Journal of the American Heart Association

ORIGINAL RESEARCH

Novel Biomarkers, ST-Elevation Resolution, and Clinical Outcomes Following Primary Percutaneous Coronary Intervention

Jay S. Shavadia, MD; Christopher B. Granger, MD; Wendimagegn Alemayehu, PhD; Cynthia M. Westerhout, PhD; Thomas J. Povsic, MD; Sean Van Diepen, MD; Christopher Defilippi, MD; Paul W. Armstrong, MD

BACKGROUND: Despite restoration of epicardial flow following primary percutaneous coronary intervention (PPCI), microvascular reperfusion as reflected by ST-elevation resolution (ST-ER) resolution remains variable and its pathophysiology remains unclear.

METHODS AND RESULTS: Using principal component analyses, we explored associations between 91 serum biomarkers drawn before PPCI clustered into 14 pathobiologic processes (including NT-proBNP [N-terminal pro-B-type natriuretic peptide] as an independent cluster), and (1) ST-ER resolution ≥50% versus <50%; and (2) 90-day composite of death, shock, and heart failure. Network analyses were performed to understand interbiomarker relationships between the ST-ER groups. Among the 1160 patients studied, 861 (74%) had ST-ER ≥50% at a median 40 (interquartile range, 23–70) minutes following PPCI, yet both groups had comparable post-PPCI TIMI (Thrombolysis in Myocardial Infarction) grade 3 flow (86.6% versus 82.9%; *P*=0.25). ST-ER ≥50% was associated with significantly lower pre-PPCI concentrations of platelet activation cluster (particularly P-selectin, von Willebrand factor, and platelet-derived growth factor A) and NT-proBNP, including after risk adjustment. Across both ST-ER groups, strong interbiomarker relationships were noted between pathways indicative of myocardial stretch, platelet activation, and inflammation, whereas with ST-ER <50% correlations between iron homeostasis and inflammation were observed. Of all 14 biomarker clusters, only NT-proBNP was significantly associated with the 90-day clinical composite.

CONCLUSIONS: Suboptimal ST-ER is common despite achieving post-PPCI TIMI grade 3 flow. The cluster of platelet activation proteins and NT-proBNP were strongly correlated with suboptimal ST-ER and NT-proBNP was independently associated with 90-day outcomes. This analysis provides insights into the pathophysiology of microvascular reperfusion in ST-segment–elevation myocardial infarction and suggests novel pre-PPCI risk targets potentially amenable to enhancing tissue-level reperfusion following PPCI.

Key Words: biomarkers ■ microvascular reperfusion ■ primary percutaneous coronary intervention ■ ST-elevation resolution ■ ST-segment–elevation myocardial infarction

ST-segment elevation represents the electrocardiographic hallmark of acute epicardial coronary occlusion. In patients presenting with ST-segment–elevation myocardial infarction (STEMI), early postreperfusion ST-segment–elevation resolution (ST-ER) provides a useful global correlate of coronary reperfusion (both epicardial and microvascular), given that restoration of TIMI (Thrombolysis in Myocardial Infarction) grade 3 coronary flow does not adequately reflect optimal microvascular reperfusion.[1,2] As such, both mechanical and pharmacologic strategies have been explored in the peri–primary percutaneous coronary intervention (PPCI) setting to facilitate optimal post-PPCI ST-ER; unfortunately, these strategies have largely been unsuccessful.[3–5] This underscores our limited ability to discriminate potentially diverse

Correspondence to: Jay S. Shavadia, MD, 103 College Drive, Saskatoon, Saskatchewan, Canada S7N 0W8. E-mail: jss372@usask.ca

Supplementary Materials for this article are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.016033

For Sources of Funding and Disclosures, see page 9.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- Higher expression levels of platelet activation proteins and NT-proBNP (N-terminal pro-B-type natriuretic peptide) appear to associate with suboptimal ST-segment–elevation resolution despite successful primary percutaneous coronary intervention.

What Are the Clinical Implications?

- These results identify novel and potential pathophysiologic risk targets aimed at enhancing microcirculatory reperfusion in ST-segment–elevation myocardial infarction.

Nonstandard Abbreviations and Acronyms

APEX AMI	Assessment of Pexelizumab in Acute Myocardial Infarction
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PDGF-A	platelet derived growth factor subunit A
PPCI	primary percutaneous coronary intervention
STEMI	ST-segment–elevation myocardial infarction
ST-ER	ST-segment–elevation resolution
vWF	von Willebrand factor

subgroups of STEMI patients with differing pathophysiology related to suboptimal ST-ER, and in whom more informed and targeted therapeutic interventions would be welcome.

With advances in cardiovascular proteomics, our mechanistic understandings of various complex cardiovascular pathways have improved.[6–9] By leveraging the application of these high-throughput discovery approaches in patients presenting with STEMI, the objectives of this study were to explore the associations between a spectrum of cardiovascular proteins and post PPCI ST-ER, as well as clinical outcomes (90-day composite of death, cardiogenic shock, and congestive heart failure).

METHODS

The authors declare that all supporting data are available within the article (and its online supplementary files).

Study Design and Patient Population

We studied patients with STEMI enrolled in the APEX AMI (Assessment of Pexelizumab in Acute Myocardial Infarction) study and in whom serum had been collected before PPCI. APEX AMI was a randomized, double-blind multinational clinical trial of an inhibitor of the terminal component of complement pexelizumab or placebo in patients with STEMI presenting within 6 hours of symptom onset treated with PPCI.[10] Details of the APEX AMI trial design, its primary outcomes, and several preplanned substudies (including electrocardiographic, imaging, and biomarker analyses) have been published previously.[10–12] The institutional review board of each participating hospital approved the protocol, and patients were required to provide written informed consent.

For the current study, our analytic sample comprised an enriched sample of 1160 patients in whom pre- and post-PPCI ECGs were available to ascertain ST-elevation measurements. The derivation of this convenience sample is described in Figure S1. Baseline characteristics of patients included and not included in the construction of the analytic cohort are described in Table S1. These 2 patient populations were largely comparable except that those included in this analytic cohort were more likely to be younger and have had prior percutaneous coronary intervention (Table S1).

Biomarker Analysis and Construction of Clusters

In the APEX AMI biomarker substudy, blood samples were collected at baseline and at 24 hours; samples were allowed to clot, centrifuged, and the resulting serum frozen immediately to –20°C and subsequently to –70°C as soon as possible.[12] Serum samples had then been shipped on dry ice and centrally stored at the Duke Center for Human Genetics (Durham, NC). For this study, the frozen baseline serum samples were thawed and 100-μL samples transported to Olink Proteomics for analyses. Ninety-one of 92 known or exploratory cardiovascular-related proteins (C-C motif chemokine 22 failed quality control and was therefore excluded from the analysis) were successfully measured simultaneously across 96 serum samples using a high-throughput, multiplex Cardiovascular III immunoassay panel using a protein extension assay technique. The Cardiovascular III immunoassay panel (as opposed to the other select panels such as inflammatory or cardiometabolic) was selected, as it encompasses proteins across the spectrum of several cardiovascular processes aligned with the exploratory aim of this analysis. Detailed descriptions of the proximal extension assay technique have been previously published.[13,14] The sensitivity, range, coefficients of variation, and calibration for each of the biomarkers

analyzed are available at www.olink.com/products/cvd-iii-panel. Biomarker levels were expressed using normalized protein expression units on a log2-scale; normalized protein expression values are developed from the cycle threshold values and presented in arbitrary units where high protein values correspond to a high protein concentration and do not represent absolute quantification. The distribution of each biomarker was then visually assessed with a histogram and box plot, all of which were found to be nonskewed.

