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Temporal changes in biomarkers and their relationships to reperfusion and to clinical outcomes among patients with ST segment elevation myocardial infarction

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Abstract Coronary plaque rupture mediating acute ST segment elevation myocardial infarction (STEMI) is associated with a systemic inflammatory response. Whether early temporal changes in inflammatory biomarkers are associated with angiographic and electrocardiographic markers of reperfusion and subsequent clinical outcomes is unclear. In the APEX-AMI biomarker substudy, 376 patients with STEMI had inflammatory biomarkers measured at the time of hospital presentation and 24 h later. The primary outcome was the 90-day composite of death, shock, or heart failure. Secondary reperfusion outcomes were (1) worst least residual ST segment elevation (ST-E: <1 mm, 1 to <2 mm, ≥2 mm) and (2) post-percutaneous coronary intervention (PCI) TIMI flow grade (0/1/2 vs 3) and TIMI myocardial perfusion grade (TMPG 0/1 vs 2/3). The 90-day incidence of death, shock or heart failure was 21.3 % in this cohort. Electrocardiographic reperfusion (worst residual ST-E <1 mm, 1 to <2 mm, ≥2 mm) was associated with differences in 24 h change in N-terminal proB-type natriuretic peptide (NT-proBNP) (1192.8, 1332.5, 1859.0 ng/mL; $p = 0.043$) and the pro-inflammatory cytokines Interleukin (IL)-6 (14.0, 13.6, 22.1 pg/mL; $p = 0.016$), IL-12 (−0.5, −0.9, −0.1 pg/mL; $p = 0.013$), and tumor necrosis factor α (TNFα) (1.0, 0.6, 3.6 pg/mL; $p = 0.023$). Angiographic reperfusion (TMPG 0/1 vs 2/3) was associated with changes in median NT-proBNP (2649.3, 1382.7 ng/mL; $p = 0.002$) and IL-6 (28.7, 15.1; $p = 0.040$). After adjustment for baseline covariates, the 24 h change in the pro-inflammatory cytokine TNFα [hazard ratio (HR) 0.49; 95 % CI 0.26–0.95; $p = 0.035$] and the anti-inflammatory cytokine IL 10 (HR 1.41; 95 % CI 1.06–1.87; $p = 0.018$) were independently associated with the primary composite outcome. Successful coronary reperfusion was associated with less systemic inflammatory response and greater temporal inflammatory changes were independently associated with higher 90-day composite of death, shock, or heart failure. These findings provide support for an association between success of reperfusion, an acute STEMI inflammatory response and subsequent clinical outcomes.

Keywords ST segment elevation myocardial infarction · Electrocardiographic reperfusion · TIMI flow grade · TIMI myocardial perfusion grade · Inflammatory biomarkers

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Background

Successful electrocardiographic and angiographic reperfusion in ST segment elevation myocardial infarction (STEMI) patients undergoing primary percutaneous coronary intervention (PCI) are predictors of cardiac morbidity and mortality [1, 2]. N-terminal proB-type natriuretic peptide (NT-proBNP) is a validated and widely used prognostic biomarker that is lower in patients with successful electrocardiographic and angiographic reperfusion [3–7]. STEMI is also associated with changes in pro-inflammatory cytokines (interleukin [IL]-6, IL-1β, IL-12 and Tumor Necrosis Factor α [TNFα]), as well as anti-inflammatory cytokines (IL-1 receptor antagonist [IL-1ra], IL-4, IL-10), and chemokines (inducible protein [IP]-10 and interferon γ [IFNγ]) [8–11]. Studies reporting outcomes associated with many of these investigational inflammatory biomarkers in myocardial infarction are conflicting and cross-study comparisons are difficult given that inflammatory mediators were measured at different points in time including pre-and post-reperfusion. Importantly, it remains unclear whether successful reperfusion or clinical outcomes are associated with temporal changes in inflammatory mediators among STEMI patients. Improving the understanding of the relationship between the inflammatory response, success of coronary reperfusion, and clinical outcomes may improve STEMI prognostication and identify potential therapeutic targets directed towards reperfusion injury.

The Assessment of Pexelizumab in Acute Myocardial Infarction (APEX-AMI) trial biomarker substudy prospectively collected inflammatory biomarker samples at baseline and 24 h to evaluate the effect of a monoclonal antibody, pexelizumab, on the inflammatory response [12, 13]. In this contemporary population of STEMI patients undergoing primary PCI, we evaluated whether early changes (baseline and 24 h) in NT-proBNP, hsCRP, and inflammatory biomarkers are independently associated with (1) 90-day incidence of death, shock or heart failure and (2) angiographic and electrocardiographic markers of reperfusion.

