Thrombus Precursor Protein and Clinical Outcomes in Patients With Acute Coronary Syndromes

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Objectives
We sought to test the prognostic performance of thrombus precursor protein (TpP) in patients presenting with an acute coronary syndrome (ACS).

Background
Because thrombus formation is a critical step in the development of ACS, a measurement of activated coagulation could yield important information. Thrombus precursor protein is a biomarker that is used to measure soluble fibrin polymers, which are the penultimate products in fibrin formation.

Methods
We measured the levels of TpP in 284 healthy volunteers and in 2,349 patients with ACS.

Results
Median TpP concentrations were 3.6 μg/ml (interquartile range 2.6 to 5.5) in the volunteers and 8.9 μg/ml (interquartile range 4.9 to 15.9) in the ACS patients (p < 0.001). Patients with ACS who had elevated TpP were older, more likely to be women, and more likely to have diabetes and pre-existing CAD (p < 0.02 for each). Thrombus precursor protein levels greater than the median were associated with a significantly increased risk for the composite of death, myocardial infarction (MI), or recurrent ischemia leading to rehospitalization or urgent revascularization through 10 months (hazard ratio [HR] 1.45, p < 0.001), as well as death or MI (HR 1.42, p = 0.02). We found that TpP correlated only weakly with cardiac troponin I, B-type natriuretic peptide, and high-sensitivity C-reactive protein (|r| < 0.15 for each). After adjusting for clinical characteristics, cardiac troponin I, high-sensitivity C-reactive protein, and B-type natriuretic peptide, we found that patients with TpP levels greater than the median remained at significantly increased risk for the composite outcome (adjusted HR 1.51, p = 0.001) and death or MI (adjusted HR 1.58, p = 0.02).

Conclusions
In patients with ACS, increased levels of TpP are associated with an increased risk of death or ischemic complications. The incorporation of a marker of activated coagulation, such as TpP, with established cardiovascular risk factors may offer valuable complementary insight into risk assessment in ACS. (J Am Coll Cardiol 2008; 51:2422–9) © 2008 by the American College of Cardiology Foundation

Acute coronary syndromes (ACS) typically are triggered by rupture or erosion of atherosclerotic plaques with subsequent formation of an obstructive thrombus. The intracoronary obstruction causes transient or persistent loss of blood flow to the myocardium, yielding ischemia and ultimately myocardial death. Biomarkers that help quantify the resultant myonecrosis, hemodynamic stress, and inflammation have been shown to provide important insights into different aspects of the pathobiology of ACS and are used currently to assist with the management of cardiovascular patients (1,2). In contrast, markers of activated coagulation

See page 2430
have not been widely adopted in this setting (3). However, because thrombus formation is usually a critical step in the development of an ACS, an effective measurement of the end products of coagulation could yield important prognostic information.

To that end, a marker of interest that is closely related to the formation of fibrin is thrombus precursor protein (TpP), a specific form of soluble fibrin (3). After thrombin cleaves fibrinopeptide A and B from fibrinogen, desAABB fibrin monomers are formed. These monomers polymerize into soluble TpP fibrin polymers, which serve as the immediate precursors to insoluble fibrin (Fig. 1) (4,5). We examined TpP levels in healthy volunteers and then investigated the prognostic performance of TpP in a large cohort of patients presenting with ACS. We also assessed the incremental information provided by TpP after adjustment for traditional clinical risk factors, presenting features, and established biomarkers.

**Methods**

**Study population.** The present analysis was conducted on 2,349 patients with ST-segment elevation myocardial infarction (STEMI), non–ST-segment elevation myocardial infarction (NSTEMI), and patients with unstable angina (UA) who were enrolled in the OPUS–TIMI 16 (Oral Glycoprotein IIb/IIIa Inhibition With Orbofiban in Patients With Unstable Coronary Syndromes–Thrombolysis In Myocardial Infarction 16) trial. The design and primary results of the study have been described (6). The TpP study included subjects who were treated with 50 mg of orbofiban twice daily and had blood samples available for analysis. Patients were followed up through 10 months and evaluated for cardiovascular events. Clinical outcomes for this analysis included death, myocardial infarction (MI), recurrent ischemia leading to rehospitalization, and recurrent ischemia leading to urgent revascularization, as well as the composite of these 4 end points. A blinded Clinical Events Committee adjudicated all suspected cases of MI and recurrent ischemia. The protocol, including the biomarker substudy, was reviewed and approved by the institutional review board/ethics committees at each participating institution, and all patients provided written informed consent.