Outcome Measurement

The primary outcome for this study was ∑ST-ER ≥50% versus <50% following PPCI, based on the previously well-established prognostic value of this ECG reperfusion metric.[15] Additional outcomes include a 90-day clinical composite of all-cause death, cardiogenic shock, and congestive heart failure. All ECGs (baseline and ≈30 minutes after PPCI) in the APEX AMI trial were evaluated centrally at the ECG core laboratories of the Canadian VIGOUR Center and Duke Clinical Research Institute blinded to treatment assignments, procedural, or clinical outcomes as has been detailed previously.[15] The clinical outcomes of cardiogenic shock and congestive heart failure were also centrally adjudicated by an events committee blinded to treatment assignment.

Statistical Analysis

Baseline characteristics are reported for patients with ST-ER ≥50% compared with <50%. Categorical variables are reported as percentages, and continuous variables reported as medians with 25th and 75th percentiles; chi-square and Wilcoxon rank-sum tests were used for the comparison of categorical and continuous variables, respectively.

Proteins measured for this analysis were categorized by their biological role into 14 clusters, as has been established within www.olink.com/products/cvd-iii-panel (including NT-proBNP [N-terminal pro-B-type natriuretic peptide] within its own cluster) and summarized in Figure S2. Of note and as highlighted in Figure S2, each protein could be classified into >1 cluster based on its biophysiologic roles. Principal component analysis was performed to determine a summary score of each biomarker within the cluster. Principal components are weighted linear combinations of the variables where the weights are chosen to account for the largest amount of variation within the data. The first principal component was then retained as the representative summary variable, as this explained the largest variability among the biomarkers belonging to the cluster. We evaluated the univariable association between each of the 14 biomarker classes and the primary outcome. For this analysis, the difference in the mean aggregate level for all biomarkers within each class were compared between patients with ST-ER ≥50% and <50% using the t test, and *P* values were adjusted for false discovery rate; this method is a well-validated adjustment for multiple testing in controlling for a low proportion of false positives.[16] For biomarker clusters with significant univariable associations with ST-ER, internal validation was performed through bootstrap resampling of 1000 samples from the derivation data set.[17]

To account for differences in patient mix and for selection bias associated with the construction of our analytic cohort, a multivariable logistic regression model was used to evaluate the adjusted association of each biomarker class and the binary ST-ER outcome. Adjustment covariates in the logistic regression model were adapted from previously used APEX AMI risk model[18] and included age, sex, chronic obstructive pulmonary disease, smoking status, diabetes mellitus, stroke, systolic blood pressure, diastolic blood pressure, time to hospital arrival from randomization, baseline white blood cell count, baseline serum creatinine, baseline heart rate, Killip class, and myocardial infarct location. Analysis for study treatment assignment (ie, pexelizumab versus placebo) was not performed given the neutral primary results. Adjusted odds ratios with corresponding 95% CI and *P* values are reported for each biomarker cluster.

Network analyses were then performed to analyze the cumulative associations between the individual biomarkers and (1) ST-ER ≥50% and (2) ST-ER <50%. For this analysis, 11 biomarkers were included as most significantly associated with ST-ER after adjustment for multiple corrections (Table S2). The graphical depiction of this network analysis illustrates 2 key findings: (1) whether the biomarkers are correlated (ie, how closely the levels of 2 biomarkers rise and fall)—the strength of each biomarker-biomarker correlation is represented by the thickness of the line connecting the biomarkers; and (2) how biomarkers correlate as a cluster (ie, how closely the levels of biomarkers correlate with multiple neighboring biomarker)—this is graphically represented by the size of the circle (or hub). For each biomarker, a summary statistic (*clustering coefficient*) is determined that is proportional to the number of (neighboring) biomarkers pairs that are also correlated to each other. No formal inference techniques were performed for this network analysis, and these results are therefore purely descriptive.

Finally, we examined the association between the biomarker clusters and the 90-day clinical composite of death, cardiogenic shock and congestive heart failure. For this analysis, Cox proportional hazard models were used, and variables included within the adjusted analysis include those previously used within

Table 1. Baseline Characteristics for Patients Across the 2 ST-ER Groups

	ST-ER (n=1160)		
	<50% (n=299)	≥50% (n=861)	*P* Value
Baseline demographics			
Age, y	60 (52–70)	59 (51–69)	0.5993
Sex (F)	67 (22.4)	195 (22.6)	0.9318
Body mass index	28 (25–30)	27 (25–31)	0.7939
History of hypertension	161 (53.8)	424 (49.2)	0.1704
History of diabetes mellitus	60 (20.1)	113 (13.1)	0.0037
History of hyperlipidemia	146 (48.8)	428 (49.7)	0.7931
History of coronary artery disease	67 (22.4)	131 (15.2)	0.0044
Prior myocardial infarction	52 (17.4)	102 (11.8)	0.0149
Prior percutaneous coronary intervention	53 (17.7)	96 (11.1)	0.0034
Prior coronary artery bypass graft	6 (2.0)	28 (3.3)	0.2714
History of congestive heart failure	10 (3.3)	19 (2.2)	0.2776
History of atrial fibrillation	7 (2.3)	43 (5.0)	0.0516
History of stroke	9 (3.0)	26 (3.0)	0.9933
History of chronic obstructive pulmonary disease	11 (3.7)	39 (4.5)	0.5326
Current smoker	117 (39.1)	396 (46.0)	0.0395
History of peripheral vascular disease	16 (5.4)	30 (3.5)	0.1541
Presenting characteristics			
Heart rate, bpm	76 (65–88)	74 (63–86)	0.0365
Systolic blood pressure, mmHg	134 (120–153)	131 (115–148)	0.0318
Diastolic blood pressure, mmHg	80 (70, 90)	80 (67, 90)	0.102
Killip class >1	44 (14.7)	84 (9.8)	0.0184
Inferior myocardial infarction	61 (20.4)	422 (49.0)	<0.0001
Hospital arrival from randomization, h	0.6 (0.3–0.9)	0.6 (0.3–0.9)	0.6187
Symptom onset to percutaneous coronary intervention, h	3.4 (2.6–4.5)	3.3 (2.4–4.3)	0.0667
Door to device, h	1.1 (0.8–1.6)	1.1 (0.8–1.6)	0.612
Sum ST-segment deviation at baseline, mm	13 (10–17)	17 (12–23)	<0.0001
Worst lead ST-segment elevation, mm	3 (2–4)	3 (2–5)	<0.0001
Baseline creatinine, μmol/L	90 (80–106)	88 (80–106)	0.5717
Baseline troponin I	56 (23–132)	50 (19–113)	0.0998
Baseline creatine kinase, IU/L	150 (89–314)	137 (91–260)	0.2468
Baseline creatine kinase myocardial band, μg/L	5 (2–15)	5 (2–13)	0.3019
Left anterior descending culprit artery	212 (71.1)	372 (43.3)	<0.0001
PPCI	292 (97.7)	844 (98.0)	0.7012
Post–percutaneous coronary intervention TIMI grade 3 flow*	131/158 (82.9)	382/441 (86.6)	0.2538
Antithrombotic agent use			
Glycoprotein IIb/IIIa inhibitor	247 (82.6)	719 (83.5)	0.7197
Thienopyridine in-hospital	279 (93.3)	823 (95.6)	0.1199
Thienopyridine at discharge	265 (88.6)	786 (91.3)	0.1744
90-d outcomes			
Death/Shock/ Congestive heart failure	46 (15.4)	74 (8.6)	0.0009
Death	15 (5.0)	18 (2.1)	0.0085
Cardiac death	11 (3.7)	14 (1.6)	0.0604
Sudden cardiac death	4 (1.3)	7 (0.8)	
Nonsudden cardiac death	7 (2.3)	7 (0.8)	
Noncardiac death	3 (1.0)	3 (0.3)	0.1807
Unknown cause of death	1 (0.3)	1 (0.3)	···
Re–myocardial infarction	10 (3.3)	24 (2.8)	0.6227
Shock	14 (4.7)	22 (2.6)	0.0676
Congestive heart failure	26 (8.7)	47 (5.5)	0.0471
Bleeding requiring transfusion	27 (9.0)	47 (5.5)	0.0295

