ORIGINAL RESEARCH

Multimarker Risk Stratification in Patients With Acute Myocardial Infarction

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***Background*—**Several biomarkers have individually been shown to be useful for risk stratification in patients with acute myocardial infarction (MI). The optimal multimarker strategy remains undefined.

***Methods and Results*—**Biomarkers representing different pathobiological axes were studied, including myocardial stress/structural changes (NT-pro B-type natriuretic peptide [NT-proBNP], midregional proatrial natriuretic peptide [MR-proANP], suppression of tumorigenicity 2 [ST2], galectin-3, midregional proadrenomedullin [MR-proADM], and copeptin), myonecrosis (troponin T), and inflammation (myeloperoxidase [MPO], high sensitivity C-reactive protein [hsCRP], pregnancy-associated plasma protein A [PAPP-A], and growth-differentiation factor-15 [GDF-15]), in up to 1258 patients from Clopidogrel as Adjunctive Reperfusion Therapy-Thrombolysis in Myocardial Infarction 28 (CLARITY-TIMI 28), a randomized trial of clopidogrel in ST-elevation MI (STEMI). Patients were followed for 30 days. Biomarker analyses were adjusted for traditional clinical variables. Forward stepwise selection was used to assess a multimarker strategy. After adjustment for clinical variables and using a dichotomous cutpoint, 7 biomarkers were each significantly associated with a higher odds of cardiovascular death or heart failure (HF) through 30 days, including NT-proBNP (adjusted odds ratio [OR_{adj}], 2.54; 95% CI, 1.47–4.37), MR-proANP (2.18; 1.27–3.76), ST2 (2.88; 1.72–4.81), troponin T (4.13; 1.85–9.20), MPO (2.75; 1.20–6.27), hsCRP (1.96, 1.17–3.30), and PAPP-A (3.04; 1.17–7.88). In a multimarker model, 3 biomarkers emerged as significant and complementary predictors of cardiovascular death or HF: ST2 (OR_{adj}, 2.87; 1.61–5.12), troponin T (2.34; 1.09–5.01 and 4.13, 1.85–9.20, respectively for intermediate and high levels), and MPO (2.49; 1.04–5.96). When added to the TIMI STEMI Risk Score alone, the multimarker risk score significantly improved the C-statistic (area under the curve, 0.75 [95% CI, 0.69–0.81] to 0.82 [0.78–0.87]; P=0.001), net reclassification index (0.93; P<0.001), and integrated discrimination index (0.09; P<0.001).

***Conclusions*—**In patients with STEMI, a multimarker strategy that combines biomarkers across pathobiological axes of myocardial stress, myocyte necrosis, and inflammation provides incremental prognostic information for prediction of cardiovascular death or HF. **(*J Am Heart Assoc*. 2016;5:e002586 doi: 10.1161/JAHA.115.002586)**

Key Words: biomarkers • multimarker • prognosis • ST-elevation myocardial infarction • Thrombolysis in Myocardial Infarction risk score

Subsequent to hospitalization for an acute myocardial infarction (MI), patients remain at increased risk of death and recurrent cardiovascular events. Several biomarkers have emerged as potential tools for patient risk stratification and provide incremental information beyond established risk predictors. To date, such markers have largely been evaluated on an individual basis, and therefore limited data are available that compare the prognostic utility of multiple candidate markers simultaneously in the setting of an acute coronary syndrome.[1–7]

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Accompanying Tables S1 through S18 are available at http://jaha.ahajournals.org/content/5/5/e002586/DC1/embed/inline-supplementary-material-1.pdf

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Given that many markers reflect different pathobiological axes of response post-MI, a multimarker strategy might provide substantially more information for risk stratification than any individual marker on its own.[3] In addition, identifying such high-risk patients could aid in early triage decisions. In particular, past studies have demonstrated that there may be additive value for combining markers including those of myonecrosis, myocardial strain or stress, and vascular inflammation.[1–7] However, the identification of more recent candidate markers that reflect alternate pathways of disease or are more sensitive markers of underlying biology offer the possibility that a novel multimarker risk score may be able to provide incremental information for risk stratification. We therefore evaluated the prognostic utility of several established and novel candidate biomarkers in a trial population of patients with an acute MI and evaluated the role of a multimarker strategy in risk stratification across several pathobiological axes of myocardial injury, including inflammation, myocardial stress, structural changes, and myonecrosis.

Methods

Study Population and Design

The design and results of Clopidogrel as Adjunctive Reperfusion Therapy-Thrombolysis in Myocardial Infarction 28 (CLARITY-TIMI 28) have previously been reported.[8,9] In brief, CLARITY-TIMI 28 was a double-blind, randomized, multicenter clinical trial of clopidogrel versus placebo in addition to fibrinolytic therapy in 3491 patients with STEMI who presented within 12 hours of symptom onset. The choice of fibrinolytic and type of heparin were left at the discretion of the managing physician. Relevant exclusion criteria included age <18 or >75 years, use of clopidogrel in the 7 days preceding randomization, contraindications to fibrinolytic drugs, planned elective angiography within 48 hours, or evidence of cardiogenic shock.

As part of the trial protocol, patients were scheduled to undergo coronary angiography 2 to 8 days after initiation of therapy to assess for late patency of the infarct-related artery. Angiography was permitted less than 48 hours only if clinically indicated. The decision for coronary revascularization was left to the discretion of the managing physician. Patients were followed for clinical outcomes, and adverse events through to 30 days after the time of randomization and vital status were obtained in 99.9% of all patients. The protocol was approved by the relevant institutional review boards, and written informed consent was obtained from all patients.

Outcomes

The prespecified clinical endpoint of interest for this analysis was the composite of cardiovascular death or heart failure (HF) based on previous prognostic assessments for the markers in acute coronary syndrome (ACS) patients. Other endpoints that were assessed in CLARITY-TIMI 28 were recurrent MI, recurrent ischemia requiring urgent revascularization, and TIMI flow grade 0 to 1 on protocol-mandated angiography. Endpoints were defined according to previously reported criteria.[8,9] All ischemic events were adjudicated by a clinical events committee that was blinded to assigned treatment arm. Information on the development of new or worsening HF was collected from the case report forms.

Blood Sampling and Analysis

A sample of blood was obtained at time of enrollment and was available for testing in up to 1258 subjects who elected to participate and were at sites that participated in this optional substudy, which represented 36% of the overall study population of CLARITY-TIMI 28. Median time from symptom onset to randomization was 2.5 hours. There were no clinically meaningful differences in baseline characteristics of the biomarker group and the overall trial cohort.[10] The serum and plasma components were frozen and shipped to the TIMI Biomarker Core Laboratory (Boston, MA), where samples were stored at −70°C. A total of 11 established and emerging biomarkers were measured in the biomarker cohort as sample volume permitted by individuals who were blinded to patient outcomes. These included biomarkers of myocardial stress or structural changes (NT-pro B-type natriuretic peptide [NT-proBNP; Roche Diagnostics, Indianapolis, IN], midregional pro-atrial natriuretic peptide [MR-proANP; B.R.A.H.M.S GmbH, Hennigsdorf, Germany], soluble suppression of tumorigenicity 2 [ST2; MBL International Corporation, Woburn, MA], galectin-3 [BG Medicine, Inc., Waltham, MA], midregional pro-adrenomedullin [MR-proADM; B.R.A.H.M.S GmbH], and copeptin [B.R.A.H.M.S GmbH]), biomarkers of myonecrosis (troponin T [third-generation assay; Roche Diagnostics]) and biomarkers of inflammation (myeloperoxidase [MPO; R&D Systems, Minneapolis, MN], high sensitivity C-reactive protein [hsCRP; Roche Diagnostics], pregnancy-associated plasma protein A [PAPP-A; Beckman Coulter, Danvers, MA], and growth differentiation factor-15 [GDF-15; R&D Systems]). Samples were assayed after 2006 with myeloperoxidase (MPO), PAPP-A, and galectin-3 completed in 2014.