Methods

Biomarker substudy and inflammatory markers of interest

The APEX-AMI (NCT00091637) trial design and results were previously reported [12, 14]. Briefly, it was an international, phase III placebo-controlled trial that compared pexelizumab (a humanized monoclonal antibody C5 complement inhibitor) versus placebo among STEMI patients undergoing primary PCI randomized within 6 h of symptom onset. The APEX-AMI biomarker substudy enrolled consenting patients from global angiographic and magnetic resonance imaging centers [13, 15]. This substudy initially planned to measure biomarkers in 4000 patients at the time of randomization and to collect 24 h samples in 1000 patients. The trial concluded earlier than planned for administrative reasons and budget considerations. The biomarker substudy population was enriched with a case–control approach wherein two controls were matched to each patient with a primary study outcome (death, shock, or heart failure) on age (within 2 years), sex, and myocardial infarct location [13]. Substudy participants had blood samples drawn: (1) immediately after randomization prior to study medication administration and PCI and (2) 24 h after randomization. After the blood clotted, samples were centrifuged and the serum was immediately frozen to −20 °C; then to −70 °C as soon as possible. Study samples were shipped on dry ice to the Duke Center for Human Genetics (Durham, NC, USA) for central storage, and then batch analyzed at a central laboratory (Montreal Heart Institute, Montreal, Quebec, Canada) at the end of the study. The results of the biomarkers substudy using samples drawn at the time of randomization were previously published [7].

Cytokines and chemokines were measured with Bio-Plex assays (LUMINEX 200, Luminex Corporation and Bio-Rad Laboratories Inc., Austin, TX). NT-proBNP and hsCRP were measured with electrochemiluminescence immunoassay (Roche Elecsys, Roche Diagnostics, Indianapolis, IN) and particle-enhanced immunonephelometry (Dade BehringNephelometer, Germany), respectively. The biomarkers of interest included NT-proBNP, hsCRP, pro-inflammatory cytokines (IL-1β, IL-6, IL-12, TNFα), anti-inflammatory cytokines (IL-1ra, IL-4 IL-10), chemokines (IP-10, IFNγ), and markers of myonecrosis (creatinine kinase [CK], CK-MB, troponin I, troponin T).

Study patients and core lab reperfusion analyses

All patients within the APEX-AMI biomarker sub-study with at least one pair (baseline and 24 h) of biomarkers of interest were included in the current study. Given no treatment related differences on clinical outcomes were observed with pexelizumab, the randomized treatment arms were pooled for this study.

All electrocardiograms were evaluated in central core labs blinded to treatment assignment and outcomes (Canadian VIGOUR Centre, Edmonton, Canada; Duke Clinical Research Institute, Durham, NC). Electrocardiographic reperfusion was evaluated as the worst least residual ST segment elevation (ST-E) on the 30 min post-PCI electrocardiogram using previously described

methodology [2]. All angiograms were independently reviewed in a central lab (Cleveland Clinic, Cleveland, OH) by two blinded, experienced reviewers. Angiographic reperfusion was evaluated with post-PCI Thrombolysis In Myocardial Infarction (TIMI) flow grade and TIMI Myocardial Perfusion Grade (TMPG) according to previously described methodologies [16].

Outcomes

The primary analysis evaluated the association between the relative change in baseline and 24 h paired biomarkers (biomarker at 24 h-biomarker at baseline/biomarker at baseline) and outcomes of interest. We used relative change for the primary outcome to control for baseline biomarker levels as many of the investigational biomarkers included in this study have been associated with infarct size [15, 17, 18]. The primary outcome of interest was the 90-day composite of all-cause death, shock or heart failure (new or worsening). This composite outcome was a pre-specified secondary endpoint of the APEX-AMI trial, and all events were centrally adjudicated by a clinical events committee according to pre-specified criteria. Secondary outcomes were electrocardiographic and angiographic evidence of reperfusion. Electrocardiographic reperfusion was evaluated with worst least residual ST-E: <1 mm, 1 to <2 mm, ≥ 2 mm. Given small numbers with poor post PCI angiographic reperfusions, post-PCI TIMI flow was grouped as 0/1/2 versus 3 and TMPG was grouped as 0/1 versus 2/3.

Statistical methods

Continuous variables were summarized as median and Inter-quartile ranges and compared with the Wilcox rank-sum test. Categorical variables were summarized as frequency and percentages and compared with the Pearson Chi square test. The median changes in biomarkers between categories of electrocardiographic and angiographic reperfusion were compared with Kruskal–Wallis one-way analysis of variance.

The association of the relative change in each of the biomarkers with the 90-day composite outcome was assessed using a Cox proportional hazards model. Modeling assumptions of normality and linearity of the relative change measures were tested and logarithmic transformation was applied to improve the distribution and relationship. Otherwise, the variable was further categorized into discrete groups. Unadjusted and adjusted associations were summarized as hazard ratios (HR) and 95 % confidence intervals (CI). To assess the adjusted association of each biomarker with the outcome of interest, the multivariable model including the set of validated covariates and as parameterized in a previous sub-study of baseline biomarkers was applied [7]. The following factors were included in the model: age, sex, baseline Q wave, hospitalization to randomization time, COPD, diabetes, stroke, systolic blood pressure, diastolic blood pressure, serum creatinine, heart rate, Killip class, baseline total ST segment deviation [7]. To account for potential differences in the likelihood of being included in this paired biomarker subset, inverse probability weighting was used in these models. In an effort to mitigate the potential influence of post-randomization clinical changes on the 24 h change in the investigational biomarker levels we conducted a parallel analysis in which both baseline and post-baseline 24 h dynamic model variables were used as adjustment factors [19]. The adjustment factors in the 24 h based model included age, baseline systolic BP, baseline Killip class, baseline heart rate, creatinine, post-PCI TIMI flow grade, worst lead residual (≥ 2 vs <2 mm), and major cardiac rhythm disturbance. Given no clinical events were observed in the first 24 h in this dataset, no patients were excluded. The data analyses were performed using the SAS (Version 9.4; SAS Institute, Cary, NC).

