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Novel multi-marker proteomics in phenotypically matched patients with ST-segment myocardial infarction: association with clinical outcomes

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Abstract

Early prediction of significant morbidity or mortality in patients with acute ST-segment elevation myocardial infarction (STEMI) represents an unmet clinical need. In phenotypically matched population of 139 STEMI patients (72 cases, 67 controls) treated with primary percutaneous coronary intervention, we explored associations between a 24-h relative change from baseline in the concentration of 91 novel biomarkers and the composite outcome of death, heart failure, or shock within 90 days. Additionally, we used random forest models to predict the 90-day outcomes. After adjustment for false discovery rate, the 90-day composite was significantly associated with concentration changes in 14 biomarkers involved in various pathophysiologic processes including: *myocardial fibrosis/remodeling* (collagen alpha-1, cathepsin Z, metalloproteinase inhibitor 4, protein tyrosine phosphatase subunits), *inflammation, angiogenesis and signaling* (interleukin 1 and 2 subunits, growth differentiation factor 15, galectin 4, trefoil factor 3), *bone/mineral metabolism* (osteoprotegerin, matrix extracellular phosphoglycoprotein and tartrate-resistant acid phosphatase), *thrombosis* (tissue factor pathway inhibitor) and *cholesterol metabolism* (LDL-receptor). Random forest models suggested an independent association when inflammatory markers are included in models predicting the outcomes within 90 days. Substantial heterogeneity is apparent in the early proteomic responses among patients with acutely reperfused STEMI patients who develop death, heart failure or shock within 90 days. These findings suggest the need to consider synergistic multi-biomarker strategies for risk stratification and to inform future development of novel post-myocardial infarction therapies.

Keywords STEMI · Biomarkers · Risk stratification · Health outcomes

Highlights

- Early prediction for death, heart failure and shock in patients presenting with ST-segment elevation myocardial infarction (STEMI) remains an unmet clinical need.
- In a phenotypically matched STEMI cohort we identified relative changes in 14 biomarkers representing across four pathophysiologic pathways 24 hours after initial presentation that were associated with death, heart failure and shock at 90-days.
- Risk stratification and future therapeutic targets in STEMI deserve consideration of synergistic multi-biomarker strategies.

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Introduction

In patients with ST-segment elevation myocardial infarction (STEMI), improvements in timely reperfusion together with advances in pharmacotherapy and percutaneous coronary intervention (PCI) have contributed to a temporal decline in mortality. However, the benefits of early, widely applied reperfusion may have been paradoxically offset by a parallel increase in new onset post-infarction heart failure, modulated in part by microcirculatory dysfunction [1, 2]. Accurately identifying which patients with STEMI are at risk for death, shock, or heart failure after timely reperfusion following either fibrinolysis or primary PCI remains a clinical challenge. Although high-risk phenotypic features, such as age, Killip class, and renal disease, have been well described and incorporated into validated clinical prediction models, their collective short-term clinical outcome discrimination remains imprecise [3, 4]. These models fail to accurately identify 16–26% of deaths and 22–27% of new onset heart failure events [3–6].

Biomarkers are biological parameters that may serve as pathophysiological indices of risk, acuity, and/or rate of disease progression and may yield incremental prognostic information beyond the traditional phenotypic presentation [7]. In STEMI, select well validated biomarkers, such as troponin and B-type natriuretic peptide, have been reported to modestly improve clinical risk prediction scores. Hence, there is need for new analytic approaches that could enhance clinical risk prediction and guide discovery of new therapies [8–10]. Advances in high-throughput multi-marker proteomic evaluation offer a contemporary approach that can facilitate the simultaneous evaluation of multiple pathobiological axes and potentially yield novel mechanistic insights into how several pathobiologic pathways related to clinical outcomes in STEMI [11, 12]. Little, however, is known about the cardiovascular proteomic heterogeneity in patients with STEMI who have been matched on the principal phenotypic risk factors for death, shock or heart failure. Accordingly, as part of the Assessment of Pexelizumab in Acute Myocardial Infarction (APEX-AMI) trial biomarker substudy of phenotypically matched patients with baseline and 24-h paired serum Olink® Cardiovascular Panel III samples, we applied a targeted discovery multi-marker approach to explore the associations between both novel proteins and established biomarkers and the APEX-AMI prespecified composite outcome of death, shock, and heart failure within 90 days.

