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The prognosis and outcomes of patients with a ST-segment elevation myocardial infarction and the biochemical and clinical inflammatory response.

by

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Abstract

Acute myocardial infarction, and specifically coronary plaque rupture, has been associated with a systemic inflammatory cascade. Using multiple large clinical trial datasets, we sought to further understand the systemic and clinical inflammatory responses in patients with ST-elevation myocardial infarction (STEMI) and have three key findings. First, the systemic inflammatory response syndrome (SIRS) was associated with 90-day clinical outcomes. Second, interleukin (IL)-6 was modestly correlated with NT-proBNP suggesting that NT-proBNP may be expressed in response to systemic inflammatory stimuli. Third, although multiple inflammatory biomarkers independently predicted 90-day clinical outcomes, they added little value to a clinical prediction model that included N-terminal B-type natriuretic peptide (NT-proBNP). These findings reinforce the importance of the inflammatory response in STEMI in relation to both clinical outcomes and possible linkages to inflammatory biomarker expression and the natriuretic peptide system.

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List of Symbols, Nomenclature, and Abbreviations

ACE: Angiotensin Converting Enzyme

ACS: Acute coronary syndrome

AF: Atrial fibrillation

APEX-AMI: Assessment of Pexelizumab in Acute Myocardial Infarction

AUC: Area under the curve

BNP: B-type natriuretic peptide

CABG: Coronary artery bypass grafting

CARDINAL: Complement and reduction of infarct size after angioplasty or lytics

CHF: Congestive heart failure

CI: Confidence interval

CK: creatine kinase

COMPLY: Complement inhibition in myocardial infarction treated with thrombolytics

COPD: Chronic obstructive pulmonary disease

ECG: electrocardiogram

IFNγ: Interferon gamma

IL: Interleukin

IL-1ra: IL-1 receptor antagonist

IP-10: Interferon inducible protein-10

IQR: Interquartile Range

MI: Myocardial infarction

NRI: Net reclassification improvement

NSTEMI: Non ST-segment elevation myocardial infarction

NT-proBNP: N-terminal B-type natriuretic peptide

PCI: percutaneous coronary intervention

r_s: Spearman correlation

SIRS: Systemic inflammatory response syndrome

STEMI: ST-segment elevation myocardial infarction

TNFα: tumor necrosis factor α

TnI: Troponin I

ULN: Upper limit of normal

WBC: White blood cell count

Σ STD: Sum ST segment deviation

Introduction

Coronary plaque rupture, which occurs in patients with ST-segment elevation myocardial infarction (STEMI), is associated with a complex systemic cytokine and chemokine inflammatory response.(1-6) Additionally, patients with ST-segment elevation myocardial infarction (STEMI) can manifest clinical physiologic changes suggestive of underlying systemic inflammation. The incidences and outcomes associated with many STEMI related biochemical and clinical inflammatory changes have been reported, but important clinical and translational research questions remain. First, the systemic inflammatory response syndrome (SIRS) is an adverse prognostic marker in multiple medical and surgical conditions, but whether SIRS is associated with mortality in STEMI remains unclear.(7,8) Second, studies reporting the independent associations between clinical outcomes and cytokines or chemokines have suggested they could potentially serve as prognostic STEMI biomarkers; however, whether they can improve the prognostic utility of ST-segment elevation myocardial infarction (STEMI) clinical risk prediction scores individually, or in a clinical environment where N-terminal B-type natriuretic peptide (NT-proBNP) is widely available, remains unknown. Third, NT-proBNP which is a validated prognostic biomarker in patients with STEMI has been reported to be elevated in patients with non-cardiac inflammatory conditions.(9-12) In vitro studies have suggested that NT-proBNP may be under inflammatory regulation, but the correlation between NT-proBNP and cytokines in a clinical population of patients with STEMI has not been reported.(13-16)

SIRS is an adverse prognostic marker in several medical and surgical conditions.(7,8) The biochemical inflammatory response to coronary plaque rupture in STEMI has been well described in pre-clinical and clinical studies, but little is known about clinical inflammatory response or its association with systemic inflammatory mediators. SIRS is

defined as ≥2 of the following criteria are present: 1) heart rate >90 per minute, 2) respiratory rate >20 per minute min, 3) body temperature >38 or <36°C, or 4) leukocyte count >12 or <4 x 10^9/L. Single center case series have reported that SIRS occurs in 11-19% of patients and outcomes associated with individual SIRS criteria in STEMI patients have been described; however the association between SIRS and clinical outcomes remain unclear because published reports have been limited by small sample sizes and a lack of multivariable adjustment.(17-23)

NT-pro BNP adds prognostic value to established STEMI risk prediction scores and plays a pathophysiologic role in STEMI.(9,10) Though BNP is commonly thought to result from myocardial stretch, emerging evidence suggests that it may also be regulated by inflammatory mediators.(24) In vitro studies have reported that the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and tumour necrosis factor alpha (TNFα) all stimulate BNP expression in cardiocytes.(13-15) This finding is supported by the recognition that elevated levels of BNP have been reported in inflammatory conditions with normal cardiac function, but the relationship between the pro-inflammatory cytokine responses and NT-proBNP levels has not yet been described in STEMI patients.(11,12) Additionally, IL-10 is an anti-inflammatory cytokine known to transiently rise following an acute coronary syndromes.(25) Pre-clinical studies have suggested that BNP can trigger an anti-inflammatory response by directly stimulating the release of IL-10, but validation of this finding in-vivo is lacking.(16) Thus, whether there is a correlation between the NT-proBNP and the systemic inflammatory response in STEMI has not yet been described.

A complex pro- and anti-inflammatory cytokine cascade accompanies coronary plaque rupture.(1-6) Studies reporting independent associations between pro-inflammatory markers (such as high sensitivity C-reactive protein [hsCRP], interleukin [IL]-6, tumor necrosis factor α [TNFα]) or anti-inflammatory cytokines (such as IL-1 receptor antagonist [IL-1ra] and IL-10) and clinical outcomes are conflicting.(25-32) Identifying the reason for the discrepancies is difficult because many of the studies were small, single-center studies with differences in study design, patient population, and timing of biomarker sampling. Thus, whether mediators of systemic inflammation are independently associated with clinical outcomes and whether they have prognostic utility in the STEMI population remains unclear. Moreover, NT-proBNP, a well validated STEMI biomarker, has been shown to add prognostic value to clinical risk prediction scores.(10,33) No studies have sought to evaluate whether the addition of inflammatory biomarkers can improve STEMI clinical prediction models either alone or in a risk prediction model that contains NT-proBNP.

This thesis seeks to understand the association between the inflammatory response, natriuretic peptides, and clinical outcomes for patients with acute STEMI. First, we described the incidence and outcomes associated with the diagnosis of SIRS at the time of STEMI presentation and at 24 hours post-admission. Second, we explored whether the inflammatory cytokines and chemokines can add prognostic value to a robust STEMI clinical risk prediction score. In addition, we evaluated whether these investigational biomarkers could improve the prognostic accuracy of a clinical model that contained NT-proBNP. Third, in an analysis, we described the correlation between the NT-proBNP and

inflammatory mediator response in an attempt to extend in-vitro studies of NT-proBNP inflammatory regulation to the clinical population. The unique objectives in the three manuscripts presented in this thesis all further the clinical or biochemical understanding of the inflammatory response in STEMI patients.

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Chapter 1:

Outcomes in ST-Segment Elevation Myocardial Infarction Patients with Systemic Inflammatory Response Syndrome[1]

[1] A version of this chapter has been accepted for publication. Van Diepen S, et al. Critical Care Medicine 2013.

Introduction

The systemic inflammatory response syndrome (SIRS) is a recognized sequela and adverse prognostic marker in a wide variety of infectious and non-infections conditions.[1-5] Coronary plaque rupture in the setting of myocardial infarctions is associated with a systemic biochemical inflammatory response, but considerably less is known about the clinical inflammatory response.[6, 7] SIRS has been reported in 11-19% of patients presenting with ST-elevation myocardial infarction (STEMI) however studies reporting associated outcomes have been limited to small case series with conflicting associations with mortality.[8-10] Thus, the association between SIRS and clinical outcome in STEMI remains unclear. In a population of STEMI patients receiving either mechanical or fibrinolytic reperfusion, we explored outcomes associated with the diagnosis of SIRS at the time of STEMI presentation and at 24 hours post-admission. We also sought to build on previous methodologies by evaluating the additive effect of individual SIRS criteria and how dynamic changes in the SIRS response over the first 24 hours influence outcomes.

Methods

Study Population

The Complement And ReDuction of INfarct size after Angioplasty or Lytics (CARDINAL) program included two international multi-center placebo-controlled phase II randomized trials that evaluated the efficacy of pexulizumab (a humanized monoclonal antibody C5 complement inhibitor) in the STEMI population.[11, 12] Patients presenting < 6 hours from symptom onset with at least 2 mm of ST-elevation or a new left bundle

branch block were eligible for enrollment. Patients randomized to the COMplement inhibition in Myocardial infarction treated with Angioplasty (COMMA) trial underwent primary percutaneous coronary intervention (PCI) and patients in the COMPlement inhibition in myocardial infarction treated with thromboLYtics (COMPLY) trial underwent fibrinolysis.[11, 12] The primary outcome of interest in both trials was the composite of death, shock, heart failure, or stroke at 90 days. The secondary endpoint was infarct size assessed by 72 hour creatine kinase (CK)-MB area under the curve. No difference was observed with pexulizumab in the primary or the composite secondary endpoints in the definitive subsequent phase III randomized trial, thus patient populations were pooled for the purposes of this analysis.[13] Informed consent was obtained from all study participants and the institutional review board at all participating hospitals approved the study protocol.

Systemic Inflammatory Response Syndrome Definition

The present analysis evaluated outcomes in patients who did and did not meet SIRS criteria at the time of presentation and 24 hours. SIRS was defined based on the American College of Chest Physicians and Society of Critical Care Medicine criteria as two or more of the following criteria: heart rate > 90 beats/minute, respiratory rate > 20 breaths/minute, leukocyte count > 12 or < 4 * 10^9/L, temperature > 38°C or **<** 36°C.[3] The percentage of immature neutrophils and PaCO2 levels were not available in the CARDINAL dataset.

Outcomes

The primary outcome was the 90 day incidence of death, cardiogenic shock, heart failure, or stroke. Secondary outcomes of interest include the individual components of the primary outcome and infarct size (assessed using 72 hour CK-MB area under the curve). The primary analysis compared the outcomes associated with and without SIRS at baseline and 24 hours. Pre-specified secondary analyses included evaluating the individual and cumulative risk of SIRS criteria, dynamic changes in SIRS criteria, and the association between SIRS at 24 hours and reperfusion success.

Statistical Analysis

Patient characteristics and outcome rates were presented by baseline SIRS diagnosis, with continuous variables summarized using the median (Interquartile Range[IQR]), and categorical data summarized by frequencies and percentages. P-values for continuous variables were obtained using the ANOVA F-test, except when the assumption of normality was not satisfied, in which case the Wilcoxon Rank Sum test was used. For categorical variables, the chi-square test was used, where appropriate; otherwise, the Fisher's Exact test was used.

Unadjusted and adjusted Cox proportional hazards models were used to assess the relationship between outcomes and the diagnosis of SIRS and the total number of SIRS criteria. These models were applied to SIRS criteria at baseline and at 24 hours post-admission. Patients experiencing events within the first 24 hours were excluded from the latter analysis. The baseline covariates used for adjustment were age, sex, prior myocardial infarction, prior heart failure, mean arterial pressure, anterior infarction, creatinine clearance, sum ST-segment elevation , symptom onset to device or

fibrinolysis time, and trial (COMMA vs. COMPLY). SIRS components were considered individually in the unadjusted model, and jointly in the multivariate model.

Tabulations of patient characteristics were applied to complete-case data. In modeling, multiple imputation (25 imputations) was employed to estimate coefficients, hazard ratios, and standard errors, after imputing missing data. Missing values of covariates were estimated based on the 90-day endpoint itself, with time-to-event, as well as available data for other covariates. The majority of candidate predictors were missing fewer than 10% of data values. Many predictors had <1% missing data, and the highest extent of missingness was 24%. Derived variables, including symptom indicators and symptom counts, were derived from the imputed data. Statistical analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, North Carolina).

Results

Study Population

Among the 1903 patients in the CARDINAL project, 1843 (96.8%) were included in the analysis. Time of event was not available in 60 patients and they were excluded. Data for all 4 SIRS criteria was available in 1186 (64.4%) of patients at the time of presentation. A total of 1761 patients were alive at 24 hours and 1142 (64.8%) of these patients had recorded data for all 4 SIRS criteria. In the complete case analysis, SIRS was present in 296 (25.0%) of study participants at the time of presentation. Among patients who survived to 24 hours, 92 (8.1%) patients met SIRS criteria. Baseline variables in patients with and without SIRS at the time of presentation are presented in

Table 1. Patients with SIRS were older and were more likely to have a history of diabetes, to smoke, to have higher mean arterial blood pressure, to have larger electrocardiographic sum ST-segment elevation, and to have higher use of anti-platelet medication. All 4 SIRS criterion were significantly different between the two groups.