All patients gave written informed consent to participate in the APEX-AMI trial and its biomarkers sub-study. The protocols were approved by the institutional review board or ethics committee of each participating site.

Results

In the APEX-AMI biomarker sub-study, 376 patients had at least one paired set of biomarker samples and outcome data. A study flow diagram is provided in Online Resource 1. Differences in baseline characteristics and 90-day clinical outcomes between APEX-AMI trial participants with and without baseline and paired biomarkers and those who did not are described in Online Resource 2. Substudy participants with paired biomarkers were older, more frequently women, and more often had a history of hypertension, Killip class >1, higher baseline creatinine, and a left anterior descending artery culprit lesion. Paired biomarker participants were less likely to be a current smoker. The 90-day composite of death, shock, or heart failure was significantly more frequent in the paired biomarker sub-study population [n = 80 (21.3 %)] compared with those who were not [n = 506 (9.4 %)].

The median baseline and 24 h biomarker levels and the absolute and relative percent change of all biomarkers of interest are provided in Table 1. Levels of NT-proBNP, hsCRP, markers of myonecrosis, pro-inflammatory biomarkers (IL-1β, IL-6, TNFα), and anti-inflammatory cytokine IL-4 increased at 24 h compared to baseline values. Conversely, pro-inflammatory cytokine IL-2, anti-

Table 1 Biomarkers levels at baseline and at 24 h post-randomization and the absolute difference

Biomarker	Baseline		24 h post-randomization		Absolute change		Relative percent change*
	N	Median(Q1,Q3)	N	Median(Q1,Q3)	N	Median(Q1,Q3)	Median(Q1,Q3)
NT-proBNP (ng/mL)	903	213 (72, 819)	374	2058 (997, 4316)	364	1466 (617, 3244)	8.98 (2.25 to 23.17)
hsCRP (mg/L)	904	4 (1, 8)	373	17 (7, 32)	360	11 (4, 23)	3.53 (1.18 to 8.62)
IL-1β (pg/mL)	913	8 (7, 9)	370	8 (6, 9)	363	0.2 (−0.6, 1.1)	0.027 (−0.078 to 0.19)
IL-6 (pg/mL)	922	13 (11, 19)	381	33 (20, 61)	376	15 (5, 40)	1.14 (0.41 to 2.88)
IL-12 (pg/mL)	902	8 (6, 10)	369	7 (5, 9)	362	−1 (−3, 1)	−0.082 (−0.25 to 0.14)
TNFα (pg/mL)	922	65 (52, 74)	380	62 (52, 71)	375	2 (−5, 10)	0.025 (−0.070 to 0.20)
IL-1ra (pg/mL)	922	204 (140, 350)	381	140 (100, 202)	376	−55 (−149, 5)	−0.30 (−0.53 to 0.028)
IL-4 (pg/mL)	922	7 (6, 8)	380	7 (6, 8)	375	0.2 (−0.5, 1.0)	0.021 (−0.077 to 0.18)
IL-10 (pg/mL)	812	7 (4, 15)	290	4 (3, 8)	267	−3 (−11, 0)	−0.41 (−0.71 to 0.007)
IFNγ (pg/mL)	922	122 (93, 147)	380	109 (81, 138)	375	−310 (−622, −64)	−0.0054 (−0.11 to 0.10)
IP-10 (pg/mL)	921	812 (484, 1220)	381	421 (271, 631)	267	−0.4 (−12, 10)	−0.46 (−0.63 to 0.13)
CK (μg/L†)	608	135 (83, 281)	643	1657 (725, 3524)	490	1541 (547, 3311)	11.05 (3.08 to 29.96)
CK-MB (μg/L†)	522	6 (2, 19)	574	156 (63, 298)	395	126 (49, 274)	20.66 (3.07 to 51.35)
Troponin I (μg/L†)	652	0.17(0.07, 0.98)	544	55 (22, 120)	498	51 (19, 119)	253 (40.24 to 1133.0)
Troponin T (μg/L†)	170	0.04 (0.01, 0.17)	165	5 (2, 9)	116	5 (2, 8)	79.00 (17.71 to 481.50)

* Relative change defined as 24 h-baseline/baseline biomarker levels 100

† The post-randomization evaluation moment for the biomarkers of myocardial necrosis (CK, CK-MB, Troponin I/T) is not at 24 h but at the peak-result through discharge or day 14(whichever comes first)

inflammatory cytokines (IL-1-ra, IL-10) and chemokines (IFNγ and IP-10) were lower at 24 h.