Methods

Study population

The APEX-AMI trial (NCT00091637) design and biomarker substudies have been previously published [13, 14]. The study population of this present case–control biomarker substudy comprises patients within the APEX-AMI trial who either experienced the 90-day composite of death, shock or heart failure (cases) or not (controls) and were matched in a 1:1study design. Case–control matching was based on a propensity/risk of outcome score derived from a logistic regression model with the following baseline patient characteristics: age, sex, history of heart failure, baseline systolic blood pressure, Killip class on presentation, baseline creatinine, and infarct location. All patients provided informed consent and the study was approved by all enrolling hospital research ethics boards and conforms with the Declaration of Helsinki.

Biomarker analysis

Baseline blood samples were collected after randomization, but prior to study drug administration and 24 h later. Blood samples were allowed to clot, then centrifuged. Serum was immediately frozen to − 20 °C, then to − 70 °C before being shipped on dry ice to the Duke Center for Human Genetics (Durham, NC, USA). The frozen baseline serum samples were thawed, and 100 µl samples transported to Olink Proteomics® for analysis. Ninety-one of 92 known or exploratory cardiovascular-related proteins (C–C motif chemokine 22 failed quality control and 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 [15, 16]. The Cardiovascular III® immunoassay panel was selected as it encompasses proteins/protein pathways that align with the key objectives of this analysis. All protein values were presented using a log2 scale. Assay characteristics including coefficients of variation and calibration are publicly available (www.olink.com/products/cvd-iii-panel).

Study outcomes

The primary analysis compared the relative difference between baseline and 24-h paired samples between the matched case–control populations. The primary study outcome was the incidence of all-cause death, cardiogenic shock, or heart failure within 90 days of randomization which was the prespecified endpoint in the APEX-AMI trial

and adjudicated by a centralized clinical events committee. Congestive heart failure (CHF) and cardiogenic shock were defined as follows: CHF was defined on the basis of the physician's decision to treat CHF with an intravenous diuretic, inotropic agent, or vasodilator and at least one of the following: presence of pulmonary edema or pulmonary vascular congestion on chest radiograph believed to be of cardiac cause; rales reaching greater than a third up the lung fields believed to be due to CHF; pulmonary capillary wedge pressure or left ventricular end-diastolic pressure greater than 18 mm Hg; or dyspnea, with documented partial pressure of oxygen less than 80 mm Hg on room air or oxygen saturation less than 90% on room air, without significant lung disease. Rehospitalization for CHF to an acute care facility primarily for the treatment of CHF had to include intravenous treatment of CHF with a diuretic, inotropic agent, or vasodilator. Cardiogenic shock was defined as hypotension of less than 90 mm Hg systolic blood pressure lasting for at least 1 h, not responsive to fluid resuscitation and/or heart rate correction, believed to be secondary to cardiac dysfunction, and associated with at least one of the following signs of hypoperfusion: cool, clammy skin, oliguria, altered sensorium, cardiac index less than or equal to 2.2 L/min/m^2 [14].

Statistical methods

Baseline patient characteristics are reported for the matched cases and controls. Categorical variables are reported as percentages, and continuous variables were 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.

The relative difference between baseline and 24-h evaluations for each biomarker was reported as a mean and standard deviation in all patients and by case or control status. We evaluated the association of the relative difference at 24 h of each of the 91 individual proteins and the primary outcome using the proportional hazard Cox regression model. The linearity assumption of the relationship of the relative differences with the outcome was assessed using the restricted cubic spline regression. The marginal Cox model for cluster data that takes into account the correlation that exists between the matched pairs was applied [17], in which the robust sandwich estimate of the standard errors of the hazard ratios (HR) were used. HR with corresponding 95% confidence intervals (CI) and *P* values were reported for each biomarker. The *P* values were adjusted for false discovery rate (FDR) with the Benjamin-Hochberg procedure. Furthermore, the mean and 95% CI of the relative differences in each biomarker were summarized for the case and control groups and were depicted in forest plots.

A random forest model was used to rank the 37 biomarkers according to their discriminative power of cases versus controls. Random forest is a non-parametric method that constructs numerous decision trees to classify a patient based on the set of predictor variables [18]. The mean decreases in accuracy when a given predictor variable was permuted and was used as a measure of predictive importance. Our random forest model was trained with 10,000 trees and 6 biomarkers were randomly selected for each tree. For all statistical analyses except the random forest model, SAS (version 9.4; SAS Institute, Cary, NC) was used; the package randomForest and R statistical software (version 3.5) were used for the random forest analysis.