Statistical Analysis

Continuous variables were compared using the Kruskal–Wallis test and categorical variables were compared using the χ^2 test for trend. The correlation between baseline biomarkers was assessed with Spearman's correlation coefficient. All biomarkers were first assessed by both categorizing the marker into

quartiles and looking at the continuous association between biomarker and risk using logistic regression models (risk per SD of log-transformed biomarker). For troponin T, all patients who had undetectable levels were categorized as the referent group and patients with detectable levels were divided into tertiles in all models. After assessing the shape of the relationship with cardiovascular events, biomarkers were subsequently assessed as dichotomous variables before multimarker selection with the threshold applied at the concentration where an inflection in the risk of cardiovascular death or HF was most apparent. For most markers, this dichotomous threshold was applied at the fourth quartile (Q) with the first through third quartile as referent. The only exceptions were MPO, GDF-15, and PAPP-A where the threshold was applied between the first and second quartile (modeled as Q2–4:1). In the case of troponin T, 3 groups were used: undetectable, the first and second tertiles combined, and the third tertile. Logistic regression models were used to assess the relationship between marker and outcomes adjusting for age, sex, past HF, diabetes mellitus, past MI, systolic blood pressure, heart rate, Killip class II to IV, anterior ST-elevation MI (STEMI), creatinine clearance <60 mL/min, time to lytic, type of lytic, and randomized treatment arm. A multimarker strategy was assessed by introducing all markers at their dichotomous thresholds (except for troponin T, which was maintained as a 3-way variable) through a forward selection process with a $P<0.05$ for retention in the final model. Final candidate markers were subsequently confirmed through backward selection. Once the final model was determined, an integeric score system was created by summing the number of elevated markers. In the case of troponin T, 1 point was assigned to patients with a detectable troponin T concentration in the first or second tertile and 2 points were assigned to patients with a detectable troponin T concentration in the top tertile. The prognostic utility of the multimarker risk score and the TIMI Risk Score for STEMI[11] and GRACE Risk Score[12] were compared through assessment of the C-statistic, integrated discrimination index (IDI), and categoryless net reclassification index (NRI) that was calculated on a continuous basis, using previously described methodology.[13] A test for the equality of the area under the receiver-operating curves (AUCs) was applied using the algorithm described by DeLong et al.[14] Because all analyses were exploratory, tests were 2-sided with a $P<0.05$ considered to be significant.

Results

Baseline characteristics for the overall biomarker cohort and by quartile of biomarker are shown in Tables S1 through S12. In general, higher concentrations of markers were associated with a higher prevalence of multiple traditional cardiovascular risk factors and a higher TIMI Risk Score for STEMI. A moderate-to-strong correlation was observed between MPO and PAPP-A ($\rho=0.60$; $P<0.001$), troponin T and NT-proBNP ($\rho=0.54$; $P<0.001$) and MR-proADM and MR-proANP ($\rho=0.67$; $P<0.001$). A modest or weak relationship was observed between all remaining markers (Table 1).

Prognostic Utility of Individual Markers

Table 2 shows the odds of cardiovascular death or HF per 1 SD increase in each biomarker as well as the incidence of cardiovascular death or HF through 30 days across quartiles

Table 1. Spearman Correlation (Rho, *P* Value) Between Candidate Biomarkers in CLARITY-TIMI 28

	hsCRP	MPO	PAPP-A	GDF-15	NT-proBNP	MR-proANP	ST2	Gal-3	TnT	Copeptin	MR-proADM
hsCRP	—	0.09	−0.06	0.12	0.31	0.04	0.05	0.19	0.18	−0.11	0.12
MPO	*P*<0.01	—	0.60	0.11	0.02	−0.16	0.05	0.31	0.09	−0.04	−0.29
PAPPA	*P*=0.09	*P*<0.01	—	0.04	−0.04	−0.22	−0.03	0.04	0.02	−0.03	−0.36
GDF-15	*P*<0.01	*P*<0.01	*P*=0.27	—	0.15	0.04	0.10	0.29	0.13	0.07	−0.03
NT-proBNP	*P*<0.01	*P*=0.55	*P*=0.21	*P*<0.01	—	0.34	0.13	0.18	0.54	−0.15	0.26
MR-proANP	*P*=0.20	*P*<0.01	*P*<0.01	*P*=0.24	*P*<0.01	—	0.07	0.15	0.19	0.25	0.67
ST2	*P*=0.08	*P*=0.10	*P*=0.46	*P*<0.01	*P*<0.01	*P*=0.02	—	0.14	0.15	0.07	0.08
Gal-3	*P*<0.01	*P*<0.01	*P*=0.33	*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01	—	0.18	0.12	0.09
TnT	*P*<0.01	*P*<0.01	*P*=0.59	*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01	—	−0.27	0.19
Copeptin	*P*<0.01	*P*=0.22	*P*=0.43	*P*=0.02	*P*<0.01	*P*<0.01	*P*=0.02	*P*<0.01	*P*<0.01	—	0.14
MR-proADM	*P*<0.01	*P*<0.01	*P*<0.01	*P*=0.27	*P*<0.01	*P*<0.01	*P*=0.01	*P*<0.01	*P*<0.01	*P*<0.01	—

Rho values in upper right and *P* values in lower left of table. Gal-3 indicates galectin-3; GDF-15, growth-differentiation factor-15; hsCRP, high-sensitivity C-reactive protein; MPO, myeloperoxidase; MR-proADM, midregional proadrenomedullin; MR-proANP, midregional proatrial natriuretic peptide; NT-proBNP, NT-pro B-type natriuretic peptide; PAPP-A, pregnancy-associated plasma protein A; ST2, suppression of tumorigenicity 2; TnT, troponin T.

Table 2. Incidence of Cardiovascular Death or HF at 30 Days per 1 standard deviation increase and by Quartile of Biomarker

Biomarker	OR (95% CI) Per 1 Standard Deviation of Log-Transformed Biomarker	*P* Value	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
NT-proBNP (n=1249)	2.27 (1.83–2.82)	<0.001	3.2%	3.5%	5.4%	16.7%	<0.001
MR-proANP (n=1121)	1.73 (1.26–2.36)	0.001	4.3%	4.3%	5.7%	16.4%	<0.001
ST2 (n=1239)	1.76 (1.40–2.21)	<0.001	4.4%	4.2%	6.2%	14.3%	<0.001
Galectin-3 (n=1034)	1.50 (1.24–1.82)	<0.001	3.8%	4.3%	8.5%	14.0%	<0.001
Copeptin (n=1126)	1.25 (0.99–1.58)	0.06	7.8%	5.0%	6.7%	11.0%	0.11
MR-proADM (n=1126)	1.19 (0.94–1.51)	0.15	7.4%	5.0%	5.0%	13.2%	0.02
Troponin T* (n=1250)	2.15 (1.75–2.63)	<0.001	3.2%	5.0%	8.2%	19.3%	<0.001
hsCRP (n=1250)	1.96 (1.61–2.39)	<0.001	4.7%	3.9%	6.4%	13.8%	<0.001
Myeloperoxidase (n=1045)	1.31 (1.04–1.65)	0.02	3.1%	10.3%	8.4%	8.4%	0.051
PAPP-A (n=876)	1.17 (0.89–1.53)	0.26	4.1%	6.8%	5.9%	7.3%	0.23
GDF-15 (n=1111)	1.26 (1.02–1.55)	0.03	5.0%	7.2%	8.3%	7.9%	0.16

GDF-15 indicates growth-differentiation factor-15; HF, heart failure; hsCRP, high-sensitivity C-reactive protein; MR-proADM, midregional proadrenomedullin; MR-proANP, midregional proatrial natriuretic peptide; NT-proBNP, NT-pro B-type natriuretic peptide; OR, odds ratio; PAPP-A, pregnancy-associated plasma protein A; ST2, suppression of tumorigenicity 2.
*All patients with undetectable troponin T levels were grouped in quartile 1, and then patients with detectable troponin T levels were divided into tertiles.