90-day clinical outcomes

The unadjusted and adjusted associations between relative biomarker change and the 90-day composite outcome of death, shock or heart failure are provided in Table 4. After adjustment for baseline covariates the pro-inflammatory cytokine, TNFα (HR 0.49; 95 % CI 0.26–0.95, p = 0.035) and the anti-inflammatory cytokine, IL-10 (HR 1.41; 95 % CI 1.06–1.87; p = 0.018), were independently associated with the primary outcome.

Electrocardiographic and angiographic reperfusion

The median absolute changes between baseline and 24 h biomarker levels stratified by worst residual ST-E are presented in Table 2 and Fig. 1. In this outcome enriched dataset, a majority of patients did not achieve complete electrocardiographic reperfusion (worst residual ST-E <1 mm [n = 103], 1 to <2 mm [n = 145] and ≥2 mm [n = 128]). Worst residual ST- E ≥2 mm was associated with significantly greater levels of NT-proBNP, and pro-inflammatory cytokines IL-6 and TNFα. Compared with those with lesser amounts of residual ST-E worst residual ST- E ≥2 mm was also associated with smaller median IL-12 reductions. All anti-inflammatory cytokines changes were numerically, but not statistically, less with residual ST-E ≥2 mm. CK and troponin I were significantly higher and CK-MB and troponin T also were numerically higher among patients with worst residual ST-E ≥2 mm. Relative changes between baseline and 24 h biomarker levels stratified by worst residual ST-E are provided in Online Resource 3.

Angiographic markers of reperfusion and their association with absolute changes in biomarkers levels are provided in Table 3. Median NT-proBNP levels were higher among patients with both poor post-PCI TIMI flow and TMPG. IL-6 was higher in participants with TMPG 0/1. All other pro-inflammatory cytokines, and hsCRP were numerically higher among patients without successful angiographic reperfusion. Markers of myonecrosis were numerically higher among patients without successful reperfusion. Boxplots of 24 h changes in NT-proBNP and IL-6 levels are displayed in Fig. 2 and median absolute changes by angiographic markers of reperfusion are provided in Online Resource 4.

Discussion

In this international dataset of STEMI patients undergoing primary PCI in whom serial measured inflammatory biomarkers were acquired, two novel finding emerged. First, successful coronary artery reperfusion by electrocardiographic

Table 2 Median absolute change between baseline and 24 h biomarker levels stratified electrocardiographic measures of reperfusion

Biomarker*	Worst lead residual ST-E			p-value
	<1 mm (n = 103)	1 to <2 mm (n = 145)	≥2 mm (n = 128)	
NT-proBNP (ng/mL)	1192.8 (574.3, 3427.8)	1332.5 (481.2, 2600.0)	1859.0 (854.8, 3986.5)	0.043
hsCRP (mg/L)	7.4 (4.0, 22.5)	8.8 (3.1, 21.0)	14.7 (6.1, 26.4)	0.067
IL-1β (pg/mL)	0.3 (−0.5, 1.0)	0.2 (−0.7, 1.1)	0.3 (−0.6, 1.4)	0.468
IL-6 (pg/mL)	14.0 (3.7, 46.5)	13.6 (4.9, 28.0)	22.1 (8.0, 51.1)	0.016
IL-12 (pg/mL)	−0.5 (−2.5, 0.8)	−0.9 (−3.1, 0.5)	−0.1 (−2.2, 1.3)	0.013
TNFα (pg/mL)	1.0 (−5.1, 9.2)	0.6 (−5.2, 7.2)	3.6 (−3.5, 13.6)	0.023
IL-1ra (pg/mL)	−46.3 (−152.5, 14.5)	−65.9 (−151.5, −12.3)	−56.9 (−143.3, 15.1)	0.414
IL-4 (pg/mL)	0.3 (−0.5, 0.9)	0.1 (−0.7, 0.8)	0.1 (−0.5, 1.3)	0.194
IL-10 (pg/mL)	−2.1 (−11.8, 0.3)	−1.9 (−7.3, −0.1)	−4.7 (−15.5, −0.5)	0.101
IP-10 (pg/mL)	−303.4 (−654.5, −123.2)	−339.0 (−693.0, −40.6)	−289.2 (−547.2, −39.7)	0.435
IFNγ (pg/mL)	−2.3 (−12.0, 7.9)	−2.6 (−13.0, 7.2)	2.4 (−11.6, 18.5)	0.136
CK (μg/L)	1255.0 (495.0, 2135.0)	1139.0 (333.0, 3084.0)	2493.0 (1132.0, 3993.0)	<0.001
CK-MB (μg/L)	124.0 (54.6, 172.9)	100.2 (50.8, 170.4)	196.6 (43.6, 364.3)	0.247
Troponin I (μg/L)	27.2 (12.2, 86.7)	48.6 (19.4, 89.1)	99.8 (16.8, 199.6)	0.007
Troponin T (μg/L)	3.2 (1.5, 7.3)	3.4 (1.9, 6.3)	6.5 (2.3, 12.3)	0.135

* All Biomarkers displayed as median (Q1,Q3)

CK creatine kinase, *hsCRP* high sensitivity C-reactive protein, *NT-proBNP* N-terminal pro B-type natriuretic peptide, *IFNγ* interferon gamma, *IL* interleukin, *IL-1ra* IL-1 receptor antagonist, *IP-10* interferon inducible protein 10, *ST-E* ST-elevation, *TNFα* tumor necrosis factor alpha

measures was associated in time with changes in NT-proBNP and multiple pro-inflammatory cytokines (IL-6, IL-12, TNFα) but not with anti-inflammatory cytokines or chemokines evaluated in this study. Additionally, post-PCI TMPG was associated with temporal changes in NT-proBNP and IL-6. Second, the temporal relative changes in the pro-inflammatory cytokine TNFα and the anti-inflammatory cytokine IL-10 were independently associated with the 90-day composite of death, shock, or heart failure.