Data presented as median (25th–75th percentiles) or percentage. PPCI indicates primary percutaneous coronary intervention; ST-ER, ST-segment–elevation resolution; and TIMI, Thrombolysis in Myocardial Infarction.

*Among patients who were included in a core-lab angiographic substudy.

the APEX AMI risk models; adjusted hazard ratios with corresponding 95% CIs and *P* values are reported for each biomarker cluster. All statistical analyses were performed using SAS (version 9.4; SAS Institute, Cary, NC) and for analysis and visualization of the network between biomarkers, Cytoscape 3.7 (http://www.cytoscape.org) was used.

Sensitivity Analyses

Following PPCI, other validated ECG metrics (such as ST-ER ≥70% versus <70% to 30% versus <30%) and angiographic indices (such as TIMI myocardial perfusion grade 3 versus 0/1/2) of reperfusion have been validated as prognostic correlates.[1,19,20] In this study, 601 patients were included in a core-lab

angiographic substudy where adjudicated analysis of TIMI myocardial perfusion grade was available following PPCI. Therefore, to supplement the primary outcomes, and further validate its robustness, we also explored the relationships between the biomarker clusters and (1) ST-ER ≥70% versus <70% to 30% versus <30%; and (2) TIMI myocardial perfusion grade 3 versus 0/1/2.

RESULTS

Cohort Characteristics

Among the 1160 patients included in this analysis, 861 (74%) had ST-ER ≥50% at a median 40 minutes (interquartile range, 23–70 minutes) after PPCI. In both ST-ER groups, the proportion of patients with post-PPCI TIMI grade 3 flow was comparable (ST-segment elevation <50% versus ≥50%, 82.9% versus 86.6%, *P*=0.25, Table 1). Patients with ST-ER <50% were more likely to present with noninferior infarcts, lower magnitude of ST-segment deviation, and have diabetes mellitus and a history of coronary artery disease. Notably, there were no differences in use of glycoprotein IIb/IIIa inhibitors or thienopyridine agents between ST-ER groups (Table 1). Patients with ST-ER <50% compared with ≥50% had a higher 90-day unadjusted risk of death/cardiogenic shock/congestive heart failure, with a trend towards an increased risk of cardiac death and a significantly higher risk of bleeding requiring transfusion (Table 1).

Associations Between Biomarker Clusters and ST-ER

Post-PPCI ST-ER ≥50% compared with <50% was associated with significantly lower mean pre-PPCI expression levels of NT-proBNP and the cluster of platelet activation proteins (Figure 1A and Table S3), including after adjustment for false discovery rate (NT-proNP, *P*=0.0007; platelet activation cluster, *P*=0.0399). Following multivariable adjustment, similar statistically significant associations between lower expression levels of the 2 biomarker clusters and higher odds of more successful ST-ER were evident (Figure 1B and Table S3). Of the 5 proteins within the platelet activation cluster (von Willebrand factor [vWF], P-selectin, PDGF-A [platelet-derived growth factor subunit A], collagen alpha-1(I) chain, and tyrosine-protein kinase receptor UFO), lower mean expression levels of P-selectin, PDGF-A, and vWF, but not collagen alpha-1(I) chain and tyrosine-protein kinase receptor UFO, remained statistically significant in their association with ≥50% ST-ER (Figure 2). After internal validation using bootstrapping, the significant relationship between the platelet activation cluster and ST-ER (*P*=0.007) was maintained.

Biomarker Correlation With Network Analysis

Figure 3 illustrates the network analyses between biomarkers and patients with ST-ER ≥50% and ST-ER <50% (Figure 3A and 3B, respectively). In patients with ST-ER ≥50%, the strongest biomarker correlations were observed between tumor necrosis factor (TNF) receptor superfamily 14,TNF receptor 1, and the junctional adhesion molecule-A. NT-proBNP, PDGF-A, vWF, and the secretoglobin family 3A member 2 had the largest hubs, suggestive of their strong clustering around their neighboring markers. NT-proBNP clustered strongly around the inflammatory molecules ST-2 protein, TNF receptor 1, osteopontin, transferrin receptor, and junctional adhesion molecule-A; PDGF-A around the thrombosis and inflammatory markers P-selectin, vWF, TNF receptor 1; vWF similarly around the thrombosis and inflammatory markers PDGF-A, P-selectin, TNF receptor 1, TNF receptor superfamily 14; and secretoglobin family 3A member 2 around the inflammatory and cell adhesion molecules junctional adhesion molecule-A, osteopontin, TNF receptor 1, and P-selectin (Figure 3A). In patients with ST-ER <50%, a similar pattern but a fewer number of biomarker correlations were evident; unique to this group, however, was the emergence of transferrin receptor as an important hub, and this regulator of iron transport was closely related to markers of inflammation such as members of the TNF family, ST2, and osteopontin (Figure 3B).

Association Between Biomarker Clusters and Clinical Outcomes

The relationships between biomarkers clusters and the 90-day clinical composite are presented in Table 2. While higher expression levels of NT-proBNP and the cluster of proteins involving inflammation, mitogen-activated protein kinase cascade, and proteolysis appear to univariably associate with the 90-day clinical composite, after multivariable adjustment, only NT-proBNP remained significantly associated with 90-day death, cardiogenic shock, and congestive heart failure.

Sensitivity Analysis

Aligned with the results of the primary outcome, lower mean expression levels of NT-proBNP and proteins within the platelet activation appeared to associate with complete (≥70%) versus partial (30% to <70%) or no ST-ER (<30%) (Table S4). Additionally, only NT-proBNP (of the 14 biomarker clusters) was significantly associated with angiographic myocardial reperfusion, with lower mean pre-PPCI NT-proBNP expression levels correlating with TIMI myocardial perfusion grade 3 following PPCI (Table S5).