Complete Case Outcomes

The primary and secondary outcomes for patients at the time of STEMI presentation and 24 hours post admission are presented in Table 2. The 90 day composite of death, cardiogenic shock, heart failure, and stroke was higher among patient with SIRS (21.6% vs. 11.9%, $p<0.001$) compared to patients without SIRS at the time of presentation. Death (10.1% vs. 6.1%, $p=0.018$), cardiogenic shock (6.8% vs. 3.6 %, $p=0.021$), and the median 72 hour CK-MB area under the curve (5008 vs. 4141; $p=0.001$) were also significantly higher among SIRS patients. At 24 hours, the primary composite outcome was higher in SIRS patients (21.7% vs. 10.1%, $p=0.001$). Among patients who survived to 24 hours, the observed 90 day composite outcome was significantly different between patients with dynamic changes in the diagnosis of SIRS within the first 24 hours of admission ($p<0.001$ across groups, Figure 1). Among patients without SIRS at presentation, 90 day outcomes were higher among patients who developed SIRS at 24 hours (21.3% vs. 8.7%). Among participants with SIRS at presentation, the resolution of SIRS at 24 hours was associated with a lower risk of 90 day outcomes compared with those with persistent SIRS (15.1% vs. 22.2%).

A total of 507 COMMA patients who survived to 24 hours had post-procedural TIMI grades recorded. The incidence of SIRS at 24 hours among patients with TIMI 3 (10.9%)

and TIMI ≤ 2 (8.6%) flow was not significantly different (p=0.535). In the COMPLY trial, ST segment resolution was available in 547 patients. There was no statistical difference in the incidence of SIRS at 24 hours in patient with ST segment resolution ≥ 70% vs. <70% (0% vs. 7.5%, p=0.614).

Multivariable and Imputed Analyses

A total of 1843 patients at baseline and 1761 patients at 24 hours were included in models that adjusted for baseline covariates and imputed missing SIRS criteria. The diagnosis of SIRS remained significantly associated with higher 90 day outcomes at the time of presentation (31.0% vs. 16.7%; adjusted Hazard Ratio [adj HR] 1.78: 95% Confidence Interval [CI], 1.35 to 2.34; p<0.001) and at 24 hours (36.7% vs. 11.1%; adj HR 2.84: 95% CI, 2.03 to 3.97; P<0.001).

The hazard ratios associated with individual and the cumulative number of SIRS criteria at baseline and at 24 hours after STEMI admission are presented in Tables 3 and 4. At both time points, heart rate, respiratory rate, and white blood cell count were all associated with a higher unadjusted risk of the 90 day composite outcomes. After multivariable adjustment, heart rate and white blood cell count remained significantly associated with a higher incidence of the primary outcome. The contribution associated with individual SIRS criteria to 90 day outcomes based on the percentage of the total adjusted chi-squared are presented in Figure 2. At the time of presentation, heart rate and white blood cell count account for 80.0% and 14.6% of the association, respectively. At 24 hours, white blood cell count (60.3%) accounted for more of the association than heart rate (30.2%).

Figure 3(a) and 3(b) display the adjusted risk associated with the cumulative number of SIRS criteria. A higher risk of death, cardiogenic shock, heart failure, or stroke was independently associated with the cumulative number of SIRS criteria at the time of hospital presentation (HR 1.41: 95% CI, 1.24 to 1.61; p<0.001, per additional SIRS criteria) and after 24 hours (HR 1.72: 95% CI, 1.47 to 2.01; p<0.001, per additional SIRS criteria). In sensitivity analyses using the complete case study population to examine associations between SIRS and individual SIRS criteria with the 90 day primary composite outcome, results were similar (data not shown).

Discussion

This analysis of STEMI patients undergoing reperfusion has three novel findings. First, the diagnosis of SIRS at the time of hospital presentation or at 24 hours post-admission was independently associated with an increased risk of death, cardiogenic shock, heart failure, or stroke at 90 days. Second, the cumulative number of individual SIRS criteria was independently associated with a higher risk of 90 day clinical outcomes. Third, the development or resolution of SIRS within the first 24 hours of admission modified the association with 90 day outcomes.

In response to calls for uniform clinical terminology, the American College of Chest Physicians and Society of Critical Care Medicine proposed a consensus definition of SIRS in 1991.[3, 14, 15] Subsequently, SIRS has been a reported marker of patient morbidity and

mortality in a variety of medical and surgical illnesses.[1, 2, 4, 5, 16] Coronary plaque rupture is associated with pro-inflammatory cytokine and chemokine responses, and independent associations between some inflammatory biomarkers and mortality have been reported.[7, 17-21] Comparatively little, however, is known about the clinical inflammatory response in patients with a myocardial infarction. Single institution case series of less than 250 patients have estimated 11-19% of STEMI patients develop SIRS, but these small sample sizes have precluded meaningful multivariable analyses.[8, 10] In this analysis, the incidence of SIRS was 25% at presentation and 8% at 24 hours. Moreover, the presence of SIRS at both time points was independently associated with higher incidence of the primary composite 90 day outcome and death alone. These findings are consistent with studies reporting adverse sequalae in SIRS patients with other cardiovascular diagnoses. In patients undergoing cardiopulmonary bypass, SIRS has been associated with adverse peri-operative hemodynamic changes and in a SHOCK trial analysis, patients with SIRS (using a modified definition of fever or leukocytosis) had lower systemic vascular resistances, longer hospitals stays, and were at a higher risk of death.[22, 23] Finally, in a recent study of 152 transcatheter aortic value patients, the diagnosis of SIRS within the first 48 post-procedural hours was associated with higher mortality at 1 year.[24] Taken together, these findings suggest that the diagnosis of SIRS is an adverse prognostic marker in a number of cardiovascular conditions.

The prognoses associated with the individual SIRS diagnostic criteria have been described in patients with myocardial infarction. Elevated heart rate, respiratory rate, and leukocytosis are all reported independent risk factors for mortality while body temperature has been correlated to infarct size.[25-29] In this analysis, only heart rate

and leukocyte count at the time of presentation and at 24 hours were independently associated with 90 day mortality, though the adjusted point estimates of body temperature and respiratory rate were non-significantly associated with risk at 24 hours. It is possible that analyzing the SIRS physiologic criterion as categorical (as opposed to continuous) variables could have diminished the statistical power to detect an independent association with these latter 2 conditions. Nonetheless, our analysis also shows that the individual components of the SIRS diagnostic criteria are cumulatively associated with an increased risk of 90 day outcomes. A previous study of patients with acute liver failure reported that the number of SIRS criteria manifested by patients was associated with higher grades of encephalopathy and mortality.[2] We hypothesize that the number of SIRS criteria manifested reflects illness severity in STEMI patients.

In this analysis, the development or resolution of SIRS within the first 24 hours of admission modified the association with risk of 90 day clinical outcomes. Moreover, the contributions associated with individual SIRS criteria to 90 day outcomes were different between the time of presentation and 24 hours. These finding suggest that the risk associated with SIRS, and its individual physiologic variables, are not constant in the first 24 hours after STEMI hospitalization. Although this analysis was not designed to assess the prognosis, our findings re-enforce the potential utility of dynamic prognostic modeling. Dynamic models have reported improved discrimination in the critical care and non ST-elevation myocardial infarction populations; however they are not routinely use in clinical care.[30, 31] The familiarity and routinely available variables in SIRS makes it

an attractive simple bedside risk assessment tool. Future studies designed to evaluate the discrimination and calibration of SIRS in a clinical prediction model are required.

Limitations

This analysis has several limitations. First, the percentage of immature neutrophils and PaCO2 levels were not available in the CARDINAL dataset, thus the exact American College of Chest Physicians and Society of Critical Care Medicine SIRS definition could not be tested. Second, this analysis describes the associations between SIRS and outcomes, thus these results cannot be used for clinical risk prediction. Missing data may have affected the ability to define associations, although imputation should limit the impact. Finally, routine surveillance cultures were not performed and infections complications were not a pre-specified endpoint in the CARDINAL project, thus the relationships between SIRS, infectious complications, and outcomes could not be explored.

Conclusion

In an international population of STEMI patients undergoing reperfusion, a diagnosis of SIRS in STEMI patients either at the time of presentation or at 24 hours post-admission was independently associated with death, shock, heart failure, or stroke at 90 days. Additionally, the number of individual SIRS criteria and dynamic changes in SIRS within the first 24 hours of admission were associated with 90 day clinical outcomes. The association of this simple composite measure with both clinical outcomes and the systemic inflammatory response underscores the importance of the clinical inflammatory response in acute myocardial infarction.

Table 1-1: Baseline characteristics of patients with and without SIRS at presentation (complete case analysis).

Baseline Demographics	**No SIRS (n=890)**	**SIRS (n=296)**	**p value**
Age, median (IQR), years	61.0 (52.0, 72.0)	59.0 (50.0, 70.0)	0.020
Women, n (%)	247 (27.8)	92 (31.1)	0.272
Weight, median (IQR), kg	80.0 (70.0, 90.0)	78.0 (68.7, 90.0)	0.277
Clinical History, n(%)			
Diabetes mellitus	131 (14.7)	67 (22.6)	0.002
Myocardial Infarction	152 (17.1)	45 (15.2)	0.453
Heart Failure	76 (8.5)	27 (9.1)	0.758
Atrial Fibrillation	57 (6.9)	16 (6.1)	0.642
Cerebrovascular Disease	49 (5.5)	19 (6.4)	0.558
Current or Past Smoker	575 (64.9)	222 (75.8)	0.001
Presenting Characteristics			
Mean Arterial Blood Pressure, median (IQR), mmHg	98.7 (87.0, 111.3)	103.7 (88.0, 117.0)	0.007
Heart Rate, median (IQR), beats/min	74.0 (65.0, 83.0)	92.0 (74.0, 101.5)	<0.001
Respiratory Rate, median (IQR), rate/min	18.0 (16.0, 20.0)	22.0 (20.0, 24.0)	<0.001
Body Temperature, median (IQR), ^{o}C	36.4 (36.1, 36.7)	36.1 (35.6, 36.6)	<0.001

White Blood Cell Count, median (IQR), 10^9/L	9.9 (8.1, 11.8)	13.2 (11.2, 15.6)	<0.001
Anterior ST-Elevation on ECG, n (%)	697 (78.3)	241 (81.4)	0.255
Symptom onset to Device or Fibrinolysis time, median (IQR), min	3.2 (2.2, 4.3)	3.0 (2.2, 4.4)	0.976
Sum ST Segment Deviation, median (IQR), mm	9.5 (5.5, 15.0)	11.0 (7.0, 17.0)	<0.001
Estimated Creatinine Clearance*, median (IQR), ml/min	92.6 (68.0, 116.7)	90.5 (69.8, 116.5)	0.719
CK-MB median, (IQR) ng/mL	6.4 (3.1, 17.6)	6.7 (3.1, 25.1)	0.165
COMMA Patients, n (%)	403 (45.3)	153 (51.7)	0.056
COMPLY Patients, n(%)	487 (54.7)	143 (48.3)	0.056
Drug Treatment, n(%)	n 837	n=283	
ASA	769 (91.9)	270 (95.4)	0.047
Beta Blocker	743 (88.8)	259 (91.5)	0.193
ACE Inhibitor	646 (77.2)	232 (82.0)	0.090
Statin	549 (65.6)	175 (61.8)	0.253
Thienopyridines (COMMA patients only)	496 (59.3)	185 (65.4)	0.069
Glycoprotein IIb/IIIa Inhibitor (COMMA patients only)	375 (44.8)	157(55.5)	0.002

*Creatinine Clearance was calculated using the Cockcroft–Gault formula

Abbreviations: ACE, Angiotensin Converting Enzyme; AUC, area under the curve; ^{o}C, degrees Celsius; hsCRP; ECG, electrocardiogram; high sensitivity C-reactive Protein; IQR, interquartile range; min; minutes; IL, Interleukin; TNF, tumor necrosis factor; SIRS, systemic inflammatory response syndrome.

Table 1-2: 90-day outcomes among patients with and without SIRS at the time of presentation and at 24 hours (complete case analysis).