The pro-and anti-inflammatory cytokine responses to myocardial infarction have been well described, but the in vivo response to coronary reperfusion has remained less clear [20–22]. We observed that NT-proBNP and the pro-inflammatory cytokines IL-6, IL-12, and TNFα absolute changes were associated with electrocardiographic reperfusion and NT-proBNP was associated with post-PCI TMPG. IL-6 and TNFα are not normally expressed in healthy myocardium and synthesis has been shown to be upregulated in infarcted, peri-infarct and non-infarcted tissue zones following myocardial infarction, while IL-12 is expressed in atherosclerotic plaques [9, 23–25]. Recognizing that IL-6 and TNFα are proposed mediators of post-infarction cardiac remodeling and that IL-12 may accelerate further cardiac atherosclerosis, our findings may have implications for future efforts aimed at understanding the pathophysiologic link between reperfusion, the systemic inflammatory response, and both post-infarction heart failure and re-infarction [9, 26]. Although a causal effect cannot be inferred from the present analysis, our results suggest that myocardial reperfusion may be associated with important mediators of the temporal inflammatory response.

There are conflicting published associations between inflammatory cytokines, chemokines and outcomes after acute coronary syndromes [7, 27, 28]. We observed that a 24 h increase in the pro-inflammatory cytokine TNFα was associated with less 90-day death, shock or heart failure while a non-significant trend was observed with the anti-inflammatory cytokine IL-4 and chemokine IFNγ. Given inflammatory markers are correlated with myocardial infarct size, this analysis controlled for biomarker levels at the time of presentation and evaluated temporal changes in inflammatory biomarkers [29, 30]. IL-4 is known to attenuate the post-infarction pro-inflammatory of IL-1 and IL-6 response. This is first study to report an association between temporal changes in these inflammatory markers and clinical outcomes [30]. IL-10, an anti-inflammatory cytokine that is expressed in ischemic and reperfused myocardium, is thought to have a protective effect by inhibiting the production of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6 < IL-8, IL-12, TNFα) and metalloproteinases [31–33]. The observed positive association between temporal changes in IL-10 and 90-day clinical outcomes should be carefully interpreted in the context of studies that have reported that the ratio of pro:anti-inflammatory cytokines (and not absolute levels) are