Results

In this study, a total of 150 patients (75 cases and 75 controls) were phenotypically matched 1:1 (eTable 1); of these, 8 samples (2 cases and 6 controls) could not be analyzed and 3 samples (2 at baseline and 1 at 24 h) did not pass quality control. Hence, the final analytic population comprised 139 paired samples (72 cases and 67 controls) (Table 1), and as described in eFigure 1 and 2, the distribution in propensity scores between cases and controls still comparable within the final analytic population. The distribution of the individual components of the primary composite were as follows: death 20/139 (14.4%), heart failure 40/139 (28.8%), and cardiogenic shock 25/139 (18%). Baseline differences among cases and controls in both the analytic and overall cohorts were well balanced, though cases had longer ischemic times and less frequently post-PCI Thrombolysis in Myocardial Infarction (TIMI) 3 flow grade.

Relative biomarker differences at 24 h and death, shock, heart failure within 90 days

A significant change in the mean relative concentration (baseline—24 h) was observed for 37 biomarkers. The directionality of the relative biomarker concentration differences (an increase or decrease in baseline—24-h concentration levels) is shown in eTable 2: this was congruent for both cases and controls across 33 markers, whereas osteoprotegerin, cystatin-B, epithelial cell adhesion molecule and transferrin receptor protein 1 markers showed a decline for controls not evident in the cases (eTable 2). Across all 37 proteins, each 10% change in the mean relative concentration difference was associated with a range between a 3–77% higher unadjusted hazard for the composite outcome (Table 2; the association between all 91 biomarkers and 90-day composite described in eTable 3). After FDR-adjustment, the mean relative difference in baseline—24-h concentrations of 14 biomarkers retained a significant association with the composite: collagen alpha-1, trefoil factor-3, interleukin-2

Table 1 Baseline characteristics for patients with (cases) and without death/cardiogenic shock/heart failure within 90 days (controls) within the analytic population

	Controls (n = 67)	Cases (n = 72)	*P* value
Age (years)[a]	66 (57, 76)	67 (58, 75)	0.77
Female sex[a]	18 (26.9)	24 (33.3)	0.41
Body mass index (kg/m^2)	26 (24, 29)	26 (24, 28)	0.64
History of hypertension	37 (55.2)	44 (61.1)	0.48
History of diabetes	12 (17.9)	12 (16.7)	0.85
History of hyperlipidemia	34 (50.7)	33 (45.8)	0.56
History of CAD	17 (25.4)	15 (20.8)	0.53
Prior MI	13 (19.4)	13 (18.1)	0.84
Prior PCI	11 (16.4)	7 (9.7)	0.24
Prior CABG	2 (3.0)	2 (2.8)	0.94
History of congestive heart failure[a]	5 (7.5)	5 (6.9)	0.91
History of atrial fibrillation	3 (4.5)	10 (13.9)	0.06
History of stroke	1 (1.5)	4 (5.6)	0.20
History of COPD	4 (6.0)	9 (12.5)	0.19
Current smoker	22 (32.8)	28 (38.9)	0.46
History of peripheral vascular disease	2 (3.0)	6 (8.3)	0.18
History of chronic inflammatory condition	3 (4.5)	1 (1.4)	0.28
Heart rate (bpm)[a]	76 (68, 90)	77 (69, 92)	0.81
Systolic BP (mmHg)[a]	118 (105, 140)	120 (108, 138)	0.83
Diastolic BP (mmHg)	70 (60, 80)	76 (66, 80)	0.08
Killip class > 1[a]	28 (41.8)	27 (37.5)	0.61
Inferior MI[a]	18 (26.9)	24 (33.3)	0.41
Sum ST segment deviation	17 (11, 24)	17 (13, 23)	0.98
Creatinine (umol/L)[a]	101 (88, 115)	98 (88, 116)	0.91
Troponin I (µg/L)[b]	42 (16, 120)	97 (40, 173)	0.09
CK (µg/L)[b]	147 (79, 238)	160 (89, 569)	0.22
CK-MB (ug/L)[b]	6 (3, 18)	10 (3, 30)	0.40
Primary PCI	61 (91.0)	67 (93.1)	0.66
Time to PCI from symptom onset (hrs)	3 (2, 4)	4 (3, 5)	0.002
Pre-PCI TIMI 3 Flow	8 (12.3)	5 (7.5)	0.35
Post-PCI TIMI 3 Flow	52 (91.2)	48 (71.6)	0.01
Left anterior descending culprit artery	43 (65.2)	45 (62.5)	0.75
NT-proBNP (ng/mL)	448 (139, 2805)	438 (109, 1572)	0.67
Pexelizumab treatment arm	30 (44.8)	34 (47.2)	0.77