Outcome	Presentation		p value	24 hours*		p value
	No SIRS (n=890)	SIRS (n=296)		No SIRS (n=1050)	SIRS (n=92)	
Death, Shock, Heart Failure, or Stroke	106 (11.9)	64 (21.6)	<0.001	106 (10.1)	20 (21.7)	0.001
Death	54 (6.1)	30 (10.1)	0.018	41 (3.9)	8 (8.7)	0.052
Cardiogenic Shock	32 (3.6)	20 (6.8)	0.021	19 (1.8)	4 (4.3)	0.107
Heart Failure	56 (6.3)	28 (9.5)	0.066	69 (6.6)	11 (12.0)	0.052
Stroke	9 (1.0)	4 (1.4)	0.747	5 (0.5)	1/92 (1.1)	0.397

*Only patients surviving to 24 hours included in this analysis

Table 1-3: Univariable and multivariable relationships between individual SIRS diagnostic criteria at the time of STEMI hospital presentation and the 90-day primary composite outcome[*]

SIRS Criteria	Univariable		Multivariable	
	Hazard Ratio (95% CI)	**p Value**	**Hazard Ratio (95% CI)**	**p Value**
Heart Rate >90/min	2.37 (1.86 to 3.03)	<0.001	2.17 (1.67 to 2.81)	<0.001
Respiratory Rate >20/min	1.33 (1.01 to 1.75)	0.041	1.24 (0.94 to 1.64)	0.125
Temperature >38°C or < 36°C	1.01 (0.73 to 1.40)	0.962	0.94 (0.68 to 1.31)	0.732
WBC >12 or <4	1.46 (1.12 to 1.91)	0.005	1.42 (1.08 to1.86)	0.012
Number of SIRS Criteria[†]				
1	1.24 (0.89 to 1.74)	0.201	1.28 (0.92 to 1.78)	0.146
2	1.63 (1.12 to2.37)	0.010	1.74 (1.19 to 2.56)	0.005
3	3.04 (1.88 to 4.91)	<0.001	2.78 (1.67 to 4.61)	<0.001
4	6.89 (3.05 to 15.58)	<0.001	5.03 (2.22 to 11.38)	<0.001

[*]Analysis using imputed data for missing SIRS variables

[†]All hazard ratios expressed compared to patients with no SIRS criteria

Abbreviations: °C, degrees Celsius; CI, confidence interval; SIRS, systemic inflammatory response syndrome; WBC, white blood cell count

Table 1-4: Univariable and multivariable relationships between individual SIRS diagnostic criteria at 24 hours after hospital admission and 90-day primary composite outcome[*]

SIRS Criteria	Univariable		Multivariable	
	Hazard Ratio (95% CI)	**p Value**	**Hazard Ratio (95% CI)**	**p Value**
Heart Rate >90/min	2.57 (1.86 to 3.56)	<0.001	1.78 (1.25 to 2.54)	0.002
Respiratory Rate >20/min	1.84 (1.29 to 2.62)	<0.001	1.28 (0.89 to 1.85)	0.186
Temperature >38°C or < 36°C	1.24 (0.72 to 2.14)	0.443	1.40 (0.81 to 2.44)	0.232
WBC >12 or <4	2.40 (1.76 to 3.27)	<0.001	2.17 (1.54 to 3.04)	<0.001
Number of SIRS Criteria[†]				
1	1.81 (1.28 to 2.56)	<0.001	1.78 (1.26 to 2.50)	0.001
2	3.66 (2.45 to 5.48)	<0.001	3.51 (2.34 to 5.26)	<0.001
≥3[‡]	5.23 (2.68 to 10.17)	<0.001	4.03 (2.06 to7.90)	<0.001

[*]Analysis using imputed data for missing SIRS variables

[†]All hazard ratios expressed compared to patients with no SIRS criteria

[‡] Only 1 patient manifested all 4 SIRS criteria at 24 hours

Abbreviations: °C, degrees Celsius; CI, confidence interval; SIRS, systemic inflammatory response syndrome; WBC, white blood cell count

Figure 1- 1: 90-day incidence of death, shock, heart failure or stroke stratified by the development and resolution of SIRS within the first 24 hours (complete case analysis).

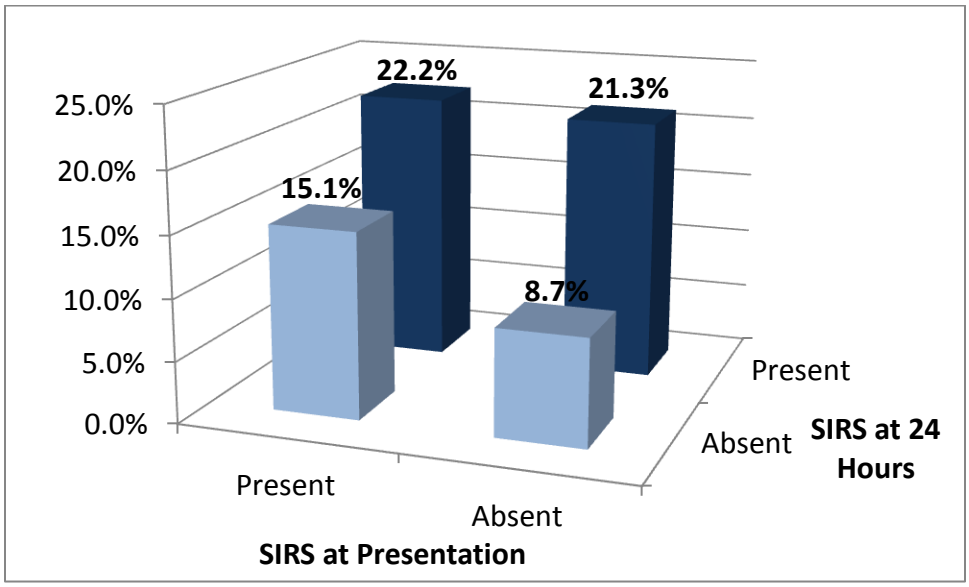


Figure 1-2: Contribution of individual SIRS criteria to 90-day outcomes based total adjusted chi-squared (imputed analysis).

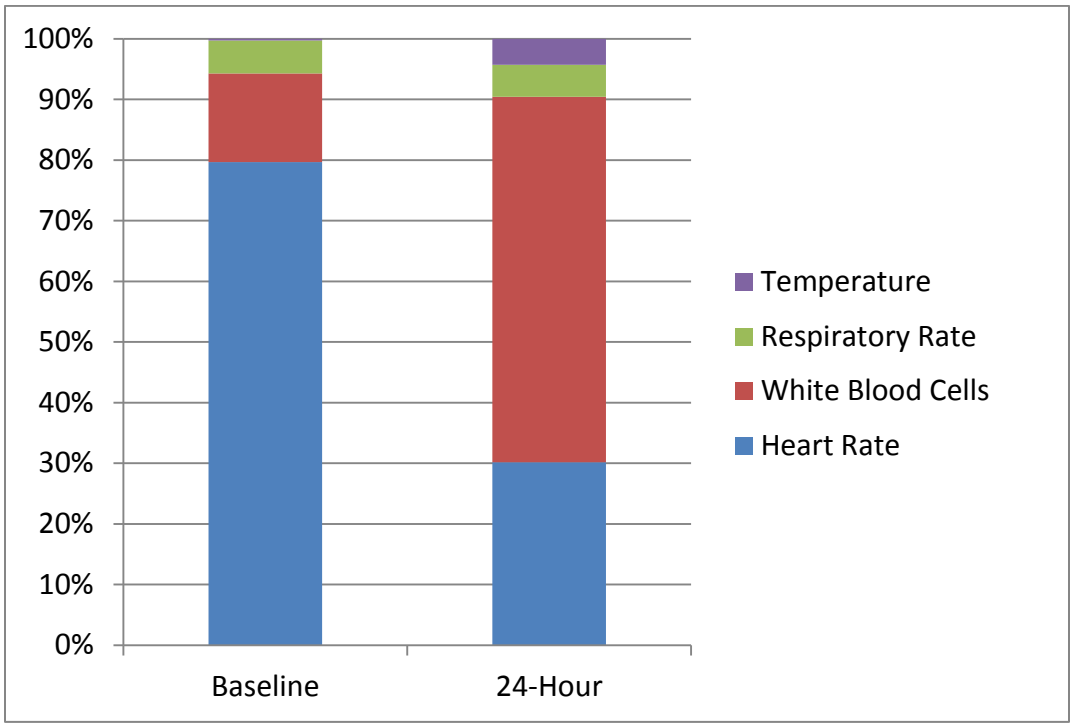
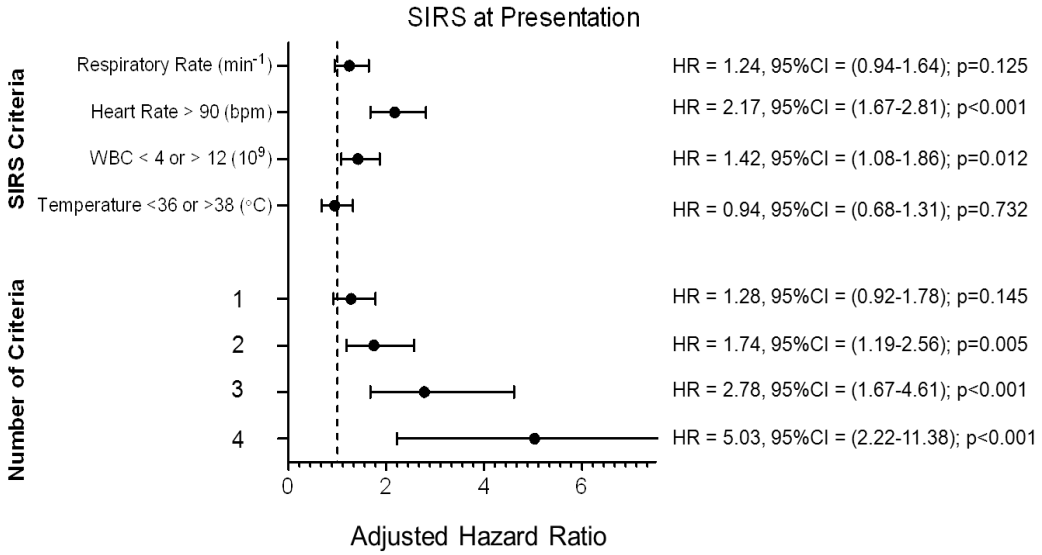
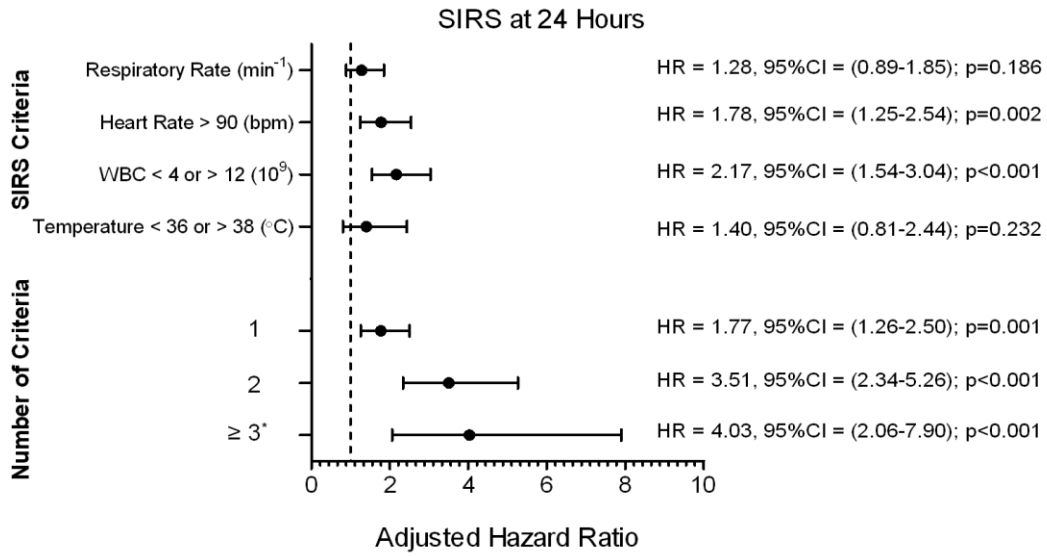


Figure 1-3: Adjusted risk of the 90-day primary outcome among for individual SIRS criteria and the cumulative number of SIRS criteria among STEMI patients at (a) Hospital Presentation (b) 24 hours post-admission (imputed analysis).





* Only 1 patient manifested all 4 SIRS criteria at 24 hours

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Chapter 2:

Prognostic Significance of Acute Phase Relevance of Baseline Pro- and Anti-Inflammatory Biomarkers in Patients with ST-Segment Elevation Myocardial Infarction[1]

[1] A version of this chapter has been pubished. Van Diepen S ,et al. International Journal of Cardiology 2013; http://dx.doi.org/10.1016/j.ijcard.2013.01.004

Introduction

There is a growing body of evidence that the complex cytokine and chemokine cascades that accompany coronary plaque rupture trigger both pro- and anti-inflammatory responses.(1-6) The prognostic significance of baseline pro-inflammatory markers (such as high sensitivity C-reactive protein [hsCRP], interleukin [IL]-6, tumor necrosis factor α [TNFα]) and anti-inflammatory biomarkers (such as IL-1 receptor antagonist [IL-1ra], IL-10) have been reported with conflicting results.(7-14) Many of these studies were small, single-center studies and differences in study design, patient population, and timing of biomarker sampling make cross-study comparisons difficult. Similarly, inflammatory chemokine levels of interferon gamma (IFNγ) and interferon inducible protein-10 (IP-10) are known to rise in acute coronary syndromes; however the prognostic significance of these biomarkers has not yet been described.(6,15) Thus, whether inflammatory biomarkers are independent prognostic markers in the ST-segment elevation myocardial infarction (STEMI) population remains unclear. Moreover, their clinical utility currently remains unknown because, unlike N-terminal B-type natriuretic peptide (NT-proBNP), no studies have evaluated whether the addition of inflammatory biomarkers can improve existing STEMI clinical prediction models.(16,17)

Since the APEX-AMI trial evaluated the anti-inflammatory strategy of complement inhibition, we prospectively designed the program to evaluate the significance of inflammatory biomarkers with clinical outcome. This substudy of an international population of STEMI patients undergoing primary percutaneous coronary intervention (PCI) sought to evaluate whether (1) baseline inflammatory biomarkers are independent predictors of a 90-day composite endpoint of death, shock, or heart failure; (2)

biomarkers can improve the prognostic accuracy of a STEMI risk prediction model that uses baseline clinical variables; (3) biomarkers can improve prognostic accuracy of a prediction model that contains NT-proBNP. The latter analysis was pre-specified given that NT-proBNP is widely available and has been shown to improve STEMI clinical risk prediction.(16)

Methods

The Assessment of Pexelizumab in Acute Myocardial Infarction (APEX-AMI) trial design and results were previously reported.(18,19) Briefly, this was a multicenter, double blind, placebo-controlled, randomized clinical trial that compared pexelizumab versus placebo in 5745 STEMI patients undergoing primary PCI. Patients over the age of 18 years were eligible if they presented for primary PCI within 6 hours of symptom onset with high-risk electrocardiographic features. Since no effect of treatment on clinical outcomes was observed, patients from the pexelizumab and placebo arms were pooled for the purposes of this study. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.(20)

Biomarker Substudy Protocol

The APEX-AMI biomarkers substudy protocol was previously described.(21) Briefly, the substudy recruited consenting patients only at US APEX-AMI study centers and global angiographic and magnetic resonance imaging APEX-AMI substudy centers. The biomarker substudy was initially designed to enroll 4000 patients; however, due to premature discontinuation of the trial and budgetary changes, the substudy size was

substantially reduced. Participants had blood samples drawn immediately after randomization, but prior to study drug administration and PCI. Samples were allowed to clot, then centrifuged and the resulting serum was frozen to -20°C immediately, then as soon as possible to -70°C. Samples were bulk shipped on dry ice to a central collection facility (Duke Center for Human Genetics, Durham, NC, USA), and at the end of the study to a central laboratory (Montreal Heart Institute, Montreal, Quebec, Canada), for batch analyses. Each participating hospital's Institutional Review Board approved the protocol and patients were required to provide written informed consent.