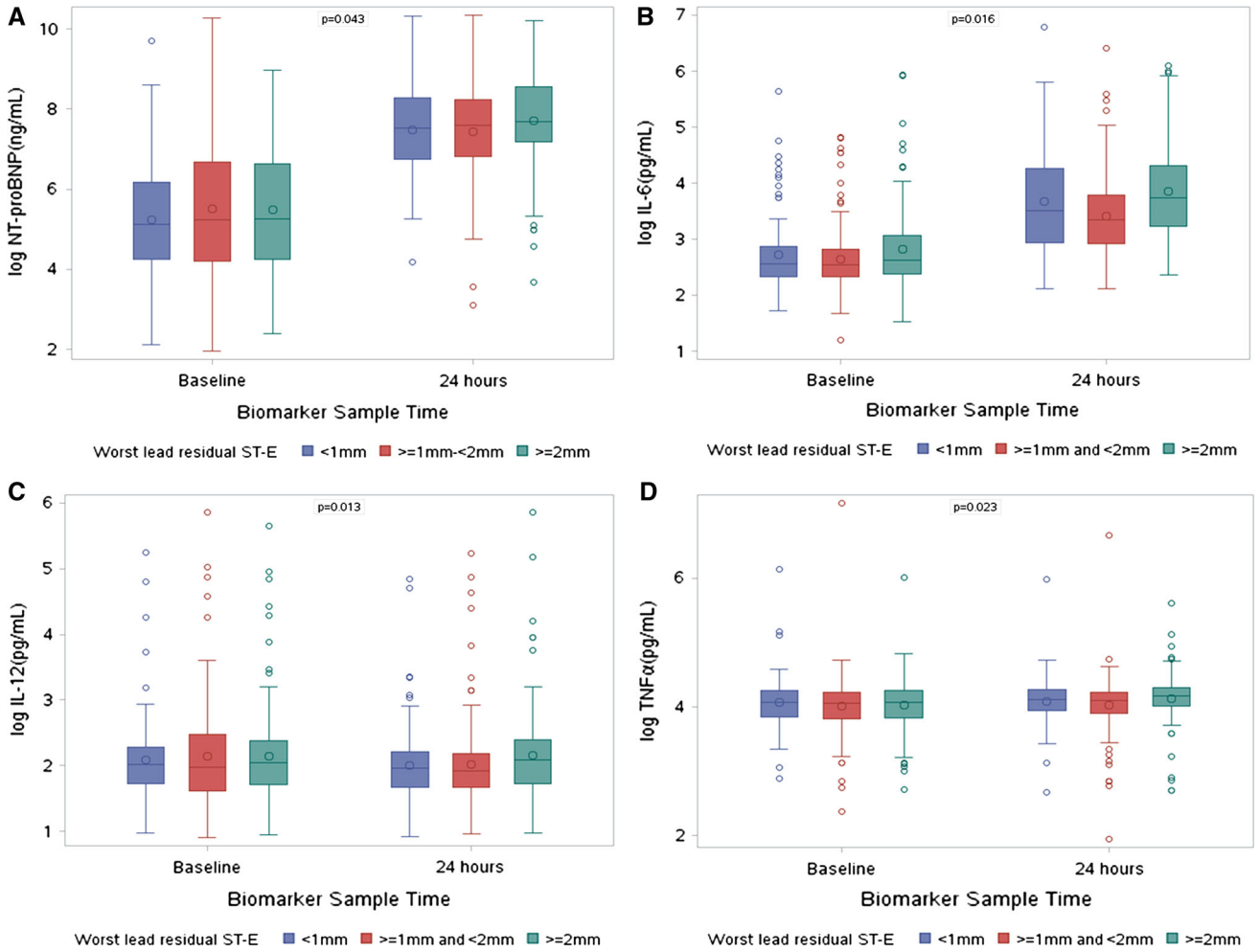

Fig. 1 Median baseline and 24 h biomarker values for **a** NT-proBNP, **b** IL-6, **c** IL-12, **d** TNFα stratified by the worst lead residual ST-Elevation. NT-proBNP, IL-6, IL-12, TNFα are temporally associated with electrocardiographic reperfusion

predictive of clinical events [34–36]. However, this inverse association with TNFα is both unexpected and potentially novel. TNFα mediates the pro-inflammatory effects through IL-6 release by vascular smooth muscle cells, which leads to increased leukocyte recruitment, platelet aggregation, apoptosis and cardiac remodeling [8, 9, 37]. This suggests that a temporal increase in TNFα should be associated with adverse clinical outcomes; however, other pre-clinical studies have suggested potential myocardial protective effects. TNFα expression has been found in both the peri-infarct and non-infarcted zone of the heart, suggesting a possible role in myocardial repair. Consistent with this hypothesis, double TNFα receptor knockout mice have increased myocardial apoptosis [32, 38, 39]. Although the pro-inflammatory TNFα has well reported deleterious physiologic effects, the in vivo effects of this inflammatory cytokine are incompletely understood and based on our results, we hypothesize that TNFα may have a net cardioprotective effect.

The present findings have implications for future biomarker research among patients with STEMI. The significant association between successful coronary reperfusion and temporal changes in inflammatory biomarker levels suggests that the timing of biomarker sampling relative to reperfusion is an important methodical consideration. We hypothesize that differences in biomarker sample timing may, in part, explain some the conflicting associations between post-infarction inflammatory biomarker levels and clinical outcomes reported in the literature. We also acknowledge that the biomarkers that were significantly associated with reperfusion were not significantly associated with clinical outcomes and the timing of biomarker sampling was remote form the 90-day clinical outcomes, thus precluding causal inferences. The modest sample size may have limited the ability to detect important associations and

Table 3 Median absolute change between baseline and 24 h biomarker levels stratified angiographic measures of reperfusion