of biomarkers. There were associations between the risk of cardiovascular death or HF and increasing levels of several biomarkers, including NT-proBNP (P_{trend}<0.001), MR-proANP (P_{trend}<0.001), ST2 (P_{trend}<0.001), galectin-3 (P_{trend}<0.001), MR-proADM (P_{trend}=0.02), troponin T (P_{trend}<0.001), MPO (P_{trend}=0.051), and hsCRP (P_{trend}<0.001). There appeared to be inflection points for risk for most of the biomarkers and, after applying such dichotomous thresholds, 7 biomarkers individually remained significantly associated with a higher odds of cardiovascular death or HF through 30 days after adjusting for traditional risk factors (Table 3). Of the biomarkers of myocardial stress/structural changes, NT-proBNP (adjusted OR, 2.54; 95% CI, 1.47–4.37), MR-proANP (adjusted OR, 2.18; 95% CI, 1.27–3.76), and

Table 3. Association Between Each Candidate Marker Individually When Modeled as a Categorical Variable and the Odds of Cardiovascular Death or HF at 30 Days After Multivariable Adjustment

	Biomarker	OR (95% CI) Adjusted for Clinical Factors Modeled Applying a Dichotomous Threshold	*P* Value
Myocardial stress/ structural changes	NT-proBNP (n=1142)	2.54 (1.47–4.37)	0.001
	MR-proANP (n=1027)	2.18 (1.27–3.76)	0.005
	ST2 (n=1133)	2.88 (1.72–4.81)	<0.001
	Galectin-3 (n=943)	1.74 (0.96–3.16)	0.07
	MR-proADM (n=1032)	1.07 (0.61–1.87)	0.82
	Copeptin (n=1031)	1.12 (0.63–1.99)	0.71
Myonecrosis (n=1142)	Troponin T (T1–T2)*	2.40 (1.22–4.71)	0.01
	Troponin T (T3)*	4.37 (2.15–8.89)	<0.001
Inflammation	MPO (n=952)	2.75 (1.20–6.27)	0.02
	hsCRP (n=1140)	1.96 (1.17–3.30)	0.01
	PAPP-A (n=794)	3.04 (1.17–7.88)	0.02
	GDF-15 (n=1019)	0.81 (0.41–1.60)	0.54

Multivariable model included age, sex, past HF, diabetes mellitus, past MI, systolic blood pressure, heart rate, Killip class II to IV, type of lytic, anterior STEMI, creatinine clearance <60 mL/min, time to lytic, treatment arm. Gal-3 indicates galectin-3; GDF-15, growth-differentiation factor-15; HF, heart failure; hsCRP, high-sensitivity C-reactive protein; MI, myocardial infarction; MPO, myeloperoxidase; MR-proADM, midregional proadrenomedullin; MR-proANP, midregional proatrial natriuretic peptide; NT-proBNP, NT-pro B-type natriuretic peptide; OR, odds ratio; PAPP-A, pregnancy-associated plasma protein A; ST2, suppression of tumorigenicity 2; STEMI, ST-elevation myocardial infarction.
*For categorical analysis, troponin T was coded as 3-way variable with undetectable troponin T as referent and detectable troponin T modeled by tertile (T).

ST2 (adjusted OR, 2.88; 95% CI, 1.72–4.81) remained significant predictors after adjusting for traditional risk factors. Of patients with detectable troponin T levels, those in tertiles 1 and 2 had more than a 2-fold higher odds of cardiovascular death or HF (adjusted OR, 2.40; 95% CI, 1.22–4.71) and those in the upper tertile had more than a 4-fold higher odds of cardiovascular death or HF at 30 days (adjusted OR, 4.37; 95% CI, 2.15–8.89). Of the inflammatory biomarkers, MPO (adjusted OR, 2.75; 95% CI, 1.20–6.27), hsCRP (adjusted OR, 1.96; 95% CI, 1.17–3.30) and PAPP-A (adjusted OR, 3.04; 95% CI, 1.17–7.88) remained significant predictors after adjusting for traditional risk factors. In general, directional consistency was observed across the individual elements of the composite outcome of cardiovascular death or HF, with a stronger relationship observed between candidate markers and the odds of cardiovascular death than for heart failure (Tables S13 and S14). In general, biomarker concentration was not associated with the odds of MI, recurrent ischemia requiring urgent revascularization or TIMI flow grade 0 to 1, except for ST2, where higher levels of which were associated with recurrent MI and TIMI flow grade 0 to 1 (Tables S15 through S17).

Multimarker Risk Stratification

When candidate markers were combined into a multimarker model, 3 biomarkers emerged as significant and complementary predictors of cardiovascular death or HF through both forward and backward selection: ST2, troponin T, and MPO. Patients with higher levels of either ST2 or MPO had more than a 2-fold higher odds of cardiovascular death or HF (adjusted OR, 2.87; 95% CI, 1.61–5.12 and adjusted OR, 2.49; 95% CI, 1.04–5.96, respectively). Similarly, patients with detectable troponin T levels in the first or second tertile had more than a 2-fold higher odds of cardiovascular death or HF (adjusted OR, 2.34; 95% CI, 1.09–5.01) and those with a troponin T concentration in the highest detectable tertile had more than a 4-fold higher odds of an event (adjusted OR, 4.13; 95% CI, 1.85–9.20; Figure 1). For patients in whom left ventricular ejection fraction (LVEF) had been assessed (n=515), directionally consistent findings were observed regarding the prognostic value of all 3 markers when LVEF was included in the model (Table S18).

When an integeric multimarker risk score was created that assigned points for elevations in ST2 (1 point), troponin T (1 or 2 points), or MPO (1 point; Figure 2), a step-wise increase in the incidence of 30 day cardiovascular death or HF was observed for patients with higher biomarker scores ($P<0.001$ for trend). Furthermore, the multimarker risk score provided incremental information for risk stratification to the TIMI Risk Score for STEMI (Figure 3). In particular, the multimarker risk score significantly improved the C-statistic for predicting cardiovascular death or HF as compared with the TIMI STEMI Risk Score alone (AUC, 0.75 [95% CI, 0.69–0.81] to 0.82 [95% CI, 0.78–0.87]; $P=0.001$), as well as the NRI (0.93; $P<0.001$) and IDI (0.09; $P<0.001$). Similarly, the multimarker score improved the C-statistic as compared with the GRACE Risk Score alone (AUC, 0.76 [95% CI, 0.72–0.80] to 0.85 [95% CI, 0.81–0.89]; $P<0.001$), as well as the NRI (0.93; $P<0.001$ and IDI (0.10; $P<0.001$).

Discussion

In a large trial population of patients with STEMI, we evaluated the prognostic utility of 11 novel and established biomarkers. We determined that a multimarker strategy that combines

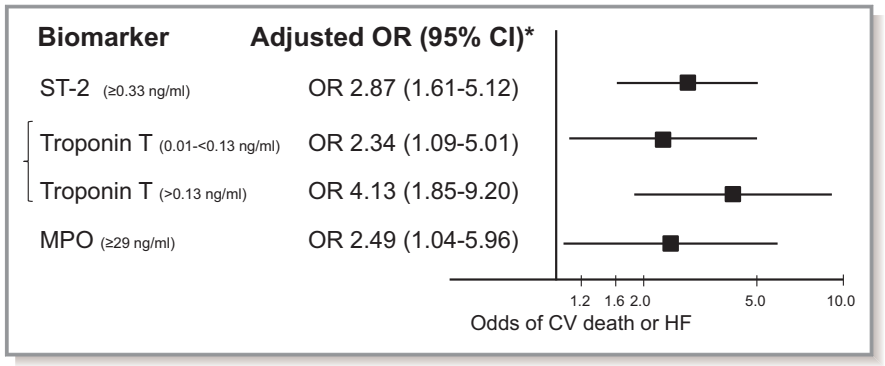

Figure 1. When all markers were combined in a model, 3 candidate markers remained significantly associated with higher odds of CV death or HF at 30 days in patients with STEMI after multivariable adjustment. Troponin T was modeled into 3 groups: undetectable as referent then tertiles (T) of detectable troponin T with tertile 1 and 2 collapsed. CV indicates cardiovascular; HF, heart failure; MPO, myeloperoxidase; OR, odds ratio; STEMI, ST-elevation myocardial infarction.