HsCRP was measured by particle-enhanced immunonephelometry (Dade Behring Nephelometer, Germany) and N-terminal pro-brain natriuretic peptide (NT-proBNP) by electrochemiluminescence immunoassay (Roche Elecsys, Roche Diagnostics, Indianapolis, IN). The remaining cytokines and chemokines were measured with Bio-Plex assays technology and kits (LUMINEX 200, Luminex Corporation, city, state and Bio-Rad Laboratories Inc., Austin, TX). They were: pro-inflammatory cytokines IL-1β, IL-6, IL-12, and TNFα; anti-inflammatory cytokines IL-1ra, IL-4, and IL-10; chemokines IFNγ and IP-10.

Outcomes

The primary outcome of interest was the 90-day composite of all-cause death, shock or heart failure (new or worsening). This composite endpoint 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.(18) The incremental prognostic value of the individual biomarkers in a clinical model for 90-day death, shock,

or heart failure was assessed using net reclassification improvement (NRI), integrated discrimination improvement (IDI), and changes in the c-index.(22)

Statistical Analysis

Baseline characteristics are displayed as medians, 25th and 75th percentiles for continuous variables and frequencies and counts for categorical variables. The Wilcoxon rank-sum test is reported for continuous factors and the Pearson chi-square for binary and categorical variables. All biomarkers were normalized by using $\log_2$ transformation. Cox proportional hazard modeling was used to assess the univariable relationship of each baseline biomarker with 90-day outcome. These biomarkers were then individually added to a multivariable model, including only baseline factors. Hazard ratios (HR) (95% confidence intervals [CI]) and p values are reported for both the univariable and multivariate relationships of biomarkers with death, shock or heart failure within 90 days of randomization. Hazard ratios refer to a doubling in these markers. The factors included in multivariable modeling were age, sex, COPD, smoking status, diabetes, history of stroke, systolic blood pressure, diastolic blood pressure, time to hospital arrival from randomization, baseline white blood cell count, baseline serum creatinine, baseline heart rate, qualifying episode Killip class, total ST-segment deviation, MI location, right bundle branch block and baseline q wave. Modeling assumptions of normally distributed continuous factors and linearity were assessed. To assess if any biomarker improved risk classification by the baseline multivariate model, the c index, net reclassification improvement result and p value along with the integrated discrimination improvement result and p values are reported.(22) This step was repeated after baseline NT-proBNP was incorporated into the clinical risk model.

Tertiles of IL-6, hsCRP and IP-10 are displayed in Kaplan-Meier curves and the log-rank test statistic was generated.

Results

Study Population

The APEX-AMI biomarker substudy included 772 patients (13.3%) from among the 5745 patients enrolled in the overall trial. In the substudy cohort there were 210 (25.4%) 90-day death, shock, or heart failure events. Baseline patient characteristics of the substudy participants and the overall trial population are shown in Table 2-1. Compared with the overall APEX-AMI population, patients in the substudy were older, more often female and from North America, and more frequently had hypertension, prior cardiovascular disease, chronic obstructive pulmonary disease, higher Killip class at presentation, anterior infarction, and higher serum creatinine. Substudy patients were less likely to smoke and had lower blood pressure and shorter symptom onset to randomization time.

Univariable Relationship of Biomarker Levels with 90-day Outcome

Biomarker levels in patients with and without death, shock or heart failure at 90 days are presented in Table 2-2. Levels of hsCRP, NT-proBNP, IL-6, 1l-10 and IL-1ra were all significantly higher among patients who had events. IP-10 levels were higher among patients who did not experience death, shock, or heart failure. No significant associations were observed with IL-1β, IL-4, IL-12, or TNFα.

Univariable associations of biomarkers with 90-day death, shock or heart failure are shown in Table 2-3. All biomarkers and PAIRs are reported in $\log_2$ units; thus, an increase in one $\log_2$ units represents a doubling in the biomarker values. Both hsCRP and NT-proBNP were associated with adverse outcomes in univariable analysis. The pro-inflammatory cytokine IL-6 and the anti-inflammatory cytokines IL-10 and IL-1ra were the only inflammatory mediators related to 90 day events in univariable assessment. The 90 day outcomes associated with tertiles of hsCRP, NT-proBNP, IL-6, and IP-10 are shown in Figures 2-1a, 2-1b, 2-1c, and 2-1d.

Multivariable Association with 90-day events in models with and without NT-proBNP

After multivariable adjustment for clinical characteristics associated with 90-day events, elevated levels of hsCRP, NT-proBNP and the pro-inflammatory cytokine IL-6 remained predictors of 90-day death, shock, or heart failure; elevated chemokine IP-10 levels were independently associated with a lower risk for 90-day events (Figure 2-1, Table 2-3). In a sensitivity analysis accounting for pexelizumab treatment assignment, hsCRP, NT-proBNP, IL-6, and IP-10 remained significantly associated with the primary outcome (data not shown). After incorporating baseline NT-proBNP levels into the multivariable model, only IL-6 (HR 1.18; 95% CI, 1.04-1.33; p=0.009) remained associated with 90-day outcomes.

Assessment of Incremental Contribution of Biomarkers to a Clinical Risk Prediction Model

The c-index of the base clinical risk prediction model was 0.778. Table 2-4 displays the effects on the c-index and the NRI and IDI resulting from adding the inflammatory markers that were significantly associated with 90-day outcomes and NT-proBNP into a

clinical prediction model for 90-day death, shock or heart failure. Although the c-index of the models increased with the addition of each individual biomarker, only baseline NT-proBNP and IL-6 improved the risk prediction model as assessed by the NRI and IDI. The addition of hsCRP modestly improved IDI but not NRI. After incorporating NT-proBNP into the model, adding either hsCRP or IL-6 did not clearly add to the risk prediction model.

Multiple pro- to anti-inflammatory ratios were also tested; however, they did not add prognostic value in addition to the ratio's numerator (Table 2-5).

Discussion

This biomarker substudy of a large contemporary population of STEMI patients treated with primary PCI has three important findings. First, IL-6 was the only pro-inflammatory biomarker that was independently associated with death, shock, or heart failure at 90 days. Second, among the anti-inflammatory biomarkers tested, only elevated levels of chemokine IP-10 were associated with 90-day outcomes. Third, the addition of baseline NT-proBNP and IL-6 levels improved the clinical risk prediction model; however, after incorporating NT-proBNP, these biomarkers added little additional predictive value.

Pro-Inflammatory Markers

Previous reports on the prognostic significance of the pro-inflammatory cytokines IL-6 and TNFα have been conflicting.(9,10,12-14) A biomarker analysis from the complement inhibition in myocardial infarction treated with angioplasty (COMMA) trial in STEMI patients showed that both IL-6 and TNFα were independent predictors of

mortality.(13) These findings were both supported and refuted by smaller case series and case-control studies.(9,10,12,14,23) The discrepancies in results among these studies could potentially be explained by differences in sample size, timing of biomarker sampling, differences in acute coronary syndrome population characteristics, or differences in study endpoints. The present analysis supports previous work that showed IL-6 was independently associated with adverse 90-day outcomes. However, in contrast with some prior studies, TNFα was not associated with outcome in our study. The strength of our study is its high quality dataset with comprehensive systematic collection of clinical factors, rigorously adjudicated outcomes, and the comprehensive clinical risk modeling of outcome that enabled assessment of incremental prognostic value of the biomarkers. Future studies could potentially be directed at examining whether a similar relationship exists in lower risk acute coronary syndrome populations and whether the change in pro-inflammatory cytokines from baseline levels adds additional prognostic information.

Anti-Inflammatory Markers

IP-10 (also referred to as CXCL10) is an inducible chemokine known to stimulate cell migration and inflammation in smooth muscle cells in pre-clinical studies.(24) It has also been postulated to have angiostatic properties, through which post-infarction down regulation may facilitate angiogenesis.(25) IP-10 has been shown to be elevated in patients with coronary artery disease and myocardial infarction.(6,26) Given these findings, IP-10 was thought to have a pro-inflammatory effect; however, a study by Koten et al. reported that pre-PCI IP-10 levels negatively correlated with infarct size, suggesting a protective and possibly anti-inflammatory effect.(6) Our findings that

elevated IP-10 levels were inversely associated with 90-day outcomes support these latter findings. Whether there is a causal link between IP-10 and outcome post STEMI remains unclear and requires further research. The anti-inflammatory cytokine, IL-10, promotes coronary plaque stability by inhibiting macrophage function, inhibiting metalloproteinases, and suppressing cytokines synthesis; and IL-1ra is a soluble anti-inflammatory protein that blocks the pro-inflammatory effects of the IL-1 (α and β) family of receptors. (27-31) The prognostic utility of these cytokines in acute coronary syndromes is uncertain.(7-9,14,32,33) Our findings suggest that neither baseline IL-10 nor IL-1ra have prognostic value in STEMI patients undergoing primary PCI.

Addition of Biomarkers to a Clinical Prediction Model

Evaluating the contribution of biomarkers to clinical risk stratification is challenging, and multiple methods have been used. The c index, which represents the area under a receiver operating curve, is a commonly used measure of model discrimination. A recognized limitation of the c index is that small statistically significant changes may not reflect a clinically relevant improvement in risk discrimination; hence, NRI and IDI have been proposed as additional clinical measures of model discrimination.(22) NRI represents the net percentage of patients who are correctly reclassified as higher or lower risk once the biomarker of interest is incorporated into a clinical model, and IDI represents the change in average sensitivity assuming no change in specificity. Clinical judgment is required in NRI result interpretation and an IDI ≥0.01 is generally accepted as significant. There is no clear scientific consensus on how to interpret discrepancies between NRI and IDI and careful clinical interpretation is required. It is our opinion that clinically robust biomarkers ideally should uniformly improve all measures of

discrimination. All of the above factored into our assessment of the contribution of inflammatory markers to clinical risk prediction.

Multiple studies have reported that inflammatory biomarkers are independently associated with adverse outcomes. However, the incremental contribution of inflammatory cytokines and chemokines to the performance of clinical risk prediction models has not been previously described. The finding that IL-6 improved clinical prediction models as assessed by NRI and IDI is consistent with prior studies that reported that IL-6 was an independent adverse prognostic marker.(13,23) Baseline hsCRP only modestly improved risk prediction by IDI, but not NRI in our analysis suggesting that it does not have strong predictive value in this clinical population. This finding contradicts studies that have shown that hsCRP is an adverse prognostic marker and it is consistent with Damman and colleagues that found that CRP did not add prognostic utility beyond clinical risk factors.(12,13,16) The observed discrepancies between this study and previous studies could potentially be explained by differences in study patient populations, endpoints, CRP assays (CRP versus hsCRP) and/or timing in marker sampling.

Finally, our finding that inflammatory biomarkers added little predictive value after NT-proBNP was incorporated into the clinical prediction model was an important observation that could potentially guide future biomarker development. This observation suggests that the inflammatory biomarkers evaluated in this study may have little routine utility in an environment in which NT-proBNP already exists as a readily available biomarker for clinical risk stratification. We acknowledge that

validation of these findings in an external dataset is required, and that it may also be reasonable to confirm that these results are consistent across other assay methodologies. Further, our results suggest that it may be a reasonable benchmark in the evaluation of future biomarkers for risk stratification in STEMI that incremental value to clinical factors and NT-proBNP is evaluated.(16) This analysis underscores that although independent associations between investigational biomarkers and outcomes are scientifically valid study endpoints, they do not necessarily equate to meaningful clinical results. In a fiscally constrained health care environment, the incurred costs of adding new biomarkers to routine clinical testing ought to only occur if they add prognostic information beyond clinical indices and widely available biomarkers.

Limitations and Strengths

The limitations of this APEX-AMI substudy warrant consideration. First, the present model only included baseline pre-PCI variables. Although post-procedural variables, such as TIMI flow and peak markers of myocardial necrosis, are included in other models, the APEX-AMI model's pre- specified purpose and focus was on presenting clinical features. Secondly, the collection and measurements of CK, CK-MB, or troponin varied by study center, thus incorporation of a single baseline marker of myocardial necrosis into the model was not possible. Thirdly, this trial population only enrolled patients presenting within 6 hours of symptom onset with a planned PCI reperfusion strategy. The results may not be applicable to patients undergoing fibrinolysis or to biomarker levels drawn from patients with longer ischemic times. Fourthly, this substudy only included 772 patients and the inflammatory biomarkers were incorporated into a STEMI prediction model that has not been externally validated. This

model however, had good discrimination performance, and was built on a robust STEMI mortality model.(34) Additionally, this dataset provided a relatively unselected population, including patients with advanced age, shock and renal insufficiency and regardless of whether PCI was successful, which may actually make it more generalizable to STEMI in clinical practice than other clinical prediction models.(35-38)

Conclusions

In a contemporary population of STEMI patients treated with primary PCI, the pro-inflammatory cytokine, IL-6, was independently associated with worse 90-day outcomes, while elevated levels of the anti-inflammatory chemokine IP-10 had a protective association. The addition of NT-proBNP and IL-6 all improved a clinical prediction model, but after NT-proBNP was incorporated into the model, the remaining biomarkers added little additional prognostic value, which if confirmed may have implications for future biomarker development for risk stratification in STEMI.

