, based on linear mixed-effect models) overcomes the regression to the mean phenomenon, a well-known limitation of observational studies investigating change.[41] Second, complete follow-up for >10 years enabled linking the ACS subphenotypes to long-term prognosis.

Some limitations also need to be acknowledged. First, the BIOMArCS study comprises a predominantly White population, and caution should be exercised when generalizing our findings to other ethnic groups. Second, during the inclusion period of BIOMArCS, there was a notable increase in the adaptation of more potent $P2Y_{12}$ inhibitors, namely, ticagrelor and prasugrel, which may affect patients' risk of adverse outcomes. Nonetheless, type of $P2Y_{12}$ inhibitor was not associated with the risk of a repeat ACS within the first year after the index ACS or long-term mortality risk during follow-up in the current study (potent $P2Y_{12}$ inhibitors versus clopidogrel, P=0.351 and P=0.674, respectively). Third, while all-cause death is an important clinical outcome, it may not entirely represent the most clinically relevant outcomes from a therapeutic perspective, given that noncardiovascular deaths will most likely not be preventable with cardiovascular secondary prevention strategies. Fourth, clinical and imaging indices of cardiac function, atherosclerotic plaque characteristics, and the degree of coronary artery disease were not routinely measured in this patient population, making it difficult to comment on the combined prognostic value of these indices with cardiovascular biomarkers. Finally, 13 of the 10 606 hs-cTnT measurements were considered outliers and were excluded from analysis. Nonetheless, subclinical events,

such as a subclinical myocardial infarction, cannot be excluded.

In conclusion, the current study demonstrates the ability to identify patients with subphenotypes of post-ACS based on repeated measurements of established cardiovascular biomarkers. These phenotypes show significant differences in all-cause death during long-term follow-up, with patients exhibiting persistently elevated biomarker concentrations having the worst outcome, regardless of whether they experienced a repeat ACS during the first year. Our findings highlight the valuable role of blood biomarkers, assessed through single or repeated measurements, in long-term ACS prognostication and personalized risk assessment.

APPENDIX

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Supplemental Material

Data S1
Table S1
Figures S1–S5

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