Data presented as median (25th, 75th percentiles) or %

CAD coronary artery disease, *MI* myocardial infarction, *PCI* percutaneous coronary intervention, *CABG* coronary artery bypass graft surgery, *COPD* chronic obstructive pulmonary disease, *bpm* beats per minute, *BP* blood pressure, *CK* creatine kinase, *TIMI* thrombolysis in myocardial infarction, *NT-proBNP* N-terminal pro-brain natriuretic peptide

[a]Phenotypic match in variables between cases and controls

[b]Core-lab derived (not olink assays)

receptor subunit alpha, growth differentiation factor 15, tyrosine-protein phosphatase non-receptor type substrate 1, osteoprotegerin, cathepsin Z, metalloproteinase inhibitor 4, interleukin-1 receptor type 2, tartrate-resistant acid phosphatase type 5, matrix extracellular phosphoglycoprotein, low-density lipoprotein receptor, galectin-4 and tissue factor pathway inhibitor (Fig. 1). The highly significant associations with tissue factor pathway inhibitor and especially collagen alpha-1 are noteworthy.

Table 2 Association between relative difference in biomarker concentrations and death, shock and heart failure within 90 days

Marker	Relative difference (%) Mean (SD)		HR[a] (95% CI)	*P* value	FDR-adjusted *P* value
	Controls (n = 67)	Cases (n = 72)			
COL1A1	1.37 (13.67)	8.16 (42.51)	1.06 (1.03–1.08)	< 0.0001	0.0005
TFF3	6.50 (8.35)	11.72 (12.52)	1.32 (1.14–1.53)	0.0003	0.013
IL2_RA	7.80 (9.76)	15.78 (16.61)	1.22 (1.08–1.38)	0.001	0.022
GDF_15	4.84 (11.14)	13.24 (14.87)	1.25 (1.09–1.43)	0.001	0.022
SHPS_1	2.25 (9.26)	8.00 (15.33)	1.20 (1.07–1.35)	0.002	0.022
OPG	− 2.95 (12.40)	3.11 (16.76)	1.25 (1.09–1.44)	0.00167	0.022
CTSZ	2.23 (7.51)	6.51 (8.65)	1.47 (1.15–1.87)	0.002	0.022
TIMP4	21.58 (19.68)	30.08 (25.34)	1.15 (1.05–1.26)	0.002	0.022
IL_1RT2	5.11 (8.62)	9.37 (12.96)	1.29 (1.10–1.52)	0.002	0.022
TR_AP	3.81 (12.83)	8.24 (22.37)	1.14 (1.05–1.25)	0.003	0.029
MEPE	3.03 (9.96)	7.19 (14.93)	1.23 (1.07–1.42)	0.004	0.032
LDL_receptor	15.43 (17.48)	23.32 (27.40)	1.10 (1.03–1.18)	0.005	0.037
Gal_4	− 7.81 (15.00)	− 0.78 (24.06)	1.16 (1.05–1.30)	0.005	0.037
TFPI	− 10.00 (7.49)	− 8.56 (7.41)	1.37 (1.09–1.72)	0.007	0.048
OPN	6.18 (8.11)	11.26 (12.09)	1.28 (1.07–1.54)	0.008	0.051
EPHB4	7.46 (6.46)	10.64 (9.59)	1.39 (1.09–1.79)	0.009	0.051
PLC	1.14 (5.08)	3.67 (7.78)	1.40 (1.09–1.81)	0.010	0.051
TNF_R2	5.46 (7.69)	9.31 (10.99)	1.31 (1.06–1.61)	0.011	0.054
CSTB	− 1.32 (14.61)	6.34 (20.83)	1.15 (1.03–1.28)	0.012	0.054
TNF_R1	4.84 (7.75)	8.95 (9.52)	1.33 (1.06–1.67)	0.012	0.054
ST2	30.42 (25.42)	45.08 (37.88)	1.10 (1.02–1.18)	0.013	0.054
SPON1	− 39.75 (22.60)	− 33.15 (23.49)	1.10 (1.02–1.18)	0.013	0.054
BLM_hydrolase	35.13 (59.44)	64.02 (70.51)	1.04 (1.01–1.08)	0.015	0.058
CHI3L1	30.13 (27.69)	43.29 (33.36)	1.08 (1.01–1.14)	0.016	0.060
Ep_CAM	− 3.55 (8.66)	1.25 (20.04)	1.16 (1.02–1.31)	0.020	0.071
IL_6RA	1.29 (2.98)	2.28 (4.30)	1.77 (1.09–2.90)	0.020	0.077
TR	− 0.21 (9.20)	1.56 (12.77)	1.26 (1.03–1.55)	0.023	0.078
RETN	5.70 (10.18)	10.79 (12.46)	1.21 (1.02–1.45)	0.030	0.096
FAS	5.95 (7.65)	8.71 (12.43)	1.20 (1.02–1.41)	0.031	0.098
CTSD	23.89 (45.92)	32.71 (53.10)	1.03 (1.00–1.06)	0.035	0.103
IL_18BP	3.30 (6.68)	6.43 (9.06)	1.32 (1.02–1.71)	0.035	0.103
IGFBP_7	1.38 (6.42)	3.40 (9.02)	1.28 (1.01–1.61)	0.038	0.108
LTBR	7.24 (9.33)	13.44 (15.41)	1.17 (1.01–1.36)	0.039	0.108
IL_1RT1	3.31 (6.17)	5.48 (8.93)	1.29 (1.01–1.64)	0.040	0.108
AP_N	0.28 (6.78)	2.83 (10.89)	1.24 (1.01–1.52)	0.044	0.114
CHIT1	6.39 (9.77)	10.11 (14.56)	1.16 (1.00–1.33)	0.047	0.119
KLK6	9.34 (23.65)	16.61 (45.25)	1.03 (1.00–1.07)	0.048	0.119