Biomarker*	Post-PCI TIMI flow grade			TIMI myocardial perfusion grade (TMPG)		
	0/1/2 (n = 35)	3 (n = 316)	p-value	0/1 (n = 36)	2/3 (n = 308)	p-value
NT-proBNP (ng/mL)	2604.5 (1464.9, 4151.2)	1366.7 (589.4, 2916.0)	0.001	2649.3 (1572.6, 5033.0)	1382.7 (621.0, 2846.4)	0.002
hsCRP (mg/L)	15.5 (4.6, 27.7)	9.7 (3.7, 22.0)	0.143	16.9 (3.5, 41.8)	10.1 (3.8, 21.9)	0.139
IL-1β (pg/mL)	0.5 (−0.3, 1.2)	0.3 (−0.6, 1.1)	0.387	0.5 (−0.3, 1.3)	0.3 (−0.6, 1.1)	0.559
IL-6 (pg/mL)	22.6 (7.2, 46.2)	15.0 (5.2, 38.2)	0.205	28.7 (9.7, 61.3)	15.1 (5.2, 37.9)	0.040
IL-12 (pg/mL)	−0.3 (−1.1, 1.1)	−0.6 (−2.5, 0.8)	0.321	0.0 (−1.7, 1.1)	−0.6 (−2.5, 0.8)	0.238
TNFα (pg/mL)	3.6 (−4.8, 13.1)	1.9 (−4.6, 9.8)	0.475	2.5 (−6.4, 8.4)	2.0 (−4.4, 10.6)	0.297
IL-1ra (pg/mL)	−54.7 (−168.3, 39.8)	−58.3 (−144.3, 3.4)	0.636	−28.4 (−127.3, 21.0)	−56.9 (−152.2, 5.9)	0.269
IL-4 (pg/mL)	0.1 (−0.6, 0.9)	0.1 (−0.5, 1.0)	0.656	0.2 (−0.6, 0.9)	0.1 (−0.5, 1.0)	0.625
IL-10 (pg/mL)	−1.4 (−8.7, 0.2)	−3.1(−10.7,−0.1)	0.586	−1.1 (−7.5, 0.5)	−3.4 (−11.2, −0.3)	0.181
IP-10 (pg/mL)	−373.3 (−976.9, −82.8)	−318.6 (−609.7, −60.4)	0.278	−309.0 (−832.7, −131.8)	−316.0 (−607.6, −55.6)	0.646
IFNγ (pg/mL)	1.8 (−11.8, 8.5)	−0.4 (−11.4, 9.9)	0.926	−1.3 (−15.5, 12.9)	0.0 (−11.2, 9.9)	0.769
CK (μg/L)	1502.0 (951.0, 3054.0)	1711.5 (633.0, 3351.0)	1.0	2089.0 (405.0, 3311.0)	1685.5 (711.5, 3367.0)	0.933
CK-MB (μg/L)	132.1 (62.9, 360.3)	117.4 (58.2, 227.8)	0.746	107.8 (62.9, 200.6)	120.0 (54.7, 240.8)	0.652
Troponin I (μg/L)	50.0 (30.8, 87.6)	51.4 (17.5, 109.8)	0.820	86.0 (37.0, 199.6)	50.0 (18.7, 100.9)	0.155
Troponin T (μg/L)	5.0 (4.4, 9.6)	5.0 (1.9, 8.6)	0.993	6.8 (3.3, 8.5)	5.0 (1.6, 8.7)	0.711

* All Biomarkers displayed as median (Q1,Q3)

PCI percutaneous coronary intervention, *TIMI* thrombolysis in myocardial infarction

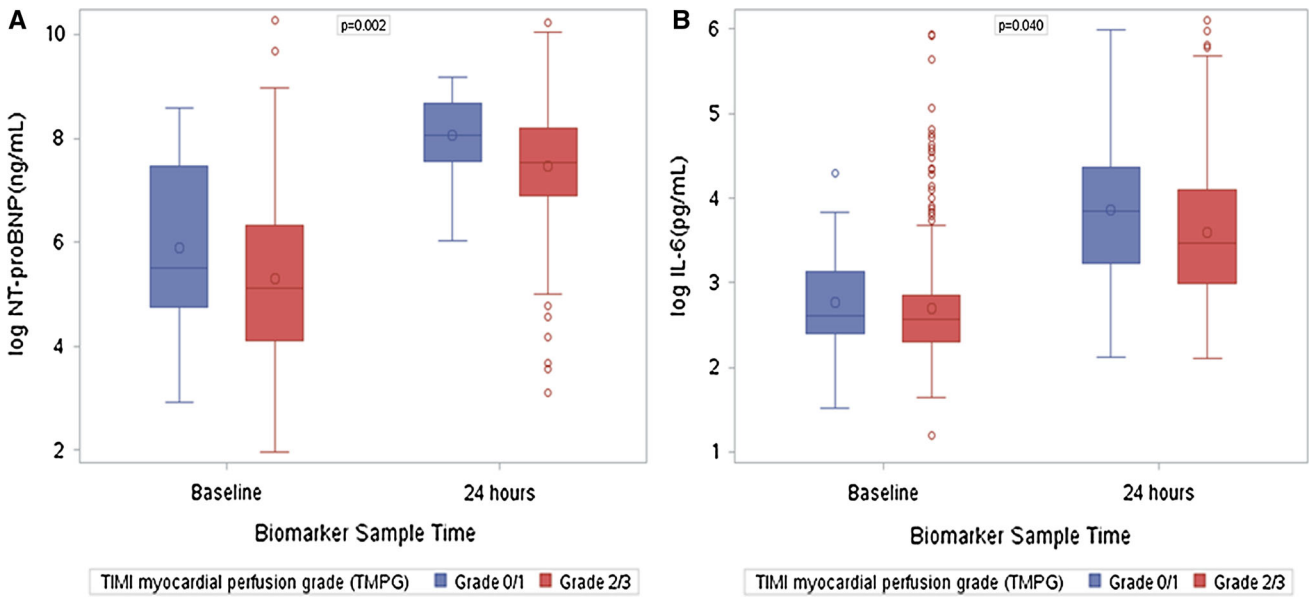

Fig. 2 Median baseline and 24 h biomarker values for **a** NT-proBNP and **b** IL-6 stratified by the Thrombolysis in Myocardial Infarction myocardial perfusion grade. NT-proBNP and IL-6 are temporally associated with successful Thrombolysis in Myocardial Infarction myocardial perfusion grade

further study in a larger dataset is required. Finally, the significant associations between changes in inflammatory biomarkers and clinical outcomes suggest that some serial evaluation of some biomarkers may contribute incremental information to dynamic clinical prediction models that have been shown to improve risk discrimination [19, 40].