**Biomarker testing.** To approximate the normal range for TpP, citrate plasma samples were collected from 284 healthy volunteers (mean age 34 years, 50% male). Citrate plasma samples were also obtained in OPUS–TIMI 16 at
the time of enrollment, which was a median of 40 h (5th percentile: 8 h, 95th percentile: 71 h) from presentation. Samples were frozen and sent to the TIMI Biomarker Laboratory (Boston, Massachusetts), where they were stored at −70°C. Samples were subsequently analyzed at Biosite, Inc. (San Diego, California), where sequential sandwich immunoassays were used to measure TpP (n = 2,349), cardiac troponin I (TnI) (n = 2,320), B-type natriuretic peptide (BNP) (n = 2,324), and high-sensitivity C-reactive protein (hsCRP) (n = 1,929) with an automated system (Tecan Genesis robotic sample processor 200/8, Durham, North Carolina).

We measured TpP using an antifibrin MH1 monoclonal antibody that binds to specific neoepitopes of soluble crosslinked and noncross-linked desAABB fibrin polymers (7). The assay system is designed to exclude detection of fibrinogen, fibrinogen–degradation products, desAA fibrin monomers, desAA fibrin polymers, desAABB fibrin monomers, crosslinked fibrinogen, desAA fibrin monomer–fibrinogen complexes, fibrin degradation products, and crosslinked fibrin degradation products. The TpP assay has a minimal detectable concentration of 0.27 μg/ml and a total imprecision of 13% and 8% at concentrations of 1 μg/ml and 20 μg/ml, respectively, in healthy volunteers. The stability of TpP during long-term storage was assessed by comparing TpP concentrations across different durations of storage in the study population; there was no difference over time (p = 0.79).

The minimal detectable concentration of the TnI assay was 0.05 ng/ml. Decision limits of 1.5 and 0.1 ng/ml were used for the TnI analyses (8). Analyses that included BNP were performed with the use of a threshold of 80 pg/ml (8,9). For hsCRP, a threshold of 15 mg/l was used in the setting of ACS (10).

Statistical analysis. All patients with available data for TpP were included in this analysis. The plasma levels of TpP were described by the median and interquartile range in the healthy volunteers and study population. In OPUS–TIMI 16, baseline characteristics of patients in the 4 TpP quartiles were compared using the Kruskal–Wallis test for trend for continuous variables and the chi-square test for trend for categorical variables. Correlations between TpP levels and other biomarkers, as well as duration of treatment with heparin, were examined using Spearman correlation coefficients. Because of nonlinearity, we analyzed TpP by using quartiles and as a dichotomous variable divided at the median. Cumulative incidence curves were plotted with the Nelson–Aalen method (11,12). When performing stratified analyses, ST-segment elevation ACS and non–ST-segment elevation ACS events (UA and NSTEMI) were assessed based on the approach used in the American College of Cardiology/American Heart Association management guidelines (13). Multivariable analyses of the association between TpP and outcomes were performed with a Cox proportional hazards survival model to adjust for traditional cardiovascular risk factors (age, gender, smoking status, diabetes, body mass index, dyslipidemia, pre-existing coronary disease, and prior aspirin use), clinical features on presentation (index diagnosis, creatinine clearance, Killip class, use of heparin, and use of beta-blockade), and other biomarkers (TnI, CRP, and BNP). Statistical heterogeneity was assessed with interaction terms within a Cox proportional hazards survival model. All analyses were performed with Stata/SE 9.2 (STATA Corporation, College Station, Texas). Two-sided p values <0.05 were considered to indicate statistical significance.