Biomarkers listed ranked by their FDR-adjusted *P* value

SD standard deviation, *HR* hazard ratio, *CI* confidence interval, *FDR* false discovery rate, *COL1A* collagen alpha-1, *TFF3* trefoil factor-3, *IL2_RA* interleukin-2 receptor subunit alpha, *GDF_15* growth differentiation factor 15, *SHPS_1* tyrosine-protein phosphatase non-receptor type substrate 1, *OPG* osteoprotegerin, *CTSZ* cathepsin Z, *TIMP4* metalloproteinase inhibitor 4, *IL_1RT2* interleukin-1 receptor type 2, *TR_AP* tartrate-resistant acid phosphatase type 5, *MEPE* matrix extracellular phosphoglycoprotein, *LDL_ receptor* low-density lipoprotein receptor, *Gal_4* galectin-4, *TFPI* tissue factor pathway inhibitor, *OPN* osteopontin, *EPHB4* ephrin type-B receptor 4, *PLC* perlecan, *TNF_R2* tumor necrosis factor receptor 2, *CSTB* cystatin-B, *TNF_R1* tumor necrosis factor receptor 1, *ST2 ST2* protein, *SPON1* spondin-1, *BLM_hydrolase* bleomycin hydrolase, *CHI3L1* chitinase-3-like protein 1, *Ep_CAM* epithelial cell adhesion molecule, *IL_6RA* interleukin-6 receptor subunit alpha, *TR* transferrin receptor protein 1, *RETN* resistin, *FAS* tumor necrosis factor receptor superfamily member 6, *CTSD* cathepsin D, *IL_18BP* interleukin-18-binding protein, *IGFBP_7* insulin-like growth factor-binding protein 7, *LTBR* lymphotoxin-beta receptor, *IL_1RT1* interleukin-1 receptor type 1, *AP_N* aminopeptidase N, *CHIT1* chitotriosidase-1, *KLK6* kallikrein-6