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research grants from Procter & Gamble for performing the laboratory analyses in this trial and was a member of the APEX-AMI steering committee

Table 2-1. Selected baseline characteristics in the overall APEX-AMI trial and its biomarker substudy population

	Entire Apex Population (n=5743)	Biomarker Substudy (n=772)	p value*
Age, median (25th, 75th %tile), years	61 (52, 71)	65 (56, 76)	<0.01
Women, n (%)	1325 (23.1)	209 (27.1)	<0.01
Body Mass Index, median (25th, 75th %tile)	27.1 (24.5, 30.1)	27.25 (24.2, 30.1)	0.97
Clinical History			
Hypertension, n (%)	2838 (49.4)	409 (53.0)	0.03
Diabetes mellitus, n (%)	912 (15.9)	127 (16.5)	0.64
Dyslipidemia, n (%)	2180 (49.7)	321 (49.7)	1.00

Prior Coronary Artery Disease, n (%)	942 (16.4)	152 (19.7)	0.01
Prior MI, n (%)	694 (12.1)	120 (15.5)	<0.01
Prior PCI, n (%)	562 (9.8)	102 (13.2)	<0.01
Prior CABG, n (%)	128 (2.2)	21 (2.7)	0.32
Prior CHF, n (%)	207 (3.6)	29 (3.8)	0.81
Prior AF, n (%)	238 (4.1)	42 (5.4)	0.05
Prior stroke, n (%)	216 (3.8)	39 (5.1)	0.04
COPD, n (%)	282 (4.9)	49 (6.3)	0.05
Past or Current Smoker, n (%)	3822 (66.6)	477 (61.9)	<0.01
Peripheral Vascular Disease, n (%)	246 (4.3)	41 (5.3)	0.13

Chronic Inflammatory Condition, n (%)	103 (1.8)	30 (3.9)	<0.01
Presenting Characteristics			
Heart rate, median (25th, 75th %tile), beats/min	75 (65, 86)	76 (65, 88)	0.06
Systolic blood pressure, median (25th, 75th %tile), mmHg	133 (117, 150)	130 (114, 148)	<0.01
Diastolic blood pressure, median (25th, 75th %tile), mmHg	80 (70, 90)	78 (66, 89)	<0.01
Killip class >I, n (%)	610 (10.6)	131 (17.0)	<0.01
High Risk Inferior (ECG), n (%)	2346 (40.8)	252 (32.6)	<0.01
Sum ST Segment Deviation, median (25th, 75th %tile), mm	13.0 (9.0, 18.5)	12.5 (9.0, 19.0)	0.68

Symptom onset to randomization, median (25th, 75th %tile), hours	2.8 (2.0, 4.0)	2.7 (1.9, 3.7)	0.01
Baseline CK µg/L, median (25th, 75th %tile)	143 (90, 278)	137 (83, 283)	0.43
Baseline Troponin I µg/L, median (25th, 75th %tile)	0.2 (0.1, 2.0)	0.2 (0.1, 0.9)	0.64
Creatinine, median µg/L, (25th, 75th %tile), µmol/L	89.0 (79.6, 106.1)	97.2 (79.6, 114.9)	<0.01
Region			<0.01
Australia and New Zealand	558 (9.7)	82 (10.6)	
Eastern Europe	1336 (23.3)	24 (3.1)	
Western Europe	1764 (30.7)	96 (12.4)	

North America	2088 (36.3)	570 (73.8)

*p value for comparison of those in substudy to those not in substudy

Abbreviations: AF, atrial fibrillation; bpm, beats per minute; CABG, coronary artery bypass grafting; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; ECG, electrocardiogram; CK, creatine kinase; min, minute; n, number; MI, myocardial infarction; PCI, percutaneous coronary intervention; ULN, upper limit of normal.

Table 2-2. Biomarker levels stratified by 90-day death, shock or heart failure

Biomarkers	90 day Death, Shock, or Heart Failure		p value
	No Event	**Event**	
hsCRP, median (25th, 75th %tile), mg/L	3.2 (1.4, 7.4)	5.8 (1.8, 18.7)	<0.001
NT-proBNP, median (25th, 75th %tile), ng/mL	173.6 (59.7, 567.7)	554.9 (147.2, 2432.0)	<0.001
IL-1β, median (25th, 75th %tile), pg/mL	8.3 (6.7, 9.5)	8.3 (6.0, 9.5)	0.734
IL-4, median (25th, 75th %tile), pg/mL	6.6 (5.6, 7.6)	6.8 (5.7, 7.7)	0.620
IL-6, median (25th, 75th %tile), pg/mL	12.8 (10.2, 16.7)	18.4 (12.2, 33.8)	<0.001
IL-10, median (25th, 75th %tile), pg/mL	7.0 (3.8, 13.1)	9.5 (4.2, 20.0)	<0.001
IL-12, median (25th, 75th %tile), pg/mL	7.5 (5.9, 10.6)	7.5(5.7, 9.8)	0.450

IL-1ra, median (25th, 75th %tile), pg/mL	191.7 (136.7, 321.3)	245.5 (155.7, 456.0)	<0.001
IFNγ, median (25th, 75th %tile), pg/mL	121.8 (92.6, 145.2)	127.1 (95.7, 153.4)	0.004
IP-10, median (25th, 75th %tile), pg/mL	814.9 (507.5, 1220.8)	797.0 (397.8, 1208.7)	0.004
TNFα, median (25th, 75th %tile), pg/mL	64.9 (52.9, 73.4)	65.2 (51.5, 75.6)	0.085

Abbreviations: 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;TNFα, tumor necrosis factor alpha.

Table 2-3. Univariable and multivariable relationships between inflammatory biomarkers and 90-day death, shock, or heart failure

Biomarker*	Univariable Analysis		Multivariable Analysis‡		Multivariable Analysis‡ Including NT-proBNP	
	Hazard Ratio (95%CI)†	**p-value**	**Hazard Ratio (95%CI)†**	**p-value**	**Hazard Ratio (95%CI)†**	**p-value**
hsCRP	1.21 (1.13, 1.30)	<0.001	1.12 (1.03, 1.21)	0.007	1.06 (0.97, 1.16)	0.202
NT-proBNP	1.22 (1.15, 1.28)	<0.001	1.14 (1.06, 1.23)	<0.001	Included	
IL-1β	1.05 (0.81, 1.37)	0.710	1.06 (0.77, 1.47)	0.709	1.09 (0.79, 1.50)	0.602
IL-4	1.15 (0.91, 1.45)	0.247	1.23 (0.89, 1.70)	0.202	1.25 (0.91, 1.70)	0.164
IL-6	1.45 (1.35, 1.57)	<0.001	1.26 (1.12, 1.41)	<0.001	1.18 (1.04, 1.33)	0.009
IL-10	1.21 (1.10, 1.32)	<0.001	1.04 (0.93, 1.16)	0.504	1.05 (0.94, 1.17)	0.386
IL-12	1.00 (0.89, 1.14)	0.947	1.00 (0.85, 1.17)	0.969	1.01 (0.86, 1.17)	0.954
IL-1ra	1.30 (1.19, 1.41)	<0.001	1.06 (0.94, 1.19)	0.325	1.05 (0.93, 1.18)	0.429
IP-10	0.92 (0.82, 1.04)	0.171	0.86 (0.76, 0.98)	0.025	0.89 (0.78, 1.02)	0.085
IFNγ	1.21 (0.99, 1.48)	0.060	1.25 (0.94, 1.66)	0.123	1.23 (0.93, 1.63)	0.155
TNFα	1.19 (0.94, 1.51)	0.156	1.15 (0.85, 1.56)	0.366	1.15 (0.85, 1.55)	0.377

* Biomarkers reported in $\log_2$

† Per doubling of the biomarker

‡ Adjusted for age, gender, 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, total ST-segment deviation, MI location, right bundle branch block, baseline q wave.

Abbreviations: CI, confidence interval; 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;TNFα, tumor necrosis factor alpha.

Table 2-4: Incremental contribution of biomarkers to regression models predicting the 90-day incidence of death, shock or heart failure.

		Discrimination			
	c index	Net Reclassification Improvement[‡]	p value	Integrated Discrimination Improvement[§]	p value
Established risk factors[*]	0.778	Reference			
Established risk factors + hsCRP	0.794	6.50	0.069	0.0178	0.004
Established risk factors + IL-6	0.797	8.83	0.012	0.0364	<0.001
Established risk factors + IP-10	0.781	-3.56	0.275	0.0074	0.031
Established risk factors + NT-proBNP	0.799	8.63	0.028	0.0303	<0.001
Established risk factors + NT-proBNP + hsCRP[†]	0.804	6.73	0.029	0.0041	0.196

Established risk factors + NT-proBNP + IL-6†	0.807	4.13	0.193	0.0187	0.006
Established risk factors + NT-proBNP + IP-10†	0.799	-0.006	0.998	0.0042	0.103

*Risk factors are: age, gender, 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, total ST-segment deviation, MI location, right bundle branch block, baseline q wave.

† Net reclassification improvement, integrated discrimination improvement, and p values compared to Established risk factors + NT-proBNP.

‡Net Reclassification Index was defined as (Pimproved_prediction_outcome + Pimproved_prediction_no outcome) - (Pworsened_prediction_outcome + Pworsened_prediction_no outcome); where P = proportion of patients and outcome = 90 day death, shock or heart failure

§The Integrated Discrimination Improvement was define as (Σ^{i}outcome ($p_{new}(i) - p_{old}(i)$)/n (outcome)) - (Σ^{j}no outcome ($p_{new}(j) - p_{old}(j)$)/n (no outcome)); where p = predicted probability of the outcome and outcome = 90 day death, shock and heart failure.

Abbreviations: hsCRP, high sensitivity C-reactive protein; NT-proBNP, n terminal pro brain natriuretic peptide; IL, Interleukin; IP-10, interferon inducible protein 10.

Table 2-5: Incremental contribution of biomarker pro- to anti-inflammatory ratios to regression models predicting the 90-day incidence of death, shock or heart failure.

	Discrimination				
	c index	Net Reclassification Improvement[‡]	p value	Integrated Discrimination Improvement[§]	p value
Established risk factors*	0.778	Reference			
Established risk factors + hsCRP/IP-10	0.788	7.67	0.033	0.0235	<0.001
Established risk factors + IL-6/IL-10	0.790	4.73	0.155	0.0186	0.003
Established risk factors + IL-6/IP-10	0.794	2.10	0.613	0.0396	<0.001
Established risk factors + NT-proBNP/ IL-10	0.792	7.10	0.067	0.0182	0.002
Established risk factors + NT-proBNP/ IP-10	0.796	7.65	0.073	0.0340	<0.001

*Risk factors are: age, gender, 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, total ST-segment deviation, MI location, right bundle branch block, baseline q wave.

[‡]Net Reclassification Index was defined as (Pimproved_prediction_outcome + Pimproved_prediction_no outcome) - (Pworsened_prediction_outcome + Pworsened_prediction_no outcome); where P = proportion of patients and outcome = 90 day death, shock or heart failure

[§]The Integrated Discrimination Improvement was define as (Σ^{i}outcome ($p_{new}(i) - p_{old}(i)$)/n (outcome)) - (Σ^{j}no outcome ($p_{new}(j) - p_{old}(j)$)/n (no outcome)); where p = predicted probability of the outcome and outcome = 90 day death, shock and heart failure

Figure2- 1: Kaplan Meier Curve of 90 day Death, Shock, and Heart Failure Stratified by Tertiles of (a) hsCRP, (b) NT-proBNP, (c) IL-6, and (d) IP-10

Figure 2-1(a):

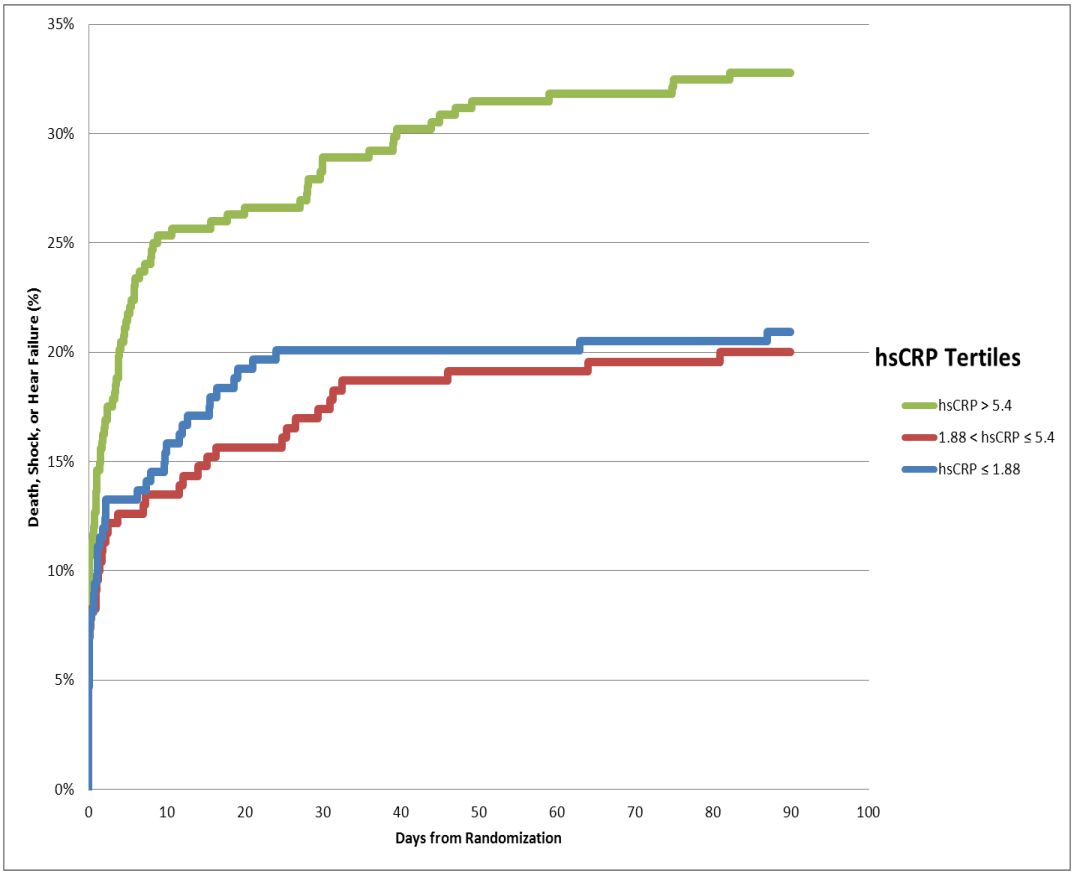


Figure 2-1(b):