Results

TpP levels. In a population of 284 healthy volunteers, the median TpP value was 3.7 μg/ml, with the 5th, 25th, 75th, and 95th percentiles at 1.6, 2.6, 5.6, and 8.9 μg/ml, respectively. In the OPUS–TIMI 16 study population, the median concentration of TpP was 8.9 μg/ml, with the 5th, 25th, 75th, and 95th percentiles at 1.4, 4.9, 15.9, and 68.1 μg/ml, respectively (p < 0.001 compared with the healthy population).

Baseline clinical characteristics and presentation. The clinical characteristics of patients in the study population, stratified by TpP quartiles, are presented in Table 1. Patients with TpP levels in the upper quartiles were modestly older and more likely to be female and have a greater body mass index, history of diabetes, pre-existing coronary artery disease, and prior use of aspirin (p < 0.02 for each). There were trends toward greater levels of TpP among patients with hypertension and with dyslipidemia.

Patients with greater TpP levels had modestly lower calculated creatinine clearances and more commonly displayed evidence of heart failure on examination (Killip class II to IV) (Table 2). Patients with TpP levels in the upper quartiles were more likely to have UA than STEMI; the median TpP levels were 9.7 μg/ml in patients with UA, 8.5 μg/ml in NSTEMI, and 7.9 μg/ml in STEMI (p < 0.001). There was no significant difference in time from symptom onset to randomization across the TpP quartiles. Similarly, median TpP levels did not differ based on time from symptom onset to randomization (0 to 24 h: 9 μg/ml, 24 to 48 h: 8.5 μg/ml, >48 h: 9.2 μg/ml, p = 0.58). Moreover, there was no significant correlation between TpP concentrations and time from symptom onset to randomization (r = 0.02, p = 0.28).

Patients in all 4 TpP quartiles underwent percutaneous coronary revascularization at similar rates. Whereas the administration of aspirin, angiotensin-converting enzyme inhibitors, and calcium channel blockers did not vary by TpP quartile, patients in the upper quartiles of TpP were less likely than patients in the lower quartiles to be treated with beta-blockers. The use of heparin in patients varied across TpP quartiles, with patients in the top quartiles less likely to have received unfractionated heparin. Similarly, the median TpP levels among patients treated with unfractionated heparin, low molecular weight heparin, or no heparin...
Within 6 h of the TpP sample acquisition, the correlation with partial thromboplastin time (PTT) values obtained was weak (r = 0.10, p = 0.001). Notably, among patients on unfractionated heparin at the time of the TpP collection (55.3% vs. 67.0%, p = 0.008). Of note, patients with UA were less likely than patients who experienced an MI to be treated with unfractionated heparin before the TpP levels were drawn (r = 0.07, p = 0.01).

### Clinical outcomes
There was a significant association between greater levels of TpP and risk for the composite outcome of death, MI, recurrent ischemia leading to rehospitalization, or recurrent ischemia leading to urgent revascularization (p = 0.0003) (Table 3). We observed directional consistency for all of the individual components of the composite outcome (Table 3). Patients in the top 2 quartiles were at comparably increased risk compared with the
bottom 2 quartiles and, therefore, the study population was further assessed using median values. Patients with TpP levels greater than the median (8.9 μg/ml) were at a 1.45-fold increased risk of the composite end point (95% confidence interval [CI] 1.20 to 1.75, \( p < 0.001 \)) (Fig. 2), and were also at increased risk of death or MI (hazard ratio [HR] 1.42, 95% CI 1.05 to 1.93, \( p = 0.02 \)) (Fig. 3).

Increased TpP levels remained associated with an increased risk of death or ischemic complications regardless of index ACS diagnosis (STEMI: HR 1.66, 95% CI 1.18 to 2.34 and NSTEMI/UA: HR 1.35, 95% CI 1.07 to 1.70) (Fig. 4). Levels of TpP greater than the median were also associated with an increased risk of the composite outcome in patients with and without pre-existing coronary artery disease. Furthermore, there was no heterogeneity in the prognostic utility of TpP in subjects stratified by time from ischemic symptoms to randomization or type of antithrombotic therapy used.