[a]HR based on a 10% change in NPX value

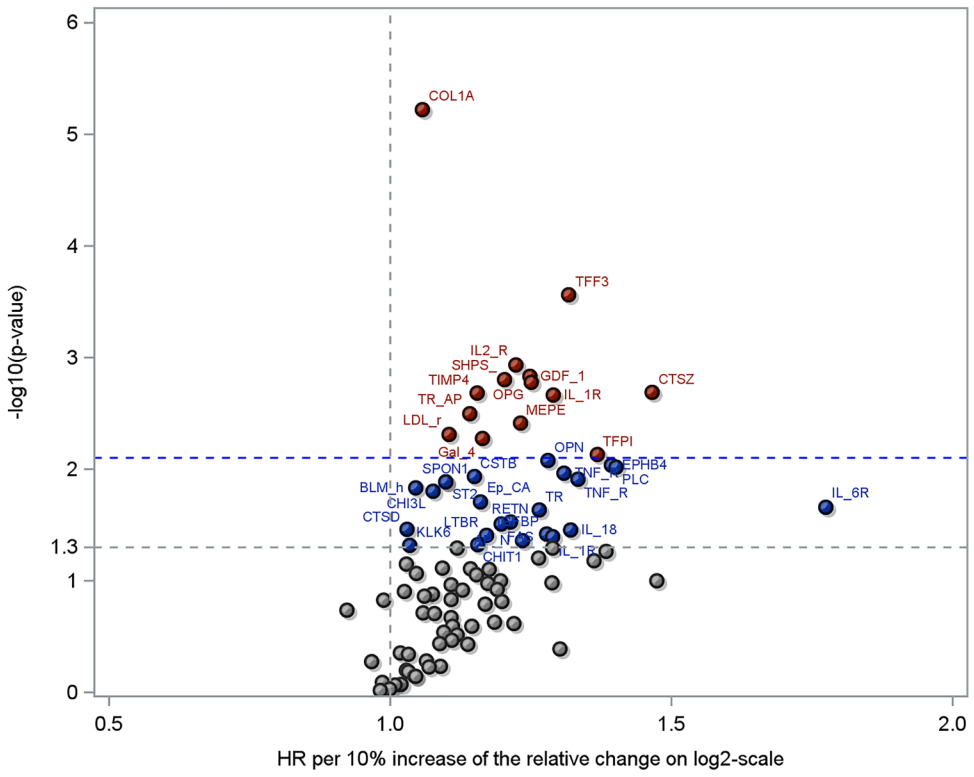

Fig. 1 Associations between biomarkers and composite endpoint to time death, shock or heart failure within 90 days (grey and blue lines represent before and after FDR adjustment, respectively). Markers above the blue line remain significantly associated with the 90-day composite; collagen alpha-1 [COL1A], trefoil factor-3 [TFF3], interleukin-2 receptor subunit alpha [IL2_RA], growth differentiation factor 15 [GDF15], tyrosine-protein phosphatase non-receptor type substrate 1 [SHPS 1], osteoprotegerin [OPG], cathepsin Z [CTSZ], metalloproteinase inhibitor 4 [TIMP4], interleukin-1 receptor type 2 [IL_1RT2], tartrate-resistant acid phosphatase type 5 [TR_AP], matrix extracellular phosphoglycoprotein [MEPE], low-density lipoprotein receptor [LDL_R], galectin-4 [GAL4] and tissue factor pathway inhibitor [TFPI]

Random forest models

The predictive importance of the temporal change in the biomarkers when considered jointly and based on the random forest analysis were ranked using the mean decrease in accuracy statistics (eTable 4). The larger the mean decrease in accuracy, the more important the biomarker. We observed that univariable and Cox regression models that included trefoil factor 3, interleukin-2 receptor subunit alpha, growth differentiation factor 15, tyrosine-protein phosphatase non-receptor type substrate 1 and cathepsin Z were among the top biomarkers in the joint random forest-based analysis, suggesting an independent association with the clinical outcome.

Discussion

In this risk-matched case–control analysis, we note that the relative change at 24 h in several proteins involved in myocardial fibrosis/remodeling, inflammation, angiogenesis and signaling, bone/mineral metabolism, thrombosis and cholesterol metabolism were associated with death, heart failure and shock within 90 days after treatment for STEMI with primary PCI. Importantly, our results highlight the absence of a single dominant pathway, strongly suggestive of potential synergistic contributions of the heterogenous pathophysiologic processes manifesting in death, cardiogenic shock or heart failure [19].