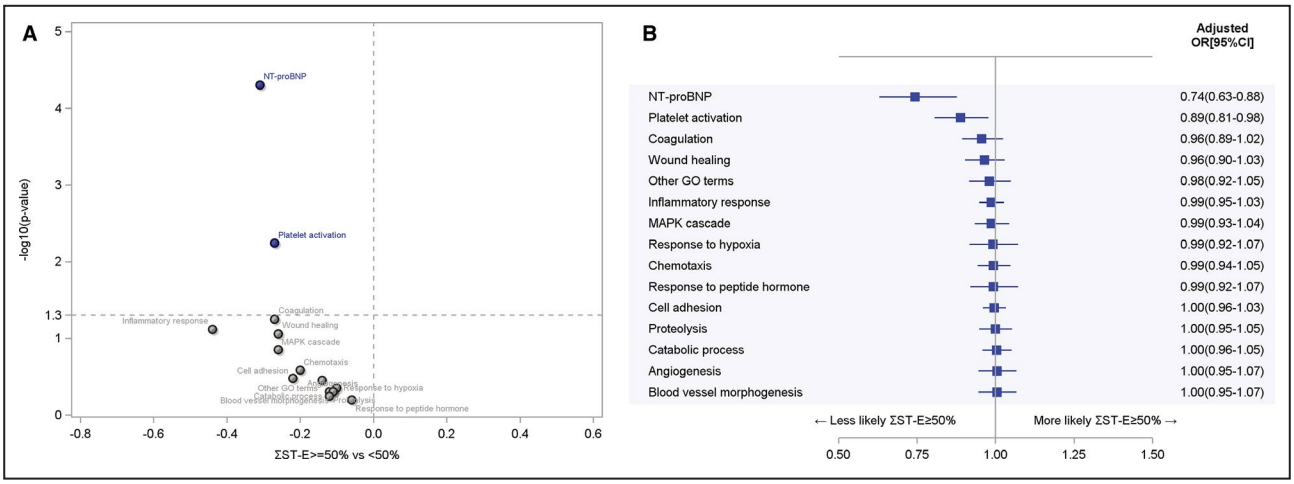

Figure 1. Associations between biomarker clusters and ST-segment–elevation resolution (univariable; A), and after multivariable adjustment (B).
A, Horizontal line at 1.3 represents the false discovery rate level of significance. GO indicates gene ontology; MAPK, mitogen activated protein kinase; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

DISCUSSION

The primary objective of this exploratory analysis was to evaluate associations and biomarker correlations with ST-ER following PPCI. Three novel findings emerged: (1) higher pre-PPCI mean expression levels of NT-proBNP and platelet activation proteins were significantly associated with less successful post PPCI ST-ER; (2) markers indicative of myocyte stretch, platelet activation, and inflammation have strong interactions across both ST-ER groups. However, the relationship between iron-transport and inflammation appears more prominent in patients with ST-ER <50% and (3) higher pre-PCI NT-proBNP concentrations was the only biomarker associated with a significantly higher 90-day risk of death, cardiogenic shock, and congestive heart failure.

Biomarker Clusters, ST-ER, and Clinical Outcomes

Following reperfusion in STEMI, ST-ER has been established as an important surrogate of tissue-level reperfusion (independent of epicardial TIMI grade 3 flow) and is well aligned with clinical outcomes.[1,15,21] Whereas the pathophysiology of optimal ST-ER is

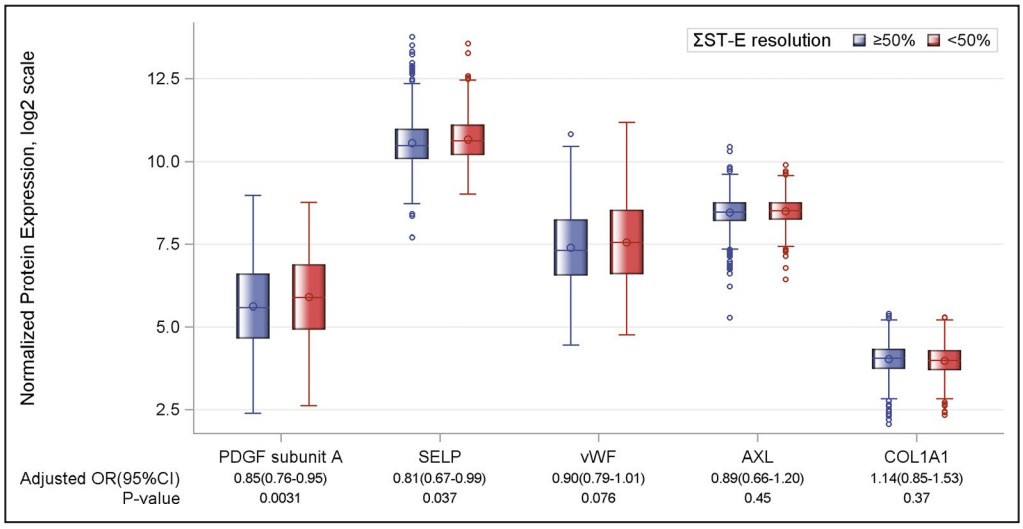

Figure 2. Associations between proteins within platelet activation cluster and ST-segment–elevation resolution (ST-ER).
AXL indicates tyrosine-protein kinase receptor UFO; COL1A1, collagen alpha-1(I) chain); PDGF, platelet-derived growth factor; SELP, P-selectin; and vWF, von Willebrand factor.

unclear, pathways involving myocardial stretch,[22,23] platelet activation,[24,25] and inflammation[26,27] have all been proposed as potential participants. Our results extend these findings by demonstrating lower pre-PPCI expression levels of NT-proBNP and 3 predominant platelet activation proteins (P-selectin, PDGF-A, and vWF) are significantly associated with more successful ST-ER following PPCI. P-selectin is known to play an integral role as an adhesion molecule facilitating endothelial-platelet-leukocyte aggregation; its inhibition after arterial wall injury in animal models resulted in a significant reduction in the adhesion between platelets and neutrophils, which suggests a role in the thrombo-inflammatory pathway following plaque rupture.[28,29] Higher P-selectin levels are therefore more likely to associate with more stable and greater coronary thrombus volume. Not surprisingly, in P-selectin knockout mice models, improved microcirculatory reperfusion and smaller infarct sizes following ischemia-reperfusion have been described.[30] Interestingly, the translations of these animal model findings have been similarly noted in a phase II trial of 544 non-STEMI patients undergoing PCI, in which preprocedural antibody-mediated (inclacumab) inhibition of P-selectin compared with placebo similarly reduced myocardial damage following percutaneous coronary intervention,[31] especially when administered within 3 hours before percutaneous coronary intervention.[32] With our results suggesting a nearly 20% greater odds of more successful ST-ER with lower pre–percutaneous coronary intervention P-selectin concentrations, the inhibition of this protein in select patients with STEMI offers a novel potential therapeutic target for enhancing microcirculatory flow in STEMI.

In acute myocardial infarction, platelet-derived growth factor has roles in regulating myocyte healing (via alpha and beta receptors) with angiogenesis and fibrous tissue deposition.[33,34] Platelet-derived growth factor is typically stored and released by activated platelets and endothelial cells, and higher PDGF-A levels have been recognized to correlate with increased collagen deposition and fibrosis.[33] Our results build on these findings, suggesting that patients with higher baseline circulating PDGF-A levels may be predisposed to a profibrotic tissue-level response, and hence more likely to have suboptimal ST-ER following PPCI.

Following atheromatous plaque rupture, vWF plays an important role in platelet adhesion and aggregation,[24,35] and its detection in fibrinolysis-resistant human coronary thrombi suggests a causal role in both thrombus stability and its growth/propagation.[36] Abundant literature also exists on its prognostic importance across the entire spectrum of patients with atherosclerotic vascular disease—not only for recurrent cardiovascular events in stable ischemic heart and carotid disease, but also as for risk of failed reperfusion in fibrinolysis-treated STEMI patients, and associated with no-reflow and consequently infarct size and clinical outcomes in those treated with PPCI.[37–42] Our findings highlight the role of vWF in tissue-level perfusion and provide impetus for evaluating selective vWF inhibition in facilitating post–myocardial infarction microcirculatory reperfusion.