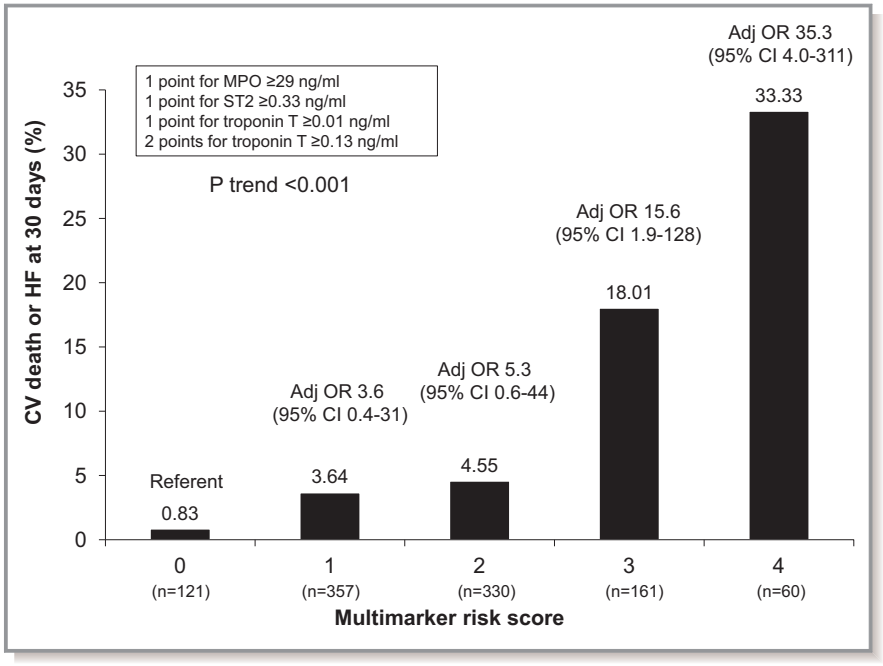

Figure 2. An integeric risk score was created using the 3 markers that were significantly associated with the odds CV death or HF including ST2, MPO, and troponin T. A step-wise increase in the incidence of 30-day CV death or HF was observed with increasing multimarker risk score. CV indicates cardiovascular; HF, heart failure; MPO, myeloperoxidase; OR, odds ratio.

biomarkers across pathobiological axes of myocardial stress, myocyte necrosis, and inflammation provides incremental prognostic information for risk stratification. In particular, by combining three markers (troponin T, ST2, and MPO) into a multimarker risk score, we were able to identify patients at increased risk of 30-day of cardiovascular death or HF with

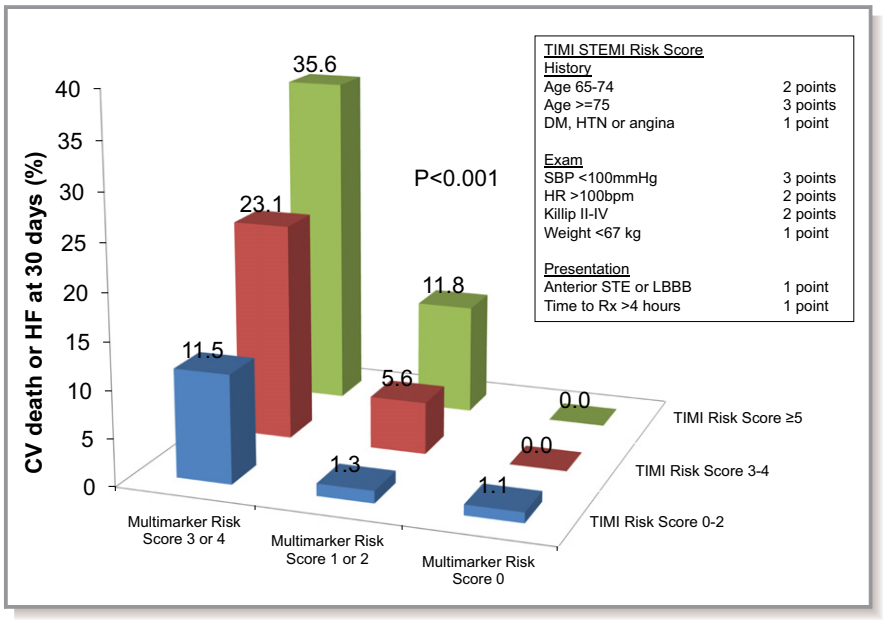

Figure 3. The multimarker risk score provided incremental information for risk stratification to the TIMI risk score for STEMI. CV indicates cardiovascular; DM, diabetes mellitus; HF, heart failure; HR, hazard ratio; HTN, hypertension; LBBB, left bundle branch block; SBP, systolic blood pressure; STE, ST elevation; STEMI, ST-elevation myocardial infarction; TIMI, Thrombolysis in Myocardial Infarction.

additive value beyond established clinical predictors and the TIMI and GRACE Risk Score for STEMI alone. These findings could therefore have implications in regards to early patient triage in the setting of acute MI.

Despite marked improvements in outcomes over the past few decades, early readmission rates and mortality remain high after hospital discharge for patients hospitalized with an acute MI.[15] Therefore, it remains of key importance to continue to develop tools that may identify individuals at heightened risk of adverse outcomes who may benefit more from enhanced monitoring or potentially directed treatment strategies. In the current analysis that included up to 1258 patients with an acute MI, we found that several candidate markers—NT-proBNP, MR-proANP, ST2, troponin T, MPO, hsCRP, and PAPP-A—were individually significantly associated with the 30-day odds of cardiovascular death or HF after adjustment for clinical predictors.

However, the rapid proliferation of candidate markers that appear to be useful for risk stratification has contributed to growing confusion among practitioners as to which marker may be more useful for clinical practice. Furthermore, few have been able to reliably provide incremental information for risk discrimination beyond established clinical tools.[16] In particular, there often exists a moderate-to-strong correlation between markers that reflect similar pathobiological axes of disease. As such, the relative prognostic value of any single candidate marker versus another may be unclear.

By assessing multiple markers simultaneously, we were able to demonstrate that only 3 candidate markers (ST2, troponin T, and MPO) remained significantly associated with the odds of cardiovascular death or HF after adjusting for both clinical predictors and other candidate markers. Interestingly, these 3 markers reflect different pathobiological axes of disease, including myocardial stress, myocyte necrosis, and inflammation, suggesting that this type of multimarker strategy may leverage the complementary prognostic utility of each marker to further optimize risk stratification and discrimination. The current findings are supported by a multimarker analysis of 7 candidate markers in a population of patients with non-ST-segment-elevation (NSTE)-ACS whereby both troponin T and MPO emerged as significant markers of long-term cardiovascular risk; however, ST2 was not evaluated.[6]

In the current study of 11 candidate markers, individuals with elevated levels of ST2, troponin T, or MPO had more than a 2-fold higher odds of cardiovascular death or HF at 30 days, and those with the highest levels of troponin T had more than a 4-fold higher odds of an event after multivariable adjustment. Moreover, by combining these markers into a multimarker risk score, we were able to demonstrate a steep stepwise increase in the 30-day incidence of cardiovascular death or HF ranging from <1% for individuals with a score of 0 to 33% for individuals with a score of 4. Of note, patients with a biomarker score of 0 had a low risk of cardiovascular death or HF regardless of their clinical features.