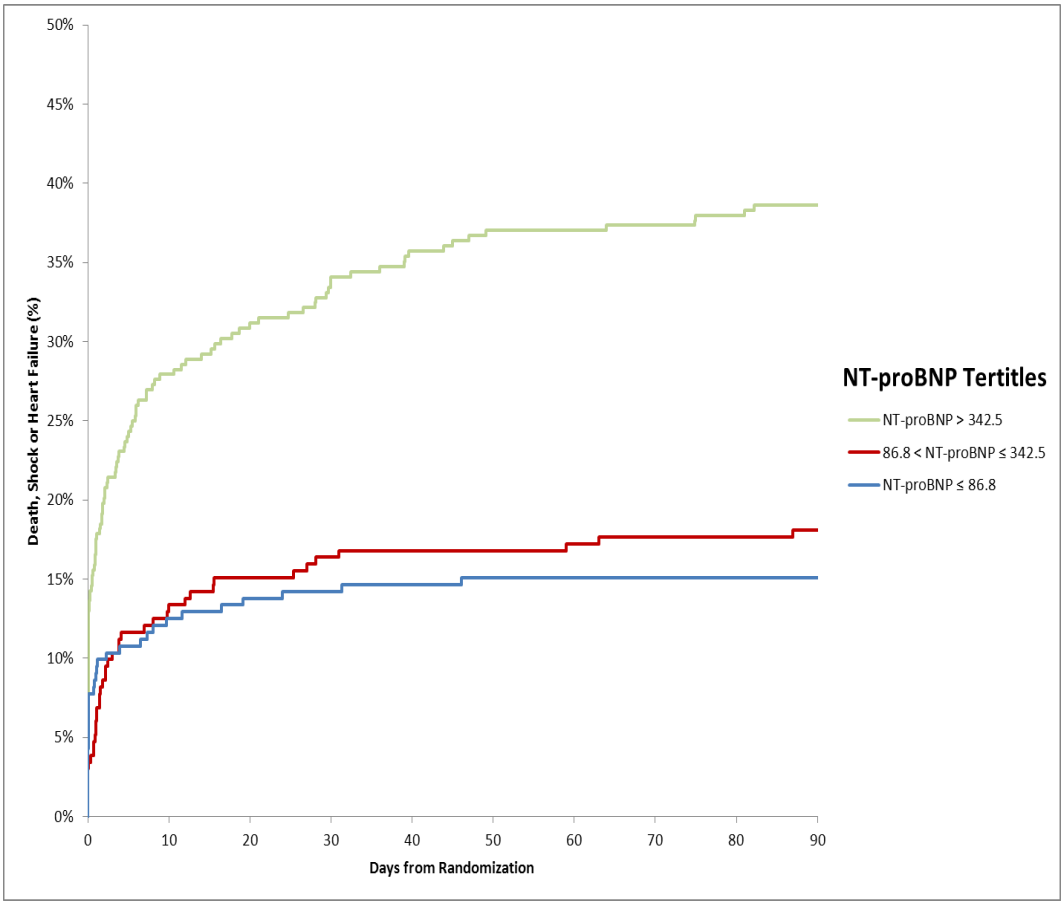

50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
Death, Shock or Heart Failure (%)
0
10
20
30
40
50
60
70
80
90
Days from Randomization
NT-proBNP Tertitles
NT-proBNP > 342.5
86.8 < NT-proBNP ≤ 342.5
NT-proBNP ≤ 86.8

Figure 2-1(c):

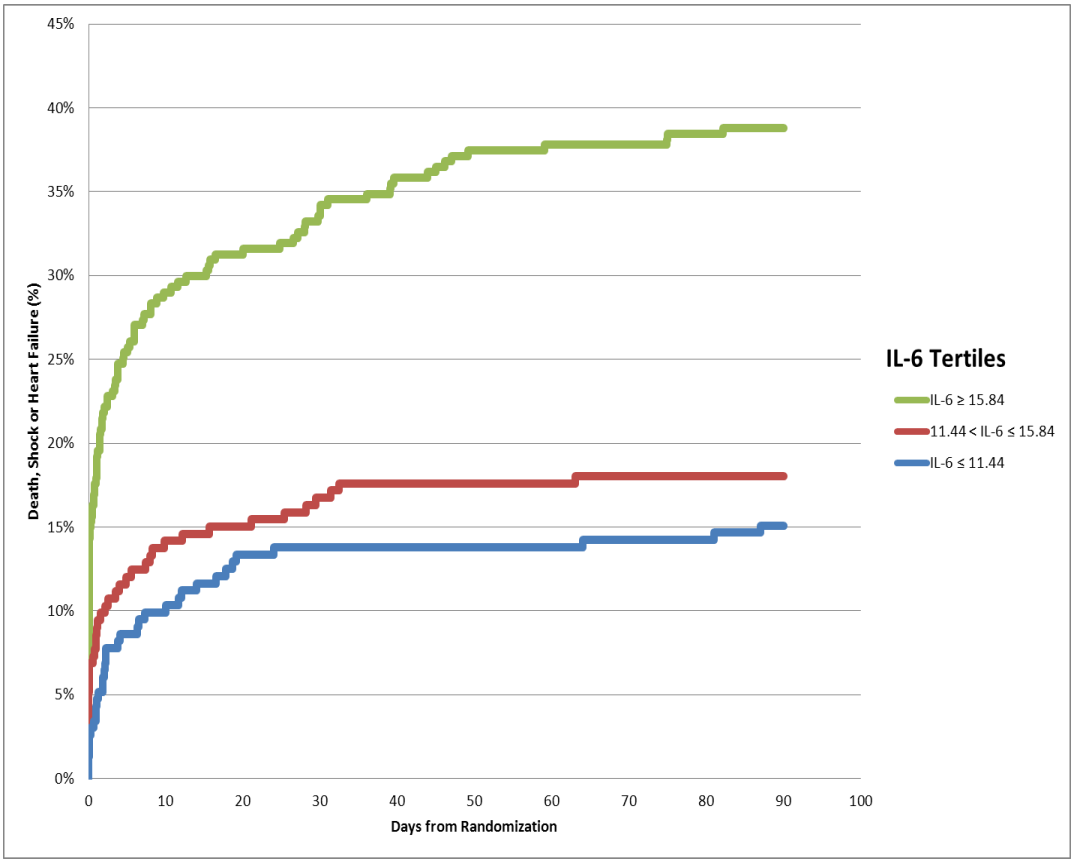
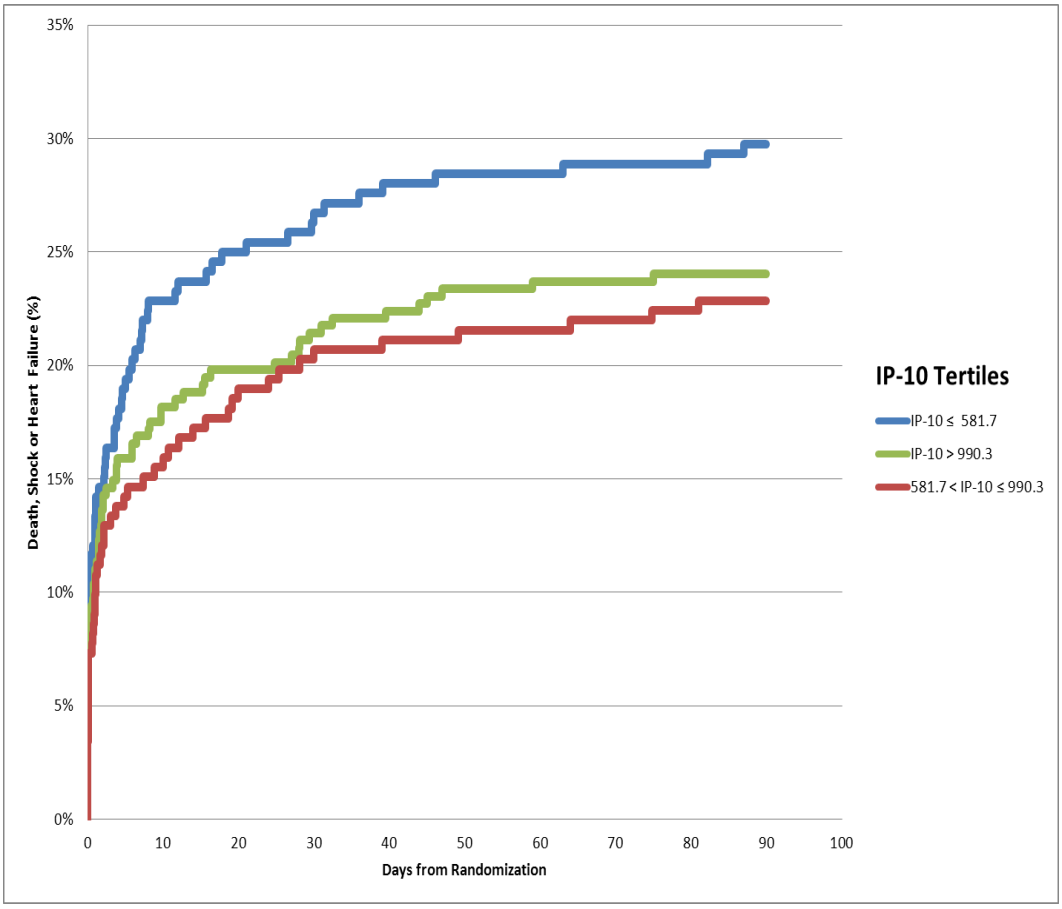

45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
Death, Shock or Heart Failure (%)
0
10
20
30
40
50
60
70
80
90
100
Days from Randomization
IL-6 Tertiles
IL-6 ≥ 15.84
11.44 < IL-6 ≤ 15.84
IL-6 ≤ 11.44

Figure 2-1(d):


35%
30%
25%
20%
15%
10%
5%
0%
Death, Shock or Heart Failure (%)
0
10
20
30
40
50
60
70
80
90
100
Days from Randomization
IP-10 Tertiles
IP-10 ≤ 581.7
IP-10 > 990.3
581.7 < IP-10 ≤ 990.3

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Chapter 3:

Baseline NT-proBNP and Biomarkers of Inflammation and Necrosis in Patients with ST-segment Elevation Myocardial Infarction[1]

[1] A version of this chapter has been published. Van Diepen S, et al. Journal of Thrombosis and Thrombolysis 2012; 34(1):106-113.

Introduction

B-type natriuretic peptide (BNP) is an important prognostic biomarker in patients with acute coronary syndrome (ACS).(1-4) N-terminal pro B-type natriuretic peptide (NT-proBNP) and markers of myocardial cell necrosis add prognostic value to established risk prediction scores. (5,6) In non ST-segment elevation myocardial infarction (NSTEMI) baseline NT-proBNP values have been shown to correlate with troponin T; however little is known about its relationship with markers of myocardial necrosis in the ST-elevation myocardial infarction (STEMI) population treated with primary percutaneous coronary intervention (PCI).(7)

There is emerging evidence that coronary plaque rupture triggers both a pro- and anti-inflammatory cytokine response.(8-13) BNP expression is commonly thought to result from myocardial stretch(14); however, it may also be regulated by systemic inflammatory mediators. In pre-clinical studies, the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and tumour necrosis factor alpha (TNFα) all stimulated cardiocyte BNP expression.(15-17) Further, BNP can be elevated in the presence of normal cardiac function in inflammatory conditions, such sepsis and burns.(18,19) IL-10, an anti-inflammatory cytokine, can transiently rise in ACS.(20) BNP has been shown to increase IL-10 release in vitro, suggesting that BNP can stimulate an anti-inflammatory response; however, this relationship has not been established in-vivo.(21) Although BNP and both pro- and anti-inflammatory mediators can be elevated after plaque rupture, the relationship between baseline N-terminal pro-brain natriuretic peptide (NT-

proBNP) and baseline inflammatory biomarkers in STEMI patients treated with primary PCI is not well described.

The purpose of this study was to describe the relationship between baseline NT-proBNP and inflammatory serum biomarkers in STEMI patients treated with primary PCI. Additionally, the relationship between NT-proBNP with markers of cell necrosis, time to revascularization, and sum ST-segment deviation were also explored.