Limitations and strengths

The study included a modest sample size and it may be underpowered to detect clinically important associations; however, this is the largest known dataset of paired inflammatory biomarkers in STEMI patients undergoing

Table 4 Associations between relative 24 h change in biomarkers and 90-day death, shock, or heart failure

Biomarker*	Unadjusted HR (95 % CI)	p-value	Adjusted† HR (95 % CI)	p-value	Adjusted‡ HR (95 % CI)	p-value
NT-proBNP	0.97 (0.82–1.14)	0.70	0.96 (0.79–1.18)	0.72	0.94 (0.78–1.14)	0.54
hsCRP¥		0.04		0.70		0.50
0	REF		REF		REF	
>0–2.3	0.79 (0.35–1.75)	0.55	0.71 (0.31–1.63)	0.42	0.90 (0.37–2.16)	0.81
>2.3	1.60 (0.69–3.68)	0.27	0.82 (0.30–2.27)	0.70	1.26 (0.50–3.17)	0.63
IL- 1β	1.18 (0.63–2.24)	0.65	0.75 (0.31–1.83)	0.52	1.02 (0.50–2.05)	0.97
IL-6	1.48 (1.16–1.89)	0.005	1.16 (0.86–1.55)	0.33	1.24 (0.95–1.62)	0.11
IL-12	1.65 (0.57–2.37)	0.62	1.19 (0.46–3.06)	0.72	0.91 (0.47–1.77)	0.77
TNFα	0.41 (0.20–0.85)	0.02	0.49 (0.27–0.87)	0.015	0.49 (0.26–0.95)	0.035
IL-1ra	1.39 (1.06–1.82)	0.02	1.01 (0.70–1.72)	0.68	1.15 (0.86–1.53)	0.35
IL-4	0.39 (0.15–1.05)	0.042	0.47(0.21–1.05)	0.066	0.45 (0.20–1.02)	0.056
IL-10	1.46 (1.10–1.94)	0.005	1.37 (0.93–2.02)	0.11	1.41(1.06–1.87)	0.018
IP-10	1.54 (1.11–2.13)	0.007	1.36 (0.90–2.05)	0.14	1.27 (0.95–1.70)	0.10
IFNγ	0.49 (0.21–1.19)	0.09	0.58 (0.28–1.23)	0.15	0.55 (0.27–1.14)	0.11

The number of patients and events for the biomarkers CK and Troponin (I/T) were too small to fit the Cox proportional hazards regression model

* The relative changes in the biomarkers were log transformed for analysis

† Adjusted for age, sex, smoking status, baseline Q, time to hospitalization from randomization, COPD, diabetes, stroke, systolic blood pressure, diastolic blood pressure, white blood cell count, serum creatinine, heart rate, Killip class, total ST segmentdeviation, MI location, right bundle branch block

‡ Adjusted for the covariates in the 24 h APEX-AMI dynamic model

¥ The log-transformation did not achieve linearity for hsCRP, thus it was analyzed categorically

reperfusion. The APEX-AMI paired biomarker substudy enrolled a high risk subset of STEMI patients, thus the results may not be generalizable to all STEMI patients. Since multiple biomarkers (and their associations) were evaluated without adjustment for multiple comparisons, statistically significant findings should be considered hypothesis generating. The 24 h markers of myonecrosis were not routinely recorded; thus, peak markers were used in the paired analyses. The present data are a subset of a biomarker substudy, given the small sample size we did not explore if previously published findings were consistent in this subset population [7]. Lastly, the study pooled treatment arms of the APEX-AMI study. Pexelizumab had neither an effect on clinical outcomes nor any of the study biomarkers with except IL-6 (24-levels were attenuated by pexelizumab treatment) [41].

Conclusions

In an international study population with centrally adjudicated outcomes, we observed that temporal changes in the pro-inflammatory cytokines IL-6, IL-12, and TNFα levels were associated with electrocardiographic evidence of reperfusion and temporal changes in IL-6 was associated with angiographic reperfusion. The composite of death, shock, or heart failure was independently and negatively associated with the 24 h change in the pro-inflammatory cytokine TNFα and positively associated with the anti-inflammatory cytokine IL-10. These findings provide potential clinical insights into the potential associations between reperfusion success and the inflammatory response in STEMI, with potential implications for post-infarction cardiac remodeling. Future research is required to elucidate whether reperfusion modulation of the systemic inflammatory response is causally linked to post-STEMI outcomes.

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Compliance with ethical standards

Conflict of interest Sean van Diepen, Wendimagegn G. Alemayehu, Yinggan Zheng: The authors declare they have no conflicts of interest; L. Kristin Newby, Christopher B. Granger: disclosures are publically available at https://www.dcri.org/about-us/conflict-of-interest; Kenneth W. Mahaffey: disclosure is publicly available at https://med.stanford.edu/profiles/kenneth-mahaffey?tab=research-and-scholarship; Paul W. Armstrong: disclosure is publically available at http://www.vigour.ualberta.ca/About/RelationshipsWithIndustry.aspx; Pierre

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