The prognostic utility of troponin in the setting of ACS was first established several years ago and has now been replicated across multiple trials,[17–22] including the current study. Consistent with many previous studies, troponin T was not associated with the risk of recurrent ischemic events, but its robust prognostic value was most apparent for the odds of cardiovascular death and HF. Given that troponin concentration reflects the extent of myonecrosis, it is also a direct correlate of infarct size.[23] However, troponin release may also occur in the setting of myocardial strain[24] and may therefore help to identify patients at increased risk of cardiovascular death or HF through alternate axes of pathobiology. To that end, the prognostic value of troponin T was maintained after adjusting for surrogates of infarct size, in addition to other clinical predictors. Similarly, ST2, a soluble interleukin-1 receptor family member, is upregulated and secreted in response to mechanical stress.[25] Yet, in the current study, we observed only a weak correlation between ST2 and other markers of myocardial strain, including BNP, galectin-3, and troponin T. Similar observations have been noted in the setting of NSTE-ACS,[26] leading investigators to speculate that ST2 may not be solely a marker of myocardial strain, but also a marker of inflammation, fibrosis, and adverse myocardial remodeling.[26]

MPO is an established marker of inflammation that may have proatherogenic and destabilizing effects on atheromatous plaque through a variety of proposed pathways, including low-density lipoprotein oxidation.[27] Inflammation is known to be not only a key mediator in atherogenesis and plaque rupture, but also plays a central role in myocardial wound healing after acute MI.[28] A maladaptive wound-healing response can contribute to adverse remodeling and may identify patients at increased risk of HF. Although markers of inflammation are sometimes considered to be strong correlates of infarct size, we observed only a weak correlation between MPO and troponin T concentration in the current study. Although the relationship has not always been consistent,[29] several studies have previously demonstrated an association between MPO and the risk of recurrent cardiovascular events when measured during hospitalization with ACS.[27,30–32] The current findings therefore lend renewed support to the use of MPO as a prognostic marker after adjusting for other established risk predictors, including a wide number of biomarkers.

Limitations to the current analysis warrant consideration. Samples were not available in the entire study cohort, and there remains the possibility of residual confounding despite extensive multivariable adjustment. Because there are no widely accepted cutpoints for many of the markers evaluated in the setting of acute MI, we applied thresholds on the basis

of their relationship with outcomes by quartiles. Therefore, the current multimarker risk score and all applied cutpoints require further validation in additional patient populations. As well, there have been reports of preanalytical issues in regards to MPO quantification in the setting of heparin exposure.[33,34] The samples used to assess MPO concentration in the current study were stored in EDTA plasma tubes, but the majority of patients were treated with systemic heparin as part of their acute MI management. However, one might expect that any assay interference attributed to heparin exposure would bias the results toward the null, which was not observed in the current study. For the assessment of troponin T, we used a current generation assay that is the current assay used in the United States, but future analyses may consider the use of newer high-sensitivity assays. As well, LVEF was only assessed in a subset of individuals, and therefore it was not possible to adjust for LVEF in all models; however, qualitatively consistent results were observed when this parameter was included as a covariate in a sensitivity analysis.

In summary, after the simultaneous assessment of 11 novel and established biomarkers of risk in patients hospitalized with an acute MI, ST2, troponin T, and MPO emerged as significant predictors of short-term cardiovascular death or HF after adjusting for clinical predictors and other candidate markers. These findings highlight the incremental utility of combining markers that reflect complementary axes of cardiovascular disease.

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SUPPLEMENTAL MATERIAL

Table S1: Baseline characteristics for patients in the biomarker substudy of CLARITY-TIMI 28.

Variable	
Number	1250
Gender (male), %	79.4%
Age (years), median (25th, 75th percentile)	58 (50, 67)
Weight (kg), median (25th, 75th percentile)	80 (70, 89)
BMI, median (25th, 75th percentile)	27.1 (24.8, 30.1)
History hypertension, %	39.4
History hyperlipidemia, %	40.8
History diabetes, %	17.4
History CHF, %	1.3
Current tobacco use, %	47.3
Prior MI, %	8.4
Prior PCI, %	4.6
Anterior MI, %	37.5
CrCl, median (25th, 75th percentile)	86.9 (70.2, 106.7)
Sx onset to lytic (hrs), median (25th, 75th percentile)	2.5 (1.7, 3.8)
Killip class 2-4, %	7.0
Fibrin-specific lytic, %	86.7
Initial heparin type, %	
None, %	7.0
UFH, %	44.9
LMWH, %	43.4
Both, %	4.7
Angiography performed during index hospitalization, %	96.0

Table S2: Baseline characteristics by NT-proBNP quartile at baseline

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Number	313	312	312	312	
Ranges (pg/ml)	< 36.4	36.4-93.8	93.8-282.4	>282.4	
Gender (male), %	88.8	83.3	76.6	68.9	<0.001
Age (years), median	51	56	62	65	<0.001
Weight (kg), median	80.0	80.0	80.0	78.0	0.03
BMI, median	27.1	26.8	27.5	27.1	0.44
History hypertension, %	31.1	30.4	44.2	51.6	<0.001
History hyperlipidemia, %	44.5	42.4	41.7	34.1	0.02
History diabetes, %	13.9	15.9	15.4	24.4	0.001
History CHF, %	0.0	0.3	1.0	3.9	<0.001
Current tobacco use, %	54.5	49.7	43.5	41.7	<0.001
Prior MI, %	7.1	5.8	8.4	12.5	0.007
Prior PCI, %	5.1	3.8	3.5	5.8	0.75
Anterior MI, %	31.9	34.0	36.5	47.8	<0.001
CrCl, median	93.2	92.5	84.8	76.6	<0.001
Sx onset to lytic (hrs), median	2.0	2.3	2.6	3.6	<0.001
Killip class 2-4, %	2.6	6.8	5.5	13.2	<0.001
Fibrin-specific lytic, %	89.5	87.2	88.1	82.1	0.01
Initial heparin type, %					
None, %	6.1	5.4	7.1	9.3	0.30
UFH, %	41.2	42.9	50.3	44.9	
LMWH, %	47.3	47.8	37.8	41.0	
Both, %	5.4	3.8	4.8	4.8	
Angiography performed during index hospitalization, %	98.1	98.1	97.1	90.7	<0.001

Table S3: Baseline characteristics by quartile of ANP at baseline

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Number	281	280	280	280	
Ranges (pmol/L)	< 56.2	56.2-108.7	108.7 - 181.7	>181.7	
Gender (male) , %	86.1	82.9	78.2	72.1	<0.001
Age (years), median	54	55	59	65.5	<0.001
Weight (kg), median	84.0	80.0	80.0	77.0	<0.001
BMI, median	27.9	27.0	27.0	26.6	<0.001
History hypertension, %	40.0	36.5	36.9	44.2	0.32
History hyperlipidemia, %	41.6	43.4	41.7	36.9	0.27
History diabetes, %	18.3	16.7	17.6	18.0	0.995
History CHF, %	0.4	0.7	1.1	3.3	0.003
Current tobacco use, %	53.4	51.3	43.0	42.1	0.002
Prior MI, %	6.8	10.0	6.1	10.4	0.35
Prior PCI, %	4.6	5.0	2.5	6.5	0.60
Anterior MI, %	29.9	36.4	37.9	48.2	<0.001
CrCl, median	96.1	95.9	85.9	74.1	<0.001
Sx onset to lytic (hrs), median	2.3	2.5	2.6	2.8	0.06
Killip class 2-4, %	3.9	4.7	7.9	11.5	<0.001
Fibrin-specific lytic, %	82.2	91.1	87.1	85.0	0.62
Initial Heparin Type, %					
None, %	12.1	5.0	5.0	6.8	0.12
UFH, %	43.4	50.4	40.4	45.4	
LMWH, %	40.6	39.6	51.4	41.4	
Both, %	3.9	5.0	3.2	6.4	
Angiography performed during index hospitalizati	95.0	98.6	96.8	92.5	0.08