While the integration of biomarkers to clinical variables has been valuable in the recognition of the complex pathophysiologic pathways related to post-infarction heart failure, the understanding of the biological basis of myocardial healing remains incomplete. Hence, our ability to stratify early patients at greatest risk for downstream adverse left ventricular remodeling/dysfunction remains an unmet need. This study expands on prior proteomic-heart failure analyses by identifying several novel and traditional proteins associated with short-term adverse cardiovascular outcomes following an acute myocardial infarction.

Early biomarker concentration change and subsequent clinical events

Myocardial fibrosis/remodeling

Fibroblast activation and scar formation represent the hallmarks of post-infarction healing and repair [20]. While collagen deposition is central to the final common pathway of scar maturation and post-infarction remodeling, animal models have suggested significant heterogeneity in the relationships between collagen subtypes, the timing of collagen deposition and the final infarct size. For instance, decreased collagen deposition and excessive collagen degradation in the early phase of post-infarct healing is suggested

to associate with increased infarct expansion yet [21, 22], over-expression of alpha 1 and 2 collagen chains have been established to contribute to enhanced myocardial fibrosis, adverse left ventricular remodeling and heart failure across the spectrum of cardiovascular diseases [23–25]. In the context of several limitations in translating animal model findings to human post-STEMI patients, our results highlight the presence of an important relationship between an early increase in collagen alpha-1 concentrations and downstream mortality, shock or heart failure. Further, these findings align with recent interesting descriptions of sacubitril/valsartan mediated inhibition of profibrotic genes and maladaptive remodeling in pressure-overloaded left ventricles [26], and losartan mediated inhibition of profibrotic changes on tethered mitral valve leaflets post-myocardial infarction [27]. Additionally, we describe over-expression of metalloproteinase inhibitor 4, cathepsin Z and tyrosine-protein phosphatase non-receptor type substrate 1 in patients with compared to without the occurrence of death, shock or heart failure; the exact biological activities of these proteins in relation to myocyte healing remain unclear, but have all been described to regulate cell signaling, angiogenesis, myofibroblast apoptosis and cardiac fibrosis [28–31].

Inflammation, angiogenesis and signaling pathways

While the role of the inflammatory cascade in prognosticating post-infarction outcomes has been long recognized, therapeutic targets have been limited, in part, by the pleiotropy in the actions, concentrations, timing of production, and targets of mediators of inflammation. We describe higher early rises in interleukin 1 and 2 subunit concentrations in patients developing downstream heart failure and shock; aligned with their recognized role in adversely affecting myocyte calcium regulation, anaerobic glycolysis and promoting neutrophilic infiltration, our findings support novel exciting endeavors being designed to target early interleukin 1 blockade aimed at mitigating infarct size and acute post-infarction heart failure [32, 33].

Aligned with acute post-myocardial infarction inflammation, the role of growth differentiation factor 15 (GDF15) in cardiovascular pathobiology has been extensively described [34–37]. Mechanistically, the transforming growth factor-beta superfamily are ubiquitous and critical regulators of the various pathways involved in myocardial repair and healing [38]. Our findings extend prior work on GDF-15 by evaluating a 24-h change from baseline concentrations in a phenotypically matched, and early presenting STEMI cohort. Furthermore, we highlight the emerging importance of galectin-4 as an acute pro-inflammatory mediator purported to result in the deposition of stiff, non-contractile collagen and adverse cardiac healing [39]. Dynamic upregulation of galectin has been described in animal model peri-infarct border zones; similarly, galectin knockout mice demonstrated reduced infarct zone macrophage infiltration [40, 41]. The animal model data appears to be similarly translated in small studies of human STEMI patients, and aligned with our results, suggest the presence of a relationship between this protein and early left ventricular systolic dysfunction [40]. The findings from our exploratory random forest analysis also appear to be congruent in highlighting the independent association between markers of the inflammatory cascade and the composite endpoint within 90 days.