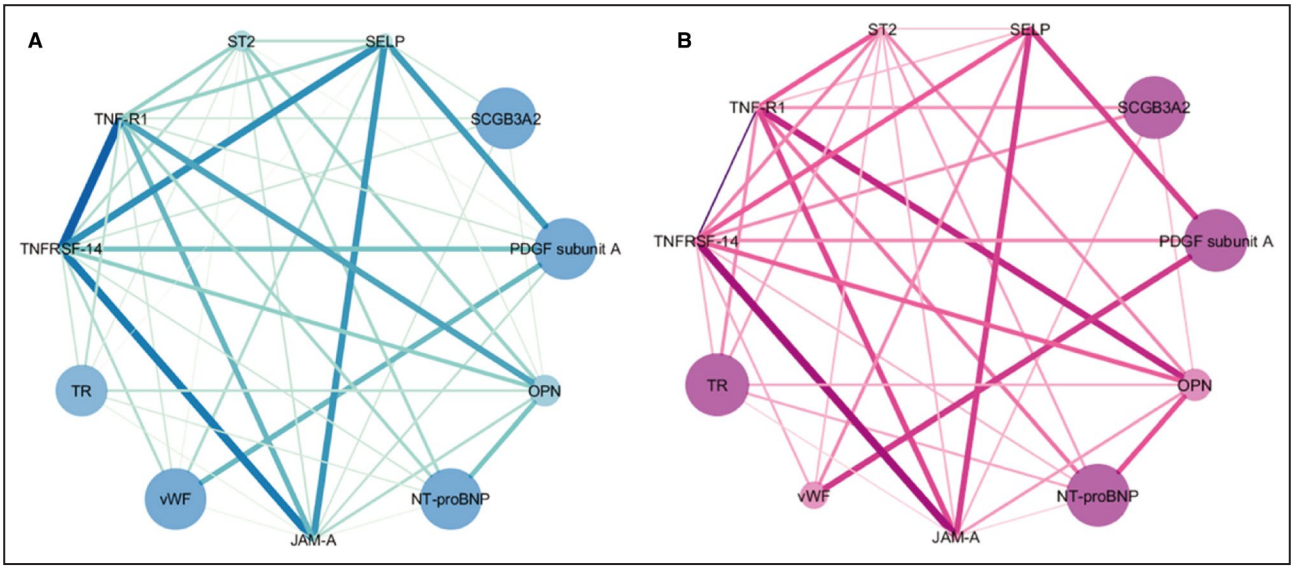

Figure 3. Network analysis between biomarkers and ST-segment–elevation resolution (ST-ER) ≥50% (A), and ST-ER <50% (B).
JAM-A indicates junctional adhesion molecule A; OPN, osteopontin; PGDF-A, platelet derived growth factor subunit A; SCGB3A2, secretoglobin family 3A member 2; SELP, P-selectin; TNF-R1, tumor necrosis factor receptor 1; TNFRSF-14, tumor necrosis factor receptor superfamily 14; TR, transferrin receptor; and vWF, von Willebrand factor.

Table 2. Associations (Unadjusted and Adjusted) Between Biomarker Clusters and the 90-Day Composite of Death, Congestive Heart Failure, and Cardiogenic Shock

Biological Process	Unadjusted Hazard Ratio (95% CI)	Unadjusted *P* Value	Adjusted Hazard Ratio (95% CI)	Adjusted *P* Value
Cell adhesion	1.07 (0.99–1.14)	0.073	1.01 (0.94–1.07)	0.865
Angiogenesis	1.09 (1.00–1.19)	0.056	1.01 (0.92–1.11)	0.774
Catabolic process	1.05 (0.98–1.13)	0.156	0.99 (0.93–1.06)	0.797
Chemotaxis	1.07 (0.98–1.15)	0.115	1.00 (0.92–1.08)	0.942
Coagulation	1.05 (0.96–1.15)	0.266	1.01 (0.92–1.11)	0.842
Response to hypoxia	1.10 (0.98–1.23)	0.115	1.02 (0.91–1.14)	0.743
Inflammatory response	1.10 (1.03–1.17)	0.003	1.02 (0.96–1.09)	0.566
Mitogen-activated protein kinase cascade	1.14 (1.05–1.24)	0.001	1.03 (0.94–1.13)	0.512
Blood vessel morphogenesis	1.09 (1.00–1.19)	0.056	1.01 (0.92–1.11)	0.774
Other gene ontology terms	1.09 (0.99–1.19)	0.083	0.99 (0.90–1.09)	0.878
Response to peptide hormone	1.11 (0.99–1.25)	0.086	1.00 (0.89–1.12)	0.964
Platelet activation	1.01 (0.89–1.14)	0.930	0.98 (0.86–1.11)	0.729
Proteolysis	1.09 (1.01–1.18)	0.036	1.02 (0.94–1.10)	0.717
Wound healing	1.05 (0.96–1.15)	0.244	1.00 (0.91–1.09)	0.994
NT-proBNP	1.96 (1.65–2.34)	<0.0001	1.54 (1.27–1.88)	<0.0001

NT-proBNP indicates N-terminal pro-B-type natriuretic peptide.

Our study also complements prior observations between higher baseline NT-proBNP concentrations and impaired myocardial reperfusion, microvascular obstruction, infarct size, and clinical outcomes in STEMI[43–45] and highlight the prognostic value of myocardial stretch and increased wall stress. Prior multibiomarker analyses have suggested that inflammation and fibrosis (with proteins such as ST2 and GDF15) independently complement NT-proBNP as prognostic predictors of clinical outcomes.[45,46] The differences between those findings and the current ones may relate in part to the substantial proportion of our study population presenting within 3 hours of symptom onset and undergoing rapid reperfusion precluding prior activation of some adverse markers. Additionally, we hypothesize that the observed relationships between NT-proBNP and platelet activation proteins with ST-ER, but only NT-proBNP with the 90-day clinical composite, may relate to in part to the use of combination antiplatelet therapy and early STEMI presentation, mitigating the rise of platelet activation proteins and their downstream association with adverse cardiovascular events.

Biomarker Correlations in ST-ER Subgroups

Across both ST-ER categories, strong interbiomarker relationships were evident between NT-proBNP and proteins associated with inflammation, suggesting close relationships between myocardial stretch and inflammatory pathways in STEMI. Similarly, the large hubs of platelet activation proteins suggest synergistic relationships with other platelet activation markers and inflammatory molecules. The close relationship between the regulator of iron transport (transferrin) and inflammation observed in patients with ST-ER <50% is, to our knowledge, unique. Redox-active iron and reactive oxygen species are recognized mediators of cellular injury in STEMI and have long been theorized to play a role in reperfusion injury. Prior smaller studies have demonstrated their deferoxamine- mediated inhibition before PPCI ameliorates markers of oxidative stress.[47] These novel interbiomarker relationships across the 2 ST-ER subgroups suggest pathophysiologic mechanistic links in STEMI worthy of further exploration in patients with suboptimal ST-ER.