Table S4: Baseline characteristics by ST2 quartile at baseline

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Number	367	259	305	308	
Ranges (ng/ml)	< 0.004	0.004-0.08	0.08-0.33	> 0.33	
Gender (male), %	79.8	80.7	80.3	77.3	0.45
Age (years), median	57	58	59	59	0.06
Weight (kg), median	80.0	80.0	79.8	79.8	0.07
BMI, median	27.4	27.2	26.8	27.0	0.42
History hypertension, %	35.9	37.4	43.7	40.8	0.08
History hyperlipidemia, %	39.3	44.4	42.3	38.3	0.81
History diabetes, %	9.9	18.9	20.5	21.9	<0.001
History CHF, %	1.7	1.5	0.7	1.0	0.28
Current tobacco use, %	50.4	44.8	47.4	45.8	0.31
Prior MI, %	8.5	9.3	9.2	6.9	0.51
Prior PCI, %	5.4	6.2	3.0	3.9	0.14
Anterior MI, %	34.1	37.8	36.4	42.9	0.03
CrCl, median	90.8	88.0	86.0	83.5	0.002
Sx onset to lytic (hrs), median	2.3	2.4	2.7	2.7	<0.001
Killip class 2-4, %	6.3	3.9	8.9	8.8	0.07
Fibrin-specific lytic, %	91.0	86.5	83.9	84.1	0.004
Initial heparin type, %					
None, %	4.4	7.7	6.6	10.1	0.11
UFH, %	42.8	46.7	47.2	43.5	
LMWH, %	46.6	42.9	42.6	41.2	
Both, %	6.3	2.7	3.6	5.2	
Angiography performed during index hospitalization, %	96.7	97.3	95.4	94.5	0.09

Table S5: Baseline characteristics by Galectin-3 quartile at baseline

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Number	260	257	259	258	
Ranges	< 11.7	11.7-14.3	14.3-18.0	> 18.0	
Gender (male) , %	85.4	80.9	81.1	70.5	<0.001
Age (years), median	56	56	60	63	<0.001
Weight (kg), median	80.0	80.0	80.0	80.0	0.48
BMI, median	27.2	26.8	26.8	27.7	0.20
History hypertension, %	31.9	34.6	40.7	47.8	<0.001
History hyperlipidemia, %	41.4	41.4	42.4	37.8	0.50
History diabetes, %	13.8	15.4	19.1	20.4	0.03
History CHF, %	0.8	0.8	1.2	2.3	0.10
Current tobacco use, %	48.5	51.0	46.1	42.0	0.08
Prior MI, %	8.9	8.2	7.0	7.8	0.55
Prior PCI, %	6.5	5.8	3.1	2.7	0.01
Anterior MI, %	36.5	37.0	34.4	43.4	0.18
CrCl, median	97.0	92.4	82.4	76.3	<0.001
Sx onset to lytic (hrs), median	2.4	2.3	2.5	2.8	0.001
Killip class 2-4, %	6.2	7.4	5.0	10.9	0.10
Fibrin-specific lytic, %	88.8	85.2	88.4	82.9	0.13
Initial heparin type, %					
None, %	8.1	9.3	4.2	5.8	0.87
UFH, %	39.2	43.2	48.3	47.3	
LMWH, %	48.5	42.4	43.6	41.5	
Both, %	4.2	5.1	3.9	5.4	
Angiography performed during index hospitalization, %	97.7	96.5	94.6	94.2	0.03

Table S6: Baseline characteristics by MR-pro ADM quartile at baseline

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Number	282	281	282	281	
Ranges (nmol/L)	< 0.13	0.13-0.47	0.47-0.62	>0.62	
Gender (male) , %	83.3	81.5	82.3	72.2	0.002
Age (years), median	59	54	56	65	<0.001
Weight (kg), median	81.0	78.0	80.0	80.0	0.63
BMI, median	27.2	26.8	27.0	27.7	0.44
History hypertension, %	39.9	32.0	33.2	51.6	0.006
History hyperlipidemia, %	36.5	41.4	43.5	41.9	0.18
History diabetes, %	17.4	11.9	17.2	24.5	0.01
History CHF, %	1.1	0.0	0.7	3.6	0.007
Current tobacco use, %	45.9	50.4	53.2	40.6	0.32
Prior MI, %	7.5	7.1	7.9	10.8	0.15
Prior PCI, %	3.9	2.8	7.1	4.6	0.25
Anterior MI, %	32.3	33.5	42.6	44.8	<0.001
CrCl, median	86.3	96.5	91.8	76.1	<0.001
Sx onset to lytic (hrs), median	2.5	2.3	2.5	2.9	<0.001
Killip class 2-4, %	4.6	6.1	6.4	10.4	0.01
Fibrin-specific lytic, %	83.3	90.4	87.9	84.0	0.96
Initial heparin type, %					
None, %	11.3	4.3	6.0	7.1	0.55
UFH, %	47.2	44.1	44.7	42.7	
LMWH, %	37.9	45.2	44.3	46.6	
Both, %	3.5	6.4	5.0	3.6	
Angiography performed during index hospitalization, %	94.3	96.1	98.2	94.3	0.70

Table S7: Baseline characteristics by copeptin quartile at baseline

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Copeptin	282	281	282	281	
Ranges (pmol/L)	< 24.4	24.4-71.2	71.2-160.4	>160.4	
Gender (male) , %	81.6	78.6	83.3	75.8	0.24
Age (years), median	57	58	58	61	<0.001
Weight (kg), median	80.0	81.0	80.0	77.0	<0.001
BMI, median	27.8	27.5	26.8	26.2	<0.001
History hypertension, %	37.9	40.5	39.1	39.0	0.87
History hyperlipidemia, %	40.3	38.5	43.9	40.8	0.62
History diabetes, %	21.4	16.8	15.1	17.6	0.19
History CHF, %	1.4	1.4	1.4	1.1	0.73
Current tobacco use, %	51.2	46.3	50.7	42.5	0.10
Prior MI, %	6.8	11.7	7.8	7.1	0.69
Prior PCI, %	6.0	5.3	3.5	3.6	0.10
Anterior MI, %	42.2	38.4	33.3	38.8	0.24
CrCl, median	95.5	91.1	87.1	77.1	<0.001
Sx onset to lytic (hrs), median	3.2	2.7	2.4	2.0	<0.001
Killip class 2-4, %	6.0	5.7	7.5	8.6	0.17
Fibrin-specific lytic, %	85.8	88.3	85.8	86.5	0.96
Initial heparin type, %					
None, %	7.8	6.0	7.4	7.1	0.89
UFH, %	42.9	47.7	42.9	46.3	
LMWH, %	46.1	40.9	45.7	40.6	
Both, %	3.2	5.3	3.9	6.0	
Angiography performed during index hospitalization, %	96.1	97.2	95.0	94.7	0.23

Table S8: Baseline characteristics by MPO quartile at baseline

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Number	262	261	261	261	
Ranges (ng/ml)	< 29.4	29.4-91.8	91.8-301.4	>301.4	
Gender (male) , %	78.2	78.9	81.2	80.5	0.42
Age (years), median	57	59	59	59	0.24
Weight (kg), median	79.7	80.0	80.0	80.0	0.44
BMI, median	26.8	26.8	27.0	27.4	0.30
History hypertension, %	31.9	39.7	37.3	45.4	0.005
History hyperlipidemia, %	37.1	41.6	44.3	39.8	0.46
History diabetes, %	14.6	20.7	16.9	16.3	0.89
History CHF, %	0.8	1.9	1.1	1.2	0.90
Current tobacco use, %	45.4	48.7	46.4	47.5	0.78
Prior MI, %	8.8	9.2	6.9	6.9	0.28
Prior PCI, %	6.1	5.4	3.4	3.1	0.054
Anterior MI, %	38.5	37.9	38.3	36.0	0.59
CrCl, median	93.3	83.3	87.6	84.7	0.06
Sx onset to lytic (hrs), median	2.4	2.5	2.5	2.5	0.08
Killip class 2-4, %	4.6	9.6	10.0	5.4	0.71
Fibrin-specific lytic, %	86.3	82.4	87.0	90.4	0.07
Initial heparin type, %					
None, %	8.0	10.3	6.5	2.3	0.34
UFH, %	40.1	38.3	34.1	67.0	
LMWH, %	48.5	49.0	53.6	23.4	
Both, %	3.4	2.3	5.7	7.3	
Angiography performed during index hospitalization, %	97.7	95.0	94.3	96.2	0.33