Bone and mineral metabolism

Our findings suggest a prognostic relationship between an early concentration change in various markers of bone and mineral metabolism [such as osteoprotegerin (OPG), matrix extracellular phosphoglycoprotein and tartrate-resistant acid phosphatase] in patients who died or developed shock or heart failure. The available data on the relationships between bone and mineral metabolism proteins and adverse cardiovascular outcomes are most robust for OPG. Elevated OPG concentrations have previously been described to independently associate with cardiac magnetic resonance imaging derived indices of microvascular obstruction and no-reflow in STEMI [42, 43], and correlate with larger infarct size [44, 45], incident and chronic heart failure [46, 47] and cardiovascular mortality [48]. Independent of acute coronary syndrome-related heart failure, elevated OPG concentrations have also been recently described for risk prediction in patients with heart failure with preserved ejection fraction, and in those with acute decompensated heart failure [49]. While the OPG-heart failure descriptions have been robust and consistent, a causal relationship is unclear, and the pathways between mediators of bone/mineral metabolism, regulation of matrix extracellular proteins, and post-infarction heart failure will require further validation.

Cholesterol metabolism

Interestingly, in patients with heart failure, the proprotein convertase subtilisin/kexin type 9 and low-density lipoprotein (LDL) receptor axis have been speculated to influence plaque stabilization, inflammation and thrombosis in mediating cardiovascular outcomes [50]. Extending these findings to STEMI patients, the results of this analysis suggest the pleiotropic importance of LDL-regulation beyond atherosclerosis alone in the regulation of downstream heart failure and shock.

Finally, N-terminal pro B-type natriuretic peptide levels have previously been validated as robust prognostic correlates post-myocardial infarction; the lack of a similar signal in this analysis likely stems from our study population (both

cases and controls) being matched for heart failure and baseline Killip class.

The random forest model provides an additional exploratory analysis in describing the independent specific association between the temporal change in markers of inflammation and the composite endpoint within 90 days, highlighting the dominance of this pathway in predicting cases from controls.

Strengths and limitations

This case–control analysis evaluated a phenotypically matched, early presenting STEMI patient population and explored both traditional and novel proteins across a spectrum of cardiovascular pathways for all patients at two timepoints. The following limitations, however, need to be considered. First, although our study population was risk matched at baseline for death, heart failure and shock, unmatched variables (such as ischemic times and post-PCI TIMI flow) and unmeasured confounding likely impact the relationship with the outcomes of interest. The impact of these differences is partially mitigated by our use of a relative (rather than absolute) baseline-24 h concentration change in our evaluation of their relationship with clinical outcomes and in addition by the balance in key prognostically relevant variables such as Killip class, heart failure and infarct location. However, the key objective of this exploratory analysis is to provide insights into potential pathobiologic pathways associated with adverse left ventricular remodeling thought to mediate heart failure and cardiogenic shock following STEMI. Second, no information pertaining to indices of left ventricular function was available in APEX-AMI or within this substudy; their inclusion may have added additional insight regarding clinical outcomes. Finally, the identified relationships between post STEMI clinical outcomes and serum proteins only apply to the 92 select cardiovascular proteins within the select Cardiovascular III® panel, and additionally, only in patients who have survived the first 24 h from index presentation; future studies aimed at expanding the proteomic spectrum and externally validating our findings would potentially enhance our understanding of these relationships.

Conclusions

In rapidly reperfused STEMI patients, early proteomic changes across processes of myocardial fibrosis/remodeling, inflammation, angiogenesis and signaling, bone/mineral metabolism, thrombosis and cholesterol metabolism appear to synergistically associate with downstream death, shock and heart failure. This exploratory analysis suggests the need to consider evaluation of multiple biomarker strategies not only for post-STEMI risk stratification, but additionally consider targeting several novel proteins/pathways synergistically, in mitigating post-STEMI death, shock or heart failure.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Assay characteristics including coefficients of variation and calibration are publicly available (www.olink.com/products/cvd-iii-panel). For all statistical analyses except the random forest model, SAS (version 9.4; SAS Institute, Cary, NC) was used; the package randomForest and R statistical software (version 3.5) were used for the random forest analysis.

Declarations

Conflict of interest Dr. deFilippi received grant support from Roche Diagnostics, Siemens Heathineers and has served as a consultant for Roche Diagnostics, Siemens Heathineers, Ortho Clinical, Abbott Diagnostics, Fiji Rebio, 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 Medicine 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, and received research grants from CSL, Boehringer Ingelheim, Bayer, and Merck. All other authors have no disclosures.

Ethical approval The study was approved by all enrolling hospital research ethics boards and conforms with the Declaration of Helsinki.

Consent to participate All patients provided informed consent.

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