Our study has both strengths and limitations. We provide mechanistic correlations with tissue-level reperfusion and identify patients at greatest risk for impaired myocardial reperfusion in a well-characterized population of early-treated patients with STEMI, with core-lab electrocardiographic and independently adjudicated clinical outcomes. This is further supported by alignment using alternate validated metrics of myocardial reperfusion. However, the markers of platelet activation, such as vWF, may have been influenced by systemic anticoagulants: the temporal relationship between the timing of baseline blood draw and the administration of systemic anticoagulants are unknown. Whereas no major baseline differences between the current population and the overall trial population were evident, we cannot exclude unknown selection bias. Although our

results are buttressed by internal validation, external validation in a replication cohort has not been performed.

CONCLUSIONS

Despite optimal epicardial coronary flow following PPCI, higher pre-PPCI expression levels of platelet activation proteins and NT-proBNP were associated with impaired post-PPCI microvascular reperfusion. Pre-PPCI NT-proBNP levels were significantly associated with 90-day clinical outcomes. This exploratory analysis provides insights into the biomarker pre-PPCI risk profile for suboptimal myocardial reperfusion and may help identify future therapeutic targets.

ARTICLE INFORMATION

Received January 21, 2020; accepted May 15, 2020.

Affiliations

From the Duke Clinical Research Institute, Durham, NC (J.S.S., C.B.G., T.J.P.); Canadian VIGOUR Centre, University of Alberta, Edmonton, Alberta, Canada (J.S.S., W.A., C.M.W., S.V.D., P.W.A.); Inova Heart and Vascular Institute, Falls Church, VA (C.D.).

Acknowledgments

The authors acknowledge Dr Jasper Tromp for consulting on this manuscript and Ms. Lisa Soulard for her editorial assistance.

Sources of Funding

Funding for this study was obtained through the Innovation and Investment Award Duke Clinical Research Institute, Canadian VIGOUR Center, and Inova Heart Institute.

Disclosures

Dr Defilippi received grant support from Roche Diagnostics and Siemens Heathineers and has served as a consultant for Roche Diagnostics, Siemens Heathineers, Ortho Clinical, Abbott Diagnostics, FijiRebio, Metanomics, Quidel, UpToDate, and WebMD. Dr Granger received grant support and consulting fees from Boehringer Ingelheim, Bayer, Bristol Myers Squibb, Daiichi Sankyo, Janssen Pharmaceutica, Pfizer, GlaxoSmithKline, and Sanofi; consulting fees and lecture fees from Boston Scientific; grant support from Merck; and consulting fees from AstraZeneca, Armetheon, Eli Lilly, Gilead, Hoffmann-La Roche, Medtronic, Takeda, and The Medicines Company. Dr Povsic has received grants from Baxter Healthcare, Caladrius Biosciences, Capricor, CSL Behring, and Janssen Pharmaceuticals; and personal fees from Eli Lilly, NovoNordisk, and Pluristem. Dr Armstrong has served as a consultant for Bayer and Merck. He has received research grants from CSL Behring, Boehringer Ingelheim, Bayer, and Merck. The remaining authors have no disclosures to report.

Supplementary Materials

Tables S1–S5
Figures S1–S2

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SUPPLEMENTAL MATERIAL

Table S1: Baseline characteristics of patients included and excluded from analysis

	Included (n=1160)	Excluded (n=4585)	P-value
Baseline demographics			
Age (years)	60 (51, 70)	62 (53, 71)	0.0004
Female sex	262 (22.6)	1063 (23.2)	0.6657
Body mass index	27 (25, 31)	27 (24, 30)	0.0002
History of hypertension	585 (50.4)	2255 (49.2)	0.4472
History of diabetes	173 (14.9)	741 (16.2)	0.2993
History of hyperlipidemia	574 (49.5)	2219 (48.4)	0.5086
History of CAD	198 (17.1)	744 (16.2)	0.4889
Prior MI	154 (13.3)	540 (11.8)	0.1618
Prior PCI	149 (12.8)	413 (9.0)	<.0001
Prior CABG	34 (2.9)	94 (2.1)	0.0697
History of CHF	29 (2.5)	179 (3.9)	0.0222
History of atrial fibrillation	50 (4.3)	188 (4.1)	0.7485
History of stroke	35 (3.0)	181 (3.9)	0.1367
History of COPD	50 (4.3)	232 (5.1)	0.2911
Current smoker	513 (44.2)	1975 (43.1)	0.4805
History of peripheral vascular disease	46 (4.0)	200 (4.4)	0.5512
Presenting characteristics			
Heart rate (bpm)	75 (64, 86)	75 (65, 86)	0.5337
Systolic BP (mmHg)	132 (116, 149)	133 (117, 150)	0.1268
Diastolic BP (mmHg)	80 (69, 90)	80 (70, 90)	0.9994
Killip class > 1	128 (11.0)	483 (10.5)	0.6314
Inferior MI	483 (41.6)	1848 (40.3)	0.5571
Sum ST segment deviation, mm	15 (11, 22)	16 (12, 23)	0.0189
Baseline creatinine (umol/L)	89 (80, 106)	89 (78, 106)	0.5818
Baseline troponin I	50 (20, 117)	51 (22, 101)	0.8551
Baseline CK (IU/L)	143 (91, 270)	143 (90, 273)	0.8271
Baseline CK-MB (ug/L)	5 (2, 14)	5 (2, 15)	0.1259
Left anterior descending culprit artery	584 (50.5)	2340 (51.4)	0.5764
Primary PCI	1136 (97.9)	4237 (92.4)	<.0001
ECG outcomes (Post-PCI ECG)			
Sum ST-deviation resolution	73 (52, 89)	69 (44, 86)	<.0001
Single lead ST-E resolution	75 (55, 100)	72 (50, 90)	<.0001
Worst lead residual ST-E			<.0001
<1 mm	364 (31.4)	1271 (27.7)	
1-2 mm	480 (41.4)	1750 (38.2)	
≥2 mm	316 (27.2)	1564 (34.1)	
90-day outcomes			
Death/Shock/CHF	120 (10.3)	466 (10.2)	0.8554
Death	33 (2.8)	238 (5.2)	0.0008
Re-MI	34 (2.9)	122 (2.7)	0.613

Shock	36 (3.1)	160 (3.5)	0.5174
CHF	42 (6.1)	233 (4.6)	0.0756

Data presented as median (25^{th}, 75^{th} percentiles) or %.

BP, blood pressure; CABG, coronary artery bypass graft surgery; CHF, congestive heart failure; CK, creatine kinase; COPD, chronic obstructive pulmonary disease; ECG, electrocardiogram; MI, myocardial infarction; PCI, percutaneous coronary intervention; ST-E, ST-elevation

Table S2: Top 11 biomarkers significantly associated (univariable, at alpha=0.05) with ST-E resolution