Table S9: Baseline characteristics by hsCRP quartile at baseline

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Number	318	307	313	312	
Ranges (mg/L)	< 1.3	1.3-2.8	2.8-6.1	> 6.1	
Gender (male) , %	80.5	88.3	77.0	72.1	<0.001
Age (years), median	56	59	57	61	0.001
Weight (kg), median	77.0	80.0	80.0	80.0	<0.001
BMI, median	26.1	26.8	27.6	28.0	<0.001
History hypertension, %	33.4	40.2	36.3	47.7	0.002
History hyperlipidemia, %	40.8	39.8	42.4	39.9	0.99
History diabetes, %	13.2	13.8	18.8	23.6	<0.001
History CHF, %	0.3	0.7	1.3	2.9	0.003
Current tobacco use, %	40.7	46.9	54.5	47.3	0.03
Prior MI, %	9.8	6.5	8.7	8.7	0.86
Prior PCI, %	6.6	2.9	4.2	4.5	0.32
Anterior MI, %	39.0	35.8	36.1	39.1	0.97
CrCl, median	85.6	87.1	89.1	84.9	0.57
Sx onset to lytic (hrs), median	2.3	2.4	2.4	3.0	<0.001
Killip class 2-4, %	5.0	4.9	7.3	10.6	0.003
Fibrin-specific lytic, %	87.4	88.3	86.9	84.3	0.21
Initial heparin type, %					
None, %	6.3	5.9	6.7	9.0	0.91
UFH, %	45.9	46.3	44.1	43.3	
LMWH, %	43.7	43.6	43.8	42.6	
Both, %	4.1	4.2	5.4	5.1	
Angiography performed during index hospitalization, %	96.9	97.7	96.5	92.9	0.009

Table S10: Baseline characteristics by PAPP-A quartile at baseline

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Number	219	219	219	219	
Ranges	< 2.4	2.4-4.4	4.4-16.2	> 16.2	
Gender (male) , %	68.5	80.4	84.9	82.6	<0.001
Age (years), median	57	58	57	60	0.25
Weight (kg), median	77.7	80.0	81.0	79.0	0.28
BMI, median	26.7	27.1	27.4	26.5	0.81
History hypertension, %	39.6	36.1	43.5	37.8	0.90
History hyperlipidemia, %	45.8	34.4	40.7	38.1	0.28
History diabetes, %	18.1	15.7	19.6	13.5	0.39
History CHF, %	0.5	0.9	0.9	1.4	0.34
Current tobacco use, %	52.1	51.1	42.0	44.5	0.04
Prior MI, %	6.9	7.3	9.2	7.8	0.59
Prior PCI, %	1.8	4.1	6.4	3.2	0.27
Anterior MI, %	33.3	47.5	42.5	33.8	0.80
CrCl, median	86.3	89.3	88.8	83.4	0.87
Sx onset to lytic (hrs), median	2.5	2.5	2.5	2.3	0.25
Killip class 2-4, %	5.9	7.3	5.0	6.0	0.76
Fibrin-specific lytic, %	85.8	79.9	87.7	95.0	<0.001
Initial Heparin type, %					
None, %	8.2	9.6	7.3	0.5	0.02
UFH, %	46.6	38.8	43.8	49.3	
LMWH, %	42.0	48.9	40.6	45.7	
Both, %	3.2	2.7	8.2	4.6	
Angiography performed during index hospitalization, %	95.9	96.8	96.3	95.0	0.59

Table S11: Baseline characteristics by GDF-15 quartile at baseline among

Variable	**Q1**	**Q2**	**Q3**	**Q4**	**p-value (trend)**
Number	278	278	278	277	
Ranges (pg/ml)	<539.6	539.6 - 784.3	784.3-1243.9	>1243.9	
Gender (male) , %	86.7	79.9	74.5	76.5	<0.001
Age (years), median	55.5	58	60	61	<0.001
Weight (kg), median	81.0	80.0	79.8	80.0	0.06
BMI, median	27.3	27.0	26.7	26.9	0.97
History hypertension, %	33.0	40.6	39.2	45.1	0.008
History hyperlipidemia, %	44.3	45.0	39.4	33.9	0.009
History diabetes, %	11.2	17.2	18.6	24.6	<0.001
History CHF, %	0.4	1.4	1.4	1.8	0.14
Current tobacco use, %	47.1	48.9	48.9	43.7	0.44
Prior MI, %	7.6	9.1	7.2	9.8	0.52
Prior PCI, %	5.0	4.0	4.3	4.0	0.61
Anterior MI, %	37.8	39.2	41.7	33.6	0.44
CrCl, median	97.7	88.5	83.3	75.9	<0.001
Sx onset to lytic (hrs), median	2.3	2.4	2.5	2.7	<0.001
Killip class 2-4, %	7.2	6.9	7.2	6.9	0.92
Fibrin-specific lytic, %	91.4	88.5	86.3	80.5	<0.001
Initial heparin type, %					
None, %	5.4	6.5	4.3	10.8	0.53
UFH, %	54.3	43.2	43.9	42.2	
LMWH, %	37.4	47.1	45.0	41.2	
Both, %	2.9	3.2	6.8	5.8	
Angiography performed during index hospitalization, %	98.2	96.4	96.0	92.1	<0.001

Table S12: Baseline characteristics by troponin T quantile at baseline

Variable	**Undetectable TnT**	**TnT Tertile 1 (if detectable)**	**TnT Tertile 2 (if detectable)**	**TnT Tertile 3 (if detectable)**	**p-value (trend)**
Number	594	219	219	218	
Ranges (ng/ml)	<0.01	0.01-0.06	0.06-0.25	>0.25	
Gender (male) , %	81.0	80.4	78.1	75.7	0.09
Age (years), median	56	59	60	62	<0.001
Weight (kg), median	80.0	80.0	80.0	78.5	0.32
BMI, median	27.1	27.1	27.3	26.8	0.70
History hypertension, %	35.7	35.9	45.2	47.2	<0.001
History hyperlipidemia, %	45.0	36.3	42.7	31.0	0.003
History diabetes, %	15.6	16.9	15.7	24.3	0.02
History CHF, %	0.8	0.9	1.4	2.8	0.05
Current tobacco use, %	50.4	44.3	46.8	42.4	0.05
Prior MI, %	10.0	6.8	7.8	6.5	0.10
Prior PCI, %	5.9	4.6	3.7	1.8	0.01
Anterior MI, %	27.4	41.1	48.4	50.5	<0.001
CrCl, median	88.9	91.5	84.5	79.8	<0.001
Sx onset to lytic (hrs), median	2.0	2.5	3.2	4.0	<0.001
Killip class 2-4, %	5.7	6.0	5.5	12.8	0.005
Fibrin-specific lytic, %	87.5	90.9	86.8	80.3	0.02
Initial Heparin type, %					
None, %	7.1	4.1	9.1	7.3	0.95
UFH, %	42.9	53.0	47.9	39.0	
LMWH, %	44.6	39.3	39.3	48.6	
Both, %	5.4	3.7	3.7	5.0	
Angiography performed during index hospitalization, %	97.6	98.6	96.3	88.5	<0.001

Table S13: The association between each candidate marker individually and the odds of CV death at 30 days after multivariable adjustment.