Methods

The Assessment of Pexelizumab in Acute Myocardial Infarction (APEX-AMI) trial was a multicenter, double blind, placebo-controlled, randomized clinical trial comparing pexelizumab versus placebo in 5745 STEMI patients undergoing primary (PCI).(22,23) Patients over the age of 18 were eligible if they presented for primary PCI within 6 hours of symptom onset with high-risk electrocardiographic features. Treatment groups were pooled for this analysis since no treatment effect on outcomes was observed.

The study population for our analyses comprised of a subset of patients presenting to an APEX-AMI biomarker study center and consented to provide blood samples for future biomarker research. Blood samples were drawn in participating patients after randomization, but prior to study drug administration and PCI.(24) Samples were allowed to clot, then centrifuged and the resultant serum frozen to -20°C then to -70°C the following day (if available). Samples were bulk shipped on dry ice to a central collection facility (Duke Center for Human Genetics, Durham, NC, USA) then to a central

laboratory (Montreal Heart Institute, Montreal, Quebec, Canada) for batched biomarker analysis.

NT-proBNP was measured using electrochemiluminescence immunoassay with Elecsys instrument and reagent kit (Roche Diagnostics, Indianapolis, IN). The analytical range was 5 to 35,000 pg/mL with an inter and intraassay variability of 4% and 8%, respectively.(25) The remaining ten biomarkers measured in up to 772 patients were: high sensitivity C reactive protein (hsCRP); pro-inflammatory cytokines (lL-1β, Il-6, IL-12); anti-inflammatory cytokines (IL-1 receptor antagonist [IL-1ra], IL-4, and IL-10); chemokines interferon gamma (IFNγ) and interferon inducible protein 10 (IP-10). These were measured using phase sandwich ELISA tests, cytometric beads assays, multiple fluorescent beads assays, and immunonephelometry.

Baseline markers of myocardial cell necrosis (creatinine kinase [CK], CK-MB, troponin I) were drawn after prior to study drug administration and analyzed at APEX-AMI study centers. The method for measuring sum ST segment deviation (Σ STD) has been previously described.(26) Briefly, blinded central electrocardiogram evaluation took place at two core laboratories (Canadian VIGOUR Centre, Edmonton, Canada; Duke Clinical Research Institute, Durham, NC). Sum STD was calculated by adding the sums of ST segment elevation and reciprocal depression.

Statistical Analysis

Categorical variables are reported using frequencies and counts and continuous variables are represented as medians (25^{th}, 75^{th} percentiles). Testing of differences

between those in the biomarker substudy and those who were not, used the Wilcoxon rand sum test for continuous variables and the χ^2 test for categorical variables.

Statistical dependence between variables was tested with the Spearman rank correlation test (rs). Since NT-proBNP is elevated in heart failure, a clinical and a hemodynamic sensitivity analysis were performed on the correlation between NT-proBNP and IL-6.(27) In the first analysis, patients with either a history of heart failure and those who developed clinical heart failure within 90 days of the index STEMI presentation were excluded. In the second, the correlation was controlled for patients with a left ventricular end diastolic pressure or pulmonary capillary wedge pressure > 16mmHg at the time of PCI.(28) Analyses were performed with SAS version 9.2 (SAS Institute Inc., Cary, NC).

Results

Study Population

Baseline patient characteristics of the 772 patient included in this analysis are presented in Table 3-1. Compared with the overall trial population, the substudy population had a higher percentage of female patients, were more likely to be older, hypertensive, have prior coronary artery disease, stroke, a chronic inflammatory condition, and have a smoking history. Patients enrolled in the biomarker substudy tended to be sicker with more anterior infarctions, higher Killip class, lower blood pressure at hospital presentation, and higher baseline creatinine.

NT-proBNP and Inflammatory Biomarkers

Table 3-2 shows the correlations between baseline inflammatory marker levels and quartiles of baseline NT-proBNP. There was significant positive correlation between hsCRP and NT-proBNP (Spearman Correlation (r_s)=0.377, p<0.001). Among all pro-inflammatory cytokines and chemokines, only IL-6 had a moderate significant positive correlation with NT-proBNP (r_s=0.317, p<0.001). No significant correlations were observed with either IL-1β or TNFα. There was a weak negative correlation between NT-proBNP and the anti-inflammatory cytokine IL-10 (r_s=-0.109, p=0.003). The relationship between NT-proBNP quartiles and IL-6, IL-10, and hsCRP is shown in Figure 3-1.

In a sensitivity analysis excluding all patients with a history of heart failure (n=29) and those who developed heart failure within 90 days of study enrolment (n=92), the correlation between baseline IL-6 and NT-proBNP remained significant (r_s=0.296, p<0.001). In a second sensitivity analysis controlling for left ventricular end diastolic pressure or pulmonary capillary wedge pressures recorded at the time of PCI, the relationship remained significant (rs=0.184, p<0.001) in the subset of patients in whom data was available (n=315).

NT-proBNP Correlation with Markers of Myocardial Necrosis and Time from Symptom Onset

The relationship between baseline markers of myocardial cell necrosis and baseline NT-proBNP are presented in Table 3-3. Total CK (r_s=0.113, p=0.011) was weakly positively

correlated with baseline NT-proBNP levels, while moderate positive correlations were observed with CK-MB (r_s=0.338, p<0.001) and troponin I (r_s =0.420, p<0.001).

Weak positive correlations with symptom onset to randomization time (r_s=0.275, p<0.001) and symptom onset to presentation time (r_s=0.197, p<0.001) were observed. There was no significant correlation with Σ STD (r_s= -0.071, p=0.051).

Discussion

This analysis from a contemporary international trial of STEMI patients undergoing primary PCI has three main findings that build upon pre-existing biomarker knowledge. First, although pre-clinical studies have reported that lL-1β, IL-6, and TNFα can stimulate BNP expression, IL-6 was the only cytokine with a modest positive correlation with baseline NT-proBNP levels.(15-17) Importantly, the relationship remained significant after excluding patients with clinical heart failure. Secondly, the small inverse relationship between NT-proBNP and IL-10 suggests there is not a clinically meaningful correlation between NT-proBNP and anti-inflammatory cytokine activation in acute coronary syndromes. Finally, baseline NT-proBNP levels were only moderately correlated with baseline CK-MB and troponin I levels.

Coronary plaque rupture triggers an inflammatory cascade.(8-12) Three pro-inflammatory cytokines have been reported to stimulate BNP release. In separate studies using rat myocytes, Tananaka et al. and Kuwukara et al. showed that IL-6

stimulates BNP expression, while a study by Ma and colleagues showed that IL-1β and TNFα stimulate BNP mRNA expression.(15-17) In the present analysis, IL-6 was the only pro-inflammatory cytokine IL-6 with a significant positive correlation with NT-proBNP, though the association was only of modest strength. Importantly, this relationship remained significant after excluding patients with a history of heart failure and those who developed clinical heart failure. This is notable given the established relationship between NT-proBNP and clinical heart failure.(27) This observation supports the hypothesis that, in addition to release in response to myocardial stretch(14), BNP expression may, in part, also be regulated by systemic inflammatory mediators.

The hypothesis that cytokines can stimulate BNP release has not been well studied in humans with ACS, but there is evidence to support this postulate. BNP rises have been reported during temporary coronary artery occlusion when cardiac filling pressures have remained unchanged.(29) Studies of critically ill septic and burn patients with normal echocardiograms have reported elevated BNP levels.(18,19) These studies did not include measurements of inflammatory cytokines, but the results are supportive of the hypothesis given that the study patients had conditions with a known systemic inflammatory response. Additionally, IL-6 and BNP levels were correlated in 15 unstable angina patients in a single center study; however, the study did not sample baseline values. Our study is the largest to report a significant positive correlation between baseline NT-proBNP and IL-6 in the STEMI population in the contemporary era. The moderate correlation supports pre-clinical studies where IL-6 has been shown to stimulate NT-pro BNP expression although we cannot infer causation from this study as other possible pathophysiologic mechanisms exist. In addition to plaque rupture,

myocardial stretch may also stimulate IL-6 expression.(30) This explanation would be consistent with the observation that both IL-6 and BNP are elevated in heart failure patients.(31,32) Future studies should be directed at elucidating the potentially complex interaction between myocardial stretch, IL-6, and NT-proBNP synthesis.

IL-10 is an anti-inflammatory cytokine that promotes coronary plaque stability by inhibiting macrophage function, suppressing cytokines production, and inhibiting of metalloproteinases.(33,343) Relatively low levels of IL-10 are thought to be a risk for coronary plaque rupture, yet levels can also transiently rise in some patients following an acute coronary syndrome.(20,35) Pre-clinical studies showed that BNP stimulates macrophage IL-10 expression leading to the hypothesis that BNP may induce an anti-inflammatory cytokine response.(21) The small inverse relationship between NT-proBNP and IL-10 observed in this study suggests that the transient rise in IL-10 levels during the acute phase of ACS is not likely due to NT-proBNP. This result is consistent with animal and human studies showing that IL-10 is decreased in heart failure following acute myocardial infarction.(36,37) Heeschen et al. have shown that found elevated IL-10 levels at the time of hospital discharge were associated with lower mortality.(20) Given the contrasting outcomes associated with elevated levels it of NT-proBNP and IL-10, it appears unlikely that there is a clinically significant correlation between these biomarkers.

Both BNP and markers of myocardial necrosis have been shown to improve baseline clinical prognostication in STEMI patients, but less is known about the relationship between these biomarkers in STEMI patients.(6) Moderate correlations between

baseline BNP and troponin were reported in STEMI patients and in a single center series of 126 STEMI patients undergoing primary PCI. (7,38) Additionally, correlations between peak CK-MB and BNP levels have been have been described previously, whereas similar correlations have not been reported for baseline levels.(39) The present study re-affirms the previously observed correlations former study's findings in an international population and, to our knowledge, this is the first study to show a moderate correlation between baseline NT-proBNP and CK-MB in STEMI patients treated with primary PCI.

Limitations

Limitations of this APEX-AMI biomarker analysis warrant consideration. First, measures of left ventricular end diastolic pressure (LVEDP) and left ventricular ejection fraction were not routinely collected. A sensitivity analysis using clinical heart failure as reflection of elevated LVEDP was performed; however subclinical heart failure cannot be excluded. Second, BNP levels are inversely related to glomerular filtration rate and this study did not exclude patients with renal failure; however the median creatinine in this study was within the normal range.(40) Lastly, these analyses should be considered hypothesis-generating, and they have not been validated in an independent data set.

Conclusions

In a cohort of contemporary STEMI patients treated with primary PCI, the pro-inflammatory cytokine IL-6 was modestly correlated with baseline NT-proBNP levels. This relationship remained significant after excluding patients with clinical heart failure

and after controlling for elevated filling pressures. These findings support pre-clinical and clinical studies that suggest that BNP may be expressed in response to systemic inflammatory stimuli. Additionally, the small inverse relationship between NT-proBNP and IL-10 suggests that a clinically relevant correlation between NT-proBNP and anti-inflammatory cytokine activation in ACS is unlikely. Finally, moderate correlations were observed between baseline NT-proBNP and CK-MB and troponin I levels. Future research should be directed at elucidating the potentially complex relationships between pro-inflammatory cytokines and BNP expression while controlling for measures of myocardial stretch and exploring how these relationships impact clinical outcomes.

Tables

Table 3-1: Selected baseline characteristics in overall trial and biomarker substudy populations

	Entire APEX Population (n=5743)	**Not in Substudy (n=4971)**	**Biomarker Substudy (n=772)** [a]	**p value** [b]
Age, median (25th, 75th %tile), years	61 (52, 71)	61 (52, 97)	65 (56, 76)	<0.01
Women, n (%)	1325 (23.1)	1116 (22.5)	209 (27.1)	<0.01
Body Mass Index, median (25th, 75th %tile), kg/m^2	27.1 (24.5, 30.1)	27.0 (24.5, 30.1)	27.2 (24.2, 30.1)	0.97
Clinical History				
Hypertension, n (%)	2838 (49.4)	2429 (48.9)	409 (53.0)	0.03
Diabetes mellitus, n (%)	912 (15.9)	785 (15.8)	127 (16.5)	0.64
Dyslipidemia, n (%)	2180 (49.7)	1859 (49.7)	321 (49.7)	1.00
Prior Coronary Artery Disease, n (%)	942 (16.4)	790 (15.9)	152 (19.7)	0.01

Prior MI, n (%)	694 (12.1)	574 (11.6)	120 (15.5)	<0.01
Prior PCI, n(%)	562 (9.8)	460 (9.3)	102 (13.2)	<0.01
Prior CABG, n (%)	128 (2.2)	107 (2.2)	21 (2.7)	0.32
Prior CHF, n (%)	207 (3.6)	178 (3.6)	29 (3.8)	0.81
Prior AF, n (%)	238 (4.1)	196 (3.9)	42 (5.4)	0.05
Prior stroke, n (%)	216 (3.8)	177 (3.6)	39 (5.1)	0.04
COPD, n (%)	282 (4.9)	233 (4.7)	49 (6.3)	0.05
Past or Current Smoker, n (%)	3822 (66.6)	3345 (67.5)	477 (61.9)	<0.01
Peripheral Vascular Disease, n (%)	246 (4.3)	205 (4.1)	41 (5.3)	0.13
Chronic Inflammatory Condition, n (%)	103 (1.8)	5 (3.6)	30 (3.9)	<0.01
Presenting Characteristics				
Heart rate, median (25th, 75th %tile), beats/min	75 (65, 86)	75 (65, 86)	76 (65, 88)	0.06
Systolic blood pressure, median (25th, 75th %tile), mm Hg	133 (117, 150)	133 (118, 150)	130 (114, 148)	<0.01
Diastolic blood pressure, median (25th, 75th %tile), mm Hg	80 (70, 90)	80 (70, 90)	78 (66, 89)	<0.01

Killip class > I, n (%)	610 (10.6)	479 (9.6)	131 (17.0)	<0.01
High Risk Inferior (ECG), n (%)	2346 (40.8)	2094 (42.1)	252 (32.6)	<0.01
Sum ST Segment Deviation, median (25th, 75th %tile), mm	13.0 (9.0, 18.5)	13.0 (9.0, 18.5)	12.5 (9.0, 19.0)	0.68
Symptom onset to randomization, median (25th, 75th %tile), hours	2.8 (2.0, 4.0)	2.8 (2.0, 4.0)	2.7 (1.9, 3.7)	0.01
Baseline CKMB median (25^{th}, 75^{th} %tile), µg/L	4.7 (2.2, 15.0)	6.7 (2.9, 21.9)	2.3 (5.3, 18.0)	0.039
Baseline CK median (25th, 75th %tile), µg/L	143 (90, 278)	144 (91, 278)	137 (83, 283)	0.43
Baseline Troponin I median (25th, 75th %tile), µg/L	0.20 (0.07, 1.0)	0.2 (0.1, 1.0)	0.17 (0.08, 0.88)	0.64
Creatinine, median (25th, 75th %tile), µmol/L	89 (80, 106)	88 (79, 106)	97 (80, 115)	<0.01
Region, n (%)				<0.01
Australia and New Zealand	558 (9.7)	496 (9.6)	82 (10.6)	
Eastern Europe	1336 (23.3)	1312 (26.4)	24 (3.1)	
Western Europe	1764 (30.7)	1668 (33.6)	96 (12.4)	
North America	2085 (36.3)	1515 (30.5)	570 (73.8)	

[a] Due to missing data, not all rows represent 100% of the substudy population

[b] p value for comparison between excluded patients and those in the biomarker substudy.