Rank	Marker, mean (SD)	Sum ST-E resolution		Unadjusted p-value	FDR[§]-adjusted p-value
		<50% (n=299)	>=50% (n=861)		
1	N-terminal prohormone brain natriuretic peptide (NT-pro BNP)	5.26 (2.03)	4.65 (1.82)	<0.0001	0.0023
2	ST2 protein (ST2)	4.01 (1.05)	3.78 (0.82)	<0.0001	0.0023
3	Transferrin receptor protein 1 (TR)	3.62 (0.68)	3.46 (0.65)	0.0005	0.0152
4	Platelet-derived growth factor subunit A (PDGF subunit A)	5.91 (1.27)	5.62 (1.29)	0.0009	0.0205
5	Secretoglobin family 3A member 2 (SCGB3A2)	3.16 (1.00)	3.41 (1.23)	0.0019	0.0346
6	Osteopontin (OPN)	8.01 (0.79)	7.89 (0.71)	0.0138	0.1800
7	Tumor necrosis factor receptor superfamily member 14 (TNFRSF14)	5.43 (0.64)	5.33 (0.58)	0.0155	0.1800
8	Junctional adhesion molecule A (JAM-A)	5.29 (0.83)	5.17 (0.70)	0.0169	0.1800
9	P-selectin (SELP)	10.67 (0.73)	10.55 (0.74)	0.0178	0.1800
10	von Willebrand factor (vWF)	7.56 (1.18)	7.39 (1.16)	0.0301	0.2739
11	Tumor necrosis factor receptor 1 (TNF-R1)	6.23 (0.68)	6.14 (0.60)	0.0433	0.3582

[§] after adjusting p-values for false discovery rate with Benjamin- Hochberg procedure

Table S3: Univariable and multivariable associations between biomarker clusters and ST-E resolution

Biological process	Sum ST-E resolution		Unadjusted p-value	Adjusted OR[§] (95 %CI)	Adj. p-value
	<50% (n=299)	>=50% (n=861)			
Cell adhesion	0.17 (3.89)	-0.08 (3.84)	0.3189	1.00(0.96-1.03)	0.8184
Angiogenesis	0.06 (2.40)	-0.05 (2.36)	0.4998	1.00(0.95-1.07)	0.8982
Catabolic process	0.08 (3.19)	-0.04 (3.04)	0.5664	1.00(0.96-1.05)	0.8895
Chemotaxis	0.14 (2.77)	-0.06 (2.72)	0.2598	0.99(0.94-1.05)	0.7963
Coagulation	0.20 (2.07)	-0.07 (2.13)	0.0561	0.96(0.89-1.02)	0.2012
Response to hypoxia	0.06 (1.86)	-0.04 (1.83)	0.4428	0.99(0.92-1.07)	0.8329
Inflammatory response	0.31 (3.83)	-0.13 (3.65)	0.0762	0.99(0.95-1.03)	0.4997
MAPK cascade	0.18 (2.79)	-0.08 (2.62)	0.1412	0.99(0.93-1.04)	0.6384
Blood vessel morphogenesis	0.06 (2.40)	-0.05 (2.36)	0.4998	1.00(0.95-1.07)	0.8982
Other GO terms	0.10 (2.25)	-0.04 (2.12)	0.354	0.98(0.92-1.05)	0.5564
Response to peptide hormone	0.04 (1.94)	-0.02 (1.87)	0.6363	0.99(0.92-1.07)	0.8508
Platelet activation	0.21 (1.43)	-0.06 (1.51)	0.0057*	0.89(0.81-0.98)	0.0159
Proteolysis	0.07 (2.85)	-0.05 (2.72)	0.4981	1.00(0.95-1.05)	0.9774
Wound healing	0.19 (2.19)	-0.07 (2.24)	0.0877	0.96(0.90-1.03)	0.266
NT proBNP	0.22 (1.06)	-0.09 (0.96)	<0.0001	0.74(0.63-0.88)	0.0004

*after adjustment for FDR: platelet activation p-value=0.0399 and NTproBNP p-value=0.0007

[+]Adjusted for age, sex, COPD, smoking status, diabetes, stroke, systolic blood pressure, diastolic blood pressure, time to hospital arrival from randomization, baseline white blood cell count, baseline serum creatinine, baseline heart rate, Killip class and MI location

GO, gene ontology; MAPK, mitogen activated protein kinases

[§] OR measures the relative increase in risk per doubling of the biomarker

Table S4: Associations between biomarker clusters and complete, partial and no ST-E resolution

Biological process	Sum ST-E resolution			Unadjusted p-value
	Complete >=70% (n=591)	Partial 70% to 30% (n=408)	No resolution < 30% (n=161)	
Cell adhesion	-0.17 (3.49)	0.10 (3.07)	0.24 (3.55)	0.2583
Angiogenesis	-0.11 (2.44)	0.08 (2.22)	0.06 (2.50)	0.4101
Catabolic process	-0.11 (3.18)	0.07 (2.82)	0.16 (3.32)	0.4891
Chemotaxis	-0.14 (2.82)	0.12 (2.55)	0.13 (2.87)	0.2623
Coagulation	-0.13 (2.18)	0.16 (2.04)	0.09 (2.04)	0.0886
Response to hypoxia	-0.10 (1.87)	0.14 (1.77)	-0.04 (1.90)	0.1215
Inflammatory response	-0.22 (3.81)	0.07 (3.32)	0.51 (4.13)	0.0751
MAPK cascade	-0.13 (2.73)	0.02 (2.38)	0.32 (3.04)	0.1527
Blood vessel morphogenesis	-0.11 (2.44)	0.08 (2.22)	0.06 (2.50)	0.4101
Other GO terms	-0.07 (2.22)	0.02 (1.97)	0.17 (2.31)	0.4291
Response to peptide hormone	-0.04 (1.93)	0.01 (1.77)	0.09 (2.03)	0.7272
Platelet activation	-0.11 (1.54)	0.13 (1.45)	0.12 (1.38)	0.0336
Proteolysis	-0.13 (2.83)	0.09 (2.56)	0.08 (2.95)	0.4005
Wound healing	-0.13 (2.30)	0.15 (2.13)	0.10 (2.21)	0.1391
NT-proBNP	-0.14 (0.94)	0.03 (0.99)	0.38 (1.10)	<0.0001

All abbreviations as in Table S3

Table S5: Associations between biomarker clusters and TIMI myocardial perfusion grade

Biological process	Post PCI TMPG		Unadjusted p-value
	0/1/2 (n=77)	3 (n=524)	
Cell adhesion	-0.30 (3.73)	0.03 (3.45)	0.4386
Angiogenesis	-0.13 (2.87)	-0.01 (2.42)	0.7036
Catabolic process	-0.32 (3.27)	0.04 (3.19)	0.362
Chemotaxis	-0.31 (3.01)	-0.00 (2.80)	0.3749
Coagulation	-0.33 (2.47)	0.04 (2.14)	0.1718
Response to hypoxia	-0.13 (2.20)	0.02 (1.90)	0.5238
Inflammatory response	-0.16 (4.02)	-0.05 (3.80)	0.8258
MAPK cascade	-0.04 (2.87)	-0.04 (2.76)	0.9894
Blood vessel morphogenesis	-0.13 (2.87)	-0.01 (2.42)	0.7036
Other GO terms	-0.11 (2.31)	0.04 (2.23)	0.5747
Response to peptide hormone	-0.17 (2.01)	-0.01 (1.95)	0.5085
Platelet activation	-0.30 (1.61)	0.07 (1.53)	0.0504
Proteolysis	-0.30 (3.01)	0.03 (2.83)	0.3421
Wound healing	-0.36 (2.57)	0.06 (2.28)	0.1369
NT-proBNP	0.21 (1.04)	-0.07 (0.98)	0.0188