	Biomarker	OR (95% CI) adjusted for clinical factors	P value
Myocardial stress/ structural changes	NT-proBNP	2.92 (1.45-5.90)	0.003
	MR-pro ANP	2.22 (1.10-4.49)	0.03
	ST-2	2.63 (1.36-5.09)	0.004
	Galectin-3	1.68 (0.83-3.42)	0.15
	MR-pro adrenomedullin	1.12 (0.55-2.28)	0.75
	Copeptin	1.49 (0.74-3.02)	0.26
Myo-necrosis	Troponin T (T1-T2)*	3.85 (1.47-10.1)	0.006
	Troponin T (T3)*	6.72 (2.51-18.0)	<0.001
Inflammation	MPO	7.15 (1.60-32.0)	0.01
	hsCRP	2.38 (1.23-4.61)	0.01
	PAPP-A	2.85 (0.86-9.46)	0.09
	GDF-15	1.01 (0.38-2.68)	0.99

All markers modeled quartile 4:quartile 1-3 except MPO, GDF-15 and PAPP-A (quartile 2-4:quartile 1). Multivariable model included age, sex, prior HF, diabetes mellitus, prior MI, systolic blood pressure, heart rate, Killip class II-IV, type of lytic, anterior STEMI, creatinine clearance <60ml/min, time to lytic, treatment arm. *Troponin T coded as 3-way variable with undetectable troponin T as referent and detectable troponin T coded by tertile (T).

Table S14: The association between each candidate marker individually and the odds of heart failure at 30 days after multivariable adjustment.

	Biomarker	OR (95% CI) adjusted for clinical factors	P value
Myocardial stress/ Structural changes	NT-proBNP	1.75 (0.83-3.71)	0.14
	MR-pro ANP	1.72 (0.81-3.64)	0.16
	ST-2	2.68 (1.35-5.36)	0.005
	Galectin-3	2.22 (1.04-4.71)	0.04
	MR-pro adrenomedullin	0.95 (0.43-2.08)	0.90
	Copeptin	0.89 (0.40-1.98)	0.77
Myo-necrosis	Troponin T (T1-T2)*	1.50 (0.63-3.55)	0.36
	Troponin T (T3)*	1.92 (0.76-4.88)	0.17
Inflammation	MPO	1.08 (0.44-2.65)	0.86
	hsCRP	1.44 (0.70-2.96)	0.32
	PAPP-A	2.57 (0.72-9.21)	0.15
	GDF-15	0.69 (0.30-1.60)	0.39

All markers modeled quartile 4:quartile 1-3 except MPO, GDF-15 and PAPP-A (quartile 2-4:quartile 1). Multivariable model included age, sex, prior HF, diabetes mellitus, prior MI, systolic blood pressure, heart rate, Killip class II-IV, anterior STEMI, type of lytic, creatinine clearance <60ml/min, time to lytic, treatment arm. *Troponin T coded as 3-way variable with undetectable troponin T as referent and detectable troponin T coded by tertile (T).

Table S15: The association between each candidate marker individually and the odds of MI at 30 days after multivariable adjustment.

	Biomarker	OR (95% CI) adjusted for clinical factors	P value
Myocardial stress/ Structural changes	NT-proBNP	0.66 (0.32-1.35)	0.25
	MR-pro ANP	1.09 (0.58-2.03)	0.79
	ST-2	1.70 (1.01-2.86)	0.046
	Galectin-3	1.02 (0.55-1.92)	0.94
	MR-pro adrenomedullin	0.85 (0.43-1.67)	0.64
	Copeptin	1.89 (1.08-3.30)	0.03
Myo-necrosis	Troponin T (T1-T2)*	0.75 (0.43-1.31)	0.31
	Troponin T (T3)*	0.57 (0.22-1.43)	0.23
Inflammation	MPO	1.00 (0.56-1.77)	0.99
	hsCRP	0.67 (0.35-1.29)	0.23
	PAPP-A	1.04 (0.51-2.11)	0.91
	GDF-15	0.32 (0.19-0.54)	<0.001

All markers modeled quartile 4:quartile 1-3 except MPO, GDF-15 and PAPP-A (quartile 2-4:quartile 1). Multivariable model included age, sex, prior HF, diabetes mellitus, prior MI, systolic blood pressure, heart rate, Killip class II-IV, anterior STEMI, type of lytic, creatinine clearance <60ml/min, time to lytic, treatment arm. *Troponin T coded as 3-way variable with undetectable troponin T as referent and detectable troponin T coded by tertile (T).

Table S16: The association between each candidate marker individually and the odds of recurrent ischemia requiring urgent revascularization at 30 days after multivariable adjustment.

	Biomarker	**OR (95% CI) adjusted for clinical factors**	**P value**
Myocardial stress/ Structural changes	NT-proBNP	0.61 (0.28-1.31)	0.20
	MR-pro ANP	1.28 (0.63-2.59)	0.50
	ST-2	1.38 (0.75-2.52)	0.30
	Galectin-3	0.66 (0.29-1.49)	0.31
	MR-pro adrenomedullin	1.22 (0.59-2.49)	0.59
	Copeptin	0.59 (0.26-1.32)	0.20
Myo-necrosis	Troponin T (T1-T2)*	1.49 (0.80-2.76)	0.21
	Troponin T (T3)*	0.69 (0.25-1.88)	0.47
Inflammation	MPO	1.01 (0.50-2.01)	0.99
	hsCRP	1.10 (0.58-2.08)	0.77
	PAPP-A	1.40 (0.59-3.33)	0.44
	GDF-15	0.42 (0.22-0.80)	0.009

All markers modeled quartile 4:quartile 1-3 except MPO, GDF-15 and PAPP-A (quartile 2-4:quartile 1). Multivariable model included age, sex, prior HF, diabetes mellitus, prior MI, systolic blood pressure, heart rate, Killip class II-IV, anterior STEMI, type of lytic, creatinine clearance <60ml/min, time to lytic, treatment arm. *Troponin T coded as 3-way variable with undetectable troponin T as referent and detectable troponin T coded by tertile (T).

Table S17: The association between each candidate marker individually and the odds of TIMI flow grade 0 or 1 at angiography after multivariable adjustment.

	Biomarker	**OR (95% CI) adjusted for clinical factors**	**P value**
Myocardial stress/ Structural changes	NT-proBNP	1.27 (0.82-1.96)	0.29
	MR-pro ANP	1.16 (0.73-1.85)	0.53
	ST-2	1.72 (1.18-2.50)	0.006
	Galectin-3	1.03 (0.65-1.64)	0.89
	MR-pro adrenomedullin	0.97 (0.61-1.56)	0.91
	Copeptin	1.55 (1.01-2.39)	0.045
Myo-necrosis	Troponin T (T1-T2)*	1.08 (0.71-1.61)	0.73
	Troponin T (T3)*	1.52 (0.88-2.56)	0.13
Inflammation	MPO	1.45 (0.90-2.33)	0.12
	hsCRP	1.41 (0.94-2.08)	0.10
	PAPP-A	1.47 (0.81-2.70)	0.21
	GDF-15	0.41 (0.27-0.62)	<0.001

All markers modeled quartile 4:quartile 1-3 except MPO, GDF-15 and PAPP-A (quartile 2-4:quartile 1). Multivariable model included age, sex, prior HF, diabetes mellitus, prior MI, systolic blood pressure, heart rate, Killip class II-IV, anterior STEMI, type of lytic, creatinine clearance <60ml/min, time to lytic, treatment arm. *Troponin T coded as 3-way variable with undetectable troponin T as referent and detectable troponin T coded by tertile (T).

Table S18: The association between ST2, troponin T and MPO and the odds of CV death or HF at 30 days after multivariable adjustment including left ventricular ejection fraction (LVEF) in the 515 subjects in whom LVEF was available.

Biomarker	**OR (95% CI) adjusted for clinical factors**
ST2	4.60 (1.69-12.5)
Troponin T (Tertile 1-2)	5.28 (1.21-23.0)
Troponin T (Tertile 3)	8.14 (1.74-38..0)
MPO	2.01 (0.53-7.67)

*Troponin T coded as 3-way variable with undetectable troponin T as referent and detectable troponin T coded by tertile (T).