Abbreviations: AF, atrial fibrillation; bpm, beats per minute; CABG, coronary artery bypass grafting; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; ECG, electrocardiogram; CK, creatine kinase; min, minute; n, number; MI, myocardial infarction; PCI, percutaneous coronary intervention; ULN, upper limit of normal.

Table 3-2: Correlation between baseline NT-proBNP quartiles and baseline inflammatory biomarkers levels

	NT-proBNP Quartile (ng/ml)				p value	Spearman Correlation
	1 (<66)	2 (66-207)	3 (208-796)	4 (>796)		
hsCRP, median (25th, 75th %tile), mg/L	2.3 (1.1, 5.6)	2.4 (1.3, 6.2)	4.0 (1.7, 8.4)	8.6 (3.8, 26.2)	<0.001	0.377
IL-1β, median (25th, 75th %tile), pg/mL	8.5 (7.0, 9.9)	8.4 (7.0, 9.5)	8.3 (7.0, 9.5)	8.0 (6.2, 9.3)	0.084	0.062
Il-4, median (25th, 75th %tile), pg/mL	6.8 (6.0, 7.7)	6.7 (5.9, 7.9)	6.8 (5.6, 7.8)	6.7 (5.5, 7.6)	0.206	0.046
Il-6, median (25th, 75th %tile), pg/mL	12.3 (10.1, 15.5)	13.3 (10.9, 17.8)	14.3 (11.1, 19.7)	18.8 (11.1, 19.7)	<0.001	0.317
Il-10, median (25th, 75th %tile), pg/mL	8.3 (4.7, 14.5)	8.1 (4.5, 15.7)	6.8 (3.7, 14.4)	5.4 (3.2, 13.4)	0.003	-0.109
IL-12, median (25th, 75th %tile), pg/mL	8.1 (6.4, 10.6)	8.1 (6.1, 10.6)	7.7 (5.9, 11.0)	7.3 (5.7, 10.0)	0.052	-0.070
IL-1ra, median (25th, 75th %tile), pg/mL	218 (147, 348)	216 (142, 347)	206 (140, 350)	217 (158, 456)	0.116	0.057
IFNγ, median (25th, 75th %tile), pg/mL	124 (98, 143)	123 (102, 146)	126 (99, 151)	122 (93, 147)	0.436	0.028
IP-10, median (25th, 75th %tile), pg/mL	818 (503, 1234)	841 (520, 1286)	802 (525, 1238)	873 (513, 1290)	0.484	0.025
TNFα, median (25th, 75th %tile), pg/mL	66.5 (54.5, 74.4)	65.5 (55.9, 73.7)	64.9 (54.3, 74.1)	64.5 (52.2, 74.2)	0.689	-0.014
WBC, median (25th, 75th %tile), $x10^9$/L	10.9 (8.6, 14.4)	10.4 (8.4, 13.4)	10.4 (8.6, 13.5)	10.6 (8.6, 13.1)	0.820	0.008

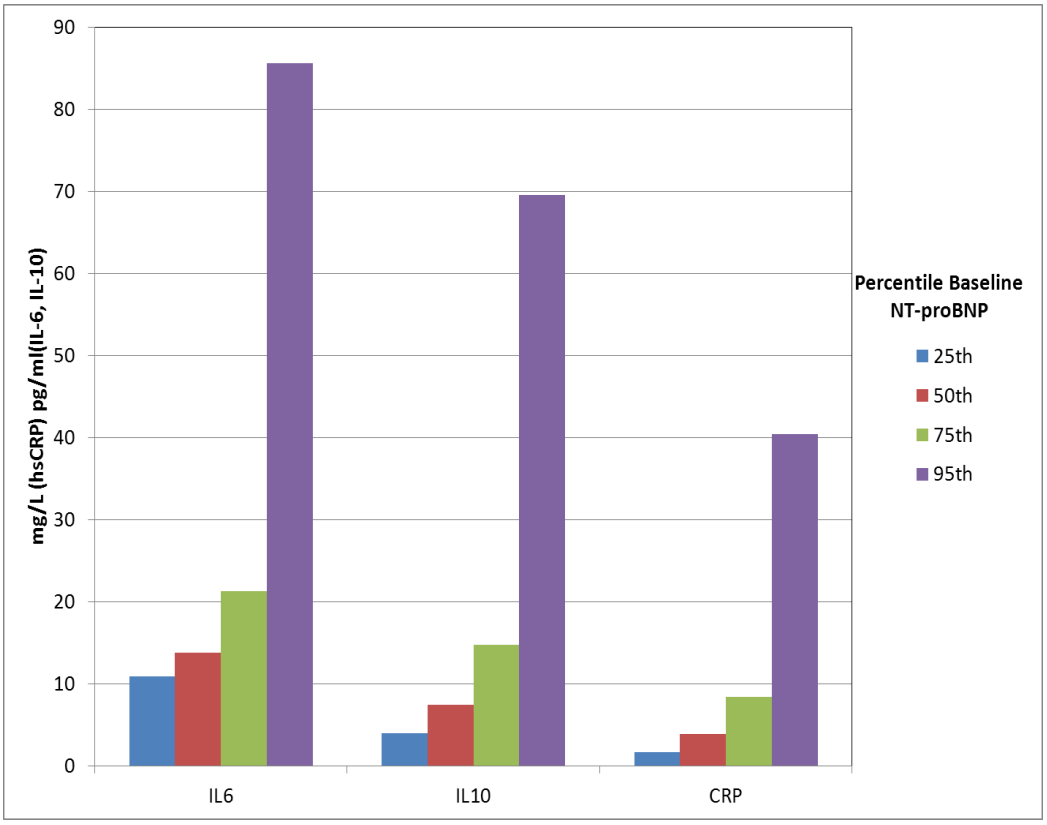
Abbreviations: hsCRP, high sensitivity C reactive protein; IFNδ, interferon gamma; IL, Interleukin; IL-1ra, IL-1 receptor antagonist ; IP-10, interferon inducible protein 10; TNFα, tumour necrosis factor alpha; WBC, White Blood Cell Count.

Table 3-3: Correlation between NT-proBNP quartiles and markers of myocardial necrosis

Baseline Biomarkers	NT-proBNP Quartile (ng/ml)				p value	Spearman Correlation
	1 (<66)	2 (66-207)	3 (208-796)	4 (>796)		
CK, median (25th, 75th %tile), µg/L	137 (80, 215)	141 (92, 276)	141 (92, 276)	121 (75, 249)	0.011	0.113
CK-MB, median (25th, 75th %tile), ug/L	3.3 (1.8, 6.2)	5.0 (2.0, 16.0)	5.3 (2.5, 11.3)	16.8 (4.6, 49.0)	<0.001	0.338
TnI, median (25th, 75th %tile), µg/L	0.1 (0.0, 0.3)	0.1 (0.1, 0.4)	0.2 (0.1, 0.6)	1.2 (0.1, 7.9)	<0.001	0.420
Σ STD, median (25th, 75th %tile), mm	13.5 (9.5, 20.0)	12.5 (9.0, 17.5)	12.5 (8.0, 18.5)	12.5 (8.0, 18.5)	0.051	-0.071

Abbreviations: CK, creatinine kinase; Σ STD, sum ST segment deviation; TnI, Troponin I.

Figure 3-1: Interleukin-6, interleukin-10, and C-reactive protein levels stratified by NT-pro brain natriuretic peptide quartiles



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Discussion and Conclusions

In several cohorts of high-risk STEMI patients undergoing reperfusion, several important findings emerge. First, the SIRS criteria were independently associated with 90 day clinical outcomes at the time of hospital presentation and at 24 hours after initial therapy. Second, the inflammatory mediators hsCRP, IL-6, and IP-10 were independently predictive of clinical outcomes, but they added little prognostic value to a clinical prediction model that contained NT-proBNP. Third, IL-6, a pro-inflammatory cytokine marker of systemic inflammation was modestly correlated with NT-proBNP, a marker of myocardial distress. This finding supports pre-clinical and clinical studies that suggest that BNP may be expressed in response to systemic inflammatory stimuli.

SIRS is a recognized sequelae and adverse prognostic marker in numerous medical and surgical conditions, but the incidence and associated outcomes in STEMI have remained unclear.(1-5) Reports of SIRS in the STEMI population have been limited to single center case series of less than 200 patients with an insufficient number of events to permit robust multivariable analyses.(6,7) In this analysis both SIRS and the cumulative number of SIRS criteria were associated with 90 days outcomes at the time of hospital presentation and at 24 hours after admission. Taken together these findings reinforce the importance of the clinical inflammatory response in STEMI. Moreover, the inflammatory response can potentially identify a high risk subset of STEMI patients and future studies aimed at identifying management and therapeutic strategies are required.

Independent associations between inflammatory biomarkers and clinical outcomes have been reported by multiple studies.(8-15) This is the first study, however, to report the incremental contribution of inflammatory cytokines and chemokines to the performance of clinical risk prediction models. This analysis' most significant finding was that although some inflammatory biomarkers improved clinical risk

prediction, they added little predictive value - assessed using c-index, NRI, and IDI - after NT-proBNP was incorporated into the clinical prediction model.

These observations suggest that the inflammatory biomarkers evaluated in this study may have little prognostic utility in a clinical practice environment where NT-proBNP is well validated and widely available. This could potentially be used as a benchmark to guide future biomarker development whereby future investigational STEMI prognostic biomarkers ought to demonstrate added prognostic value to clinical risk prediction models containing validated and available markers. Similarly, relying solely on the change in c-index does not provide clinicians with a clinically meaningful measures prognostic improvement.(16) Thus, this analysis utilized a comprehensive set of model discrimination measures to better describe the potential utility of inflammatory biomarkers in STEMI risk prediction.

BNP expression is traditionally thought to results from myocardial stretch, but studies suggest that systemic inflammation may mediate its expression.(17) Elevated BNP levels have been reported in patients with inflammatory conditions and normal cardiac function.(18,19) Additionally, in pre-clinical studies the pro-inflammatory cytokines, such as IL-6, have been shown to stimulated cardiocyte BNP expression.(17,20-22) No study has previously reported the correlation between the inflammatory mediators and NT-proBNP in patients with STEMI. In this analysis the pro-inflammatory cytokine IL-6 was modestly correlated with baseline NT-proBNP levels and this relationship remained significant after excluding patients with clinical heart failure and after controlling for elevated filling pressures. These findings support pre-clinical and clinical studies that suggest that systemic inflammatory stimuli can stimulate BNP expression. Future studies should be directed at elucidating the complex relationships

between pro-inflammatory cytokines and BNP expression to identify potential therapeutic targets and understand the thus far neutral results seen in modulators of the inflammatory cascade in STEMI.(23,24)

Conclusion

In two contemporary populations of patients with STEMI, we have demonstrated that the diagnosis of SIRS and cumulative number of SIRS criteria were independently associated with 90-day clinical outcomes among STEMI patients. Second, among the inflammatory biomarkers we evaluated only the pro-inflammatory cytokine IL-6 was modestly correlated with baseline NT-proBNP levels and this association remained significant after excluding HF patients and after controlling for elevated filling pressures. This finding suggests a possible link between the natriuretic peptide and inflammatory systems insofar as it supports previous clinical and pre-clinical studies reporting that BNP may be expressed in response to systemic inflammatory stimuli. Finally, although this correlation was demonstrated and multiple inflammatory biomarkers independently predicted 90-day death, shock or heart failure, they added little value to a clinical prediction model that included NT-proBNP.

Future studies are required to further elucidate the complex relationships between systemic inflammatory mediators, cardiac biomarkers, and the clinical inflammatory response. It also underscores the need to identify specific management strategies to address the high risk subset of patients who develop significant inflammatory responses after a myocardial infarction.

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