All abbreviations as in Table S3

Figure S1: Derivation of the analytic cohort

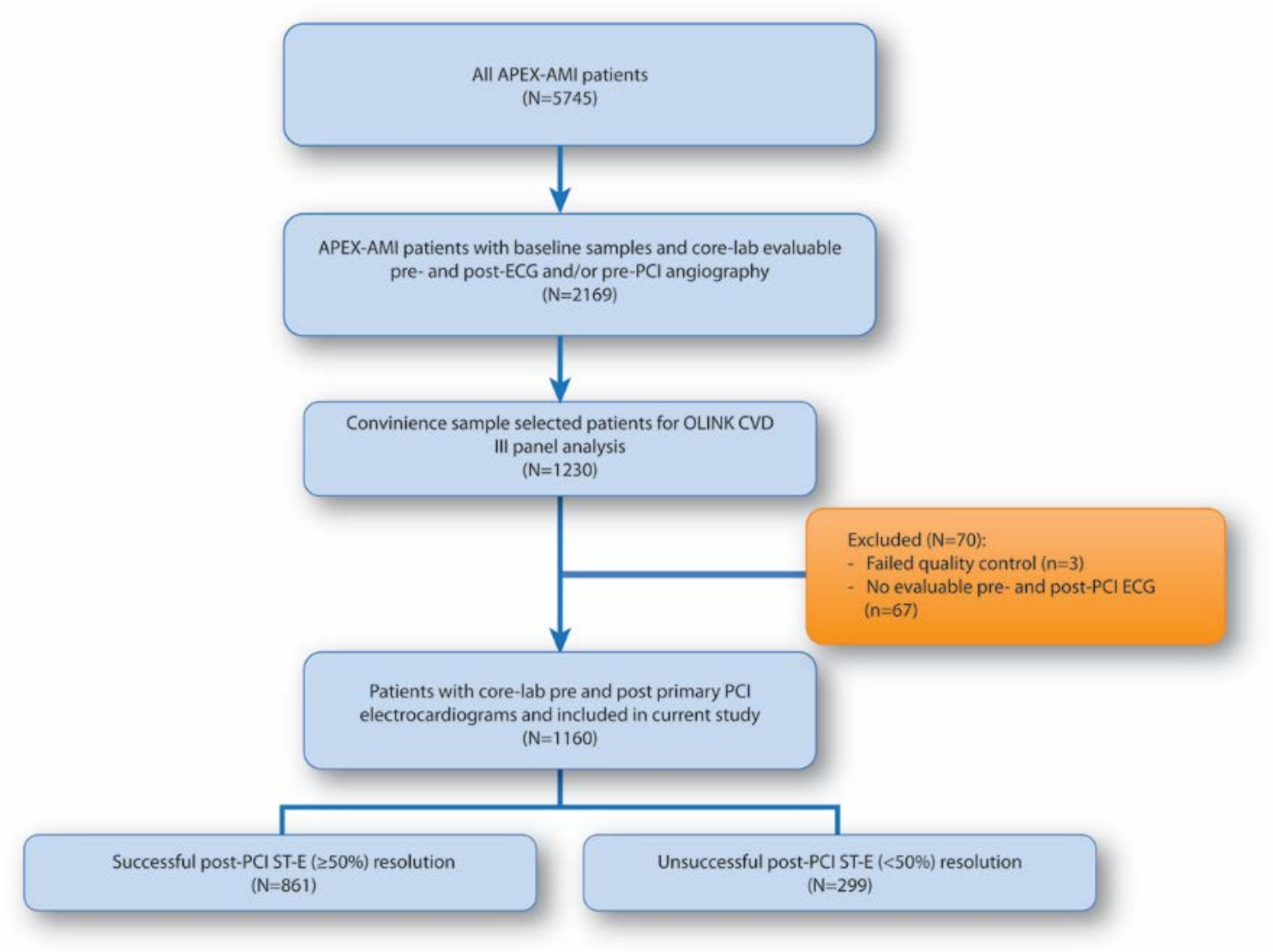
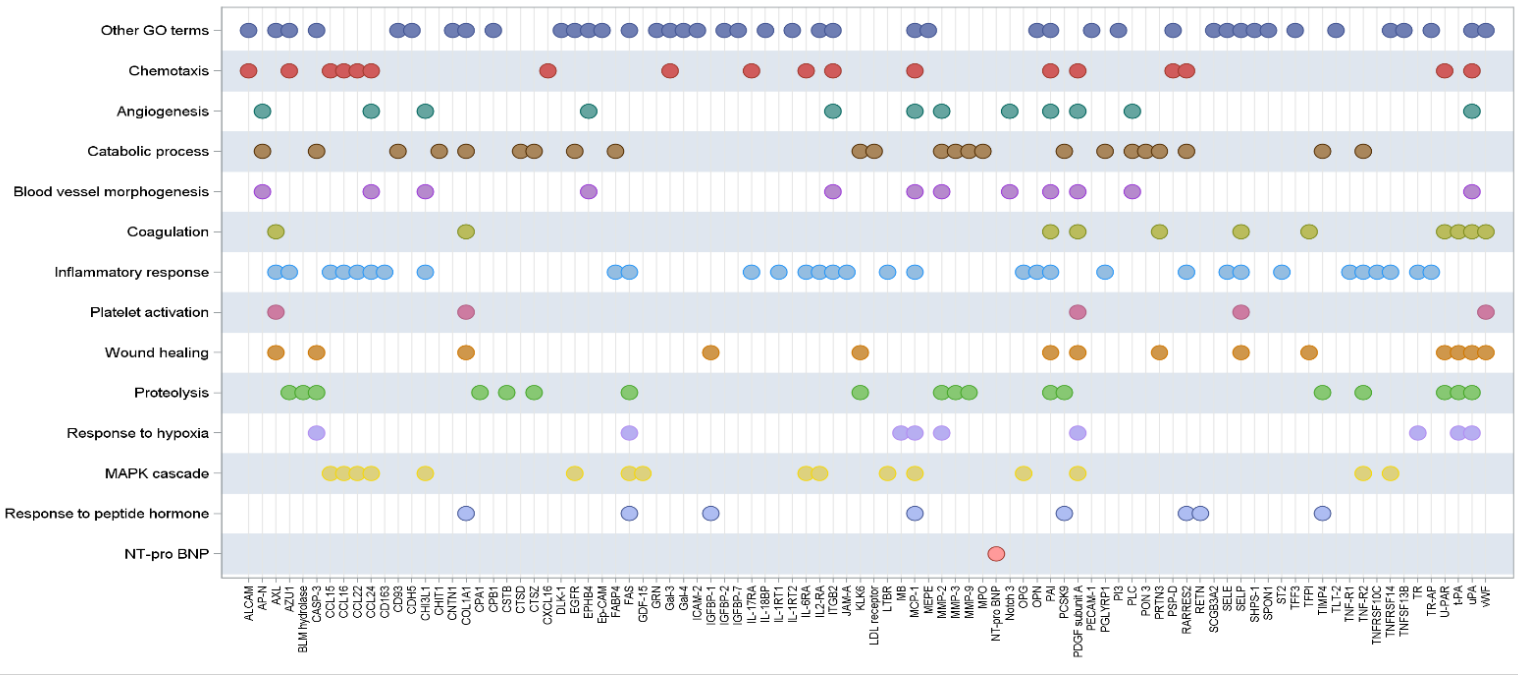


Figure S2: Summary of the evaluated proteins by their biological classes



The x-axis describes (abbreviated) each of the 91 proteins, the y-axis describes the 14 biophysiologic clusters (based on the CVD III panel, details available at [www.olink.com/products/cvd-iii-panel)](www.olink.com/products/cvd-iii-panel)

All abbreviations as in Table S3