**Multivariable analysis.** After adjusting for baseline characteristics and clinical features on presentation (including age, gender, smoking status, hypertension, dyslipidemia, body mass index, diabetes, pre-existing coronary disease, prior aspirin use, index diagnosis, creatinine clearance, Killip class, use of heparin, and use of beta-blockers), we found that patients with TpP greater than the median continued to be at increased risk for death and ischemic complications (HR 1.43, 95% CI 1.16 to 1.75, \( p = 0.001 \)) (Table 4). The levels of TpP correlated only weakly with TnI, hsCRP, and BNP (|r| < 0.15 for each). There was no statistically significant heterogeneity in the prognostic ability of TpP for the composite outcome in patients stratified by the presence or absence of elevated levels of TnI, hsCRP, or BNP (\( p = \text{NS} \) for all interaction terms). After adding TnI, hsCRP, and BNP to the multivariable model, the relationship between TpP and long-term cardiovascular outcomes, as assessed by the composite outcome, remained significant (HR 1.51, 95% CI 1.19 to 1.91, \( p = 0.001 \)). Adjusting for baseline characteristics, features on presentation, and biomarker status, patients with TpP levels greater than the median were also at greater risk of death or MI (HR 1.58, 95% CI 1.09 to 2.30, \( p = 0.02 \)).

**Discussion**

A number of biochemical markers have been shown to provide important insights into different aspects of the pathobiology of ACS, and they have been used to assist with

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**Table 3** Association Between TpP and Clinical Events Through 10 Months

<table>
<thead>
<tr>
<th>TpP Quartiles (μg/ml)</th>
<th>Clinical Outcomes</th>
<th>Composite outcome (n = 434)</th>
<th>Death (n = 77)</th>
<th>Myocardial infarction (n = 113)</th>
<th>Ischemia requiring urgent revascularization (n = 137)</th>
<th>Ischemia requiring rehospitalization (n = 160)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4.9 (n = 596)</td>
<td>17.5</td>
<td>3.5</td>
<td>4.9</td>
<td>5.2</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>4.9–8.9 (n = 590)</td>
<td>20.3</td>
<td>2.9</td>
<td>5.5</td>
<td>7.3</td>
<td>19.4</td>
<td></td>
</tr>
<tr>
<td>9–15.9 (n = 577)</td>
<td>30.6</td>
<td>4.5</td>
<td>7.2</td>
<td>11.9</td>
<td>21.2</td>
<td></td>
</tr>
<tr>
<td>&gt;15.9 (n = 586)</td>
<td>31.4</td>
<td>6.0</td>
<td>7.0</td>
<td>13.3</td>
<td>23.8</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as Nelson-Aalen event rates. Composite outcome indicates death, myocardial infarction, ischemia requiring revascularization, or ischemia requiring rehospitalization. TpP = thrombus precursor protein.

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![Figure 2: Cumulative Incidence of the Composite Outcome by TpP Level](image)

Cumulative incidence of the composite outcome (death, myocardial infarction, ischemia requiring revascularization, or ischemia requiring rehospitalization) through 10 months stratified by TpP levels ≤ median (8.9 μg/ml) or > median. CI = confidence interval; HR = hazard ratio; TpP = thrombus precursor protein.

![Figure 3: Rates of Death or MI by TpP Level](image)

Nelson-Aalen event rates through 10 months for death or myocardial infarction for patients with TpP levels ≤ median (8.9 μg/ml) or > median. MI = myocardial infarction; other abbreviations as in Figure 2.
After plaque rupture in the coronary artery, tissue factor is exposed and activates the coagulation pathway. Prothrombin is converted into thrombin; thrombin then cleaves fibrinopeptide A and fibrinopeptide B from fibrinogen, yielding desAABB fibrin monomer units with newly exposed amino-terminals. These desAABB monomers start to polymerize via D and E domains creating still-soluble desAABB fibrin polymers. The specific TpP assay used in this analysis detects these species of soluble fibrin polymers, which serve as the immediate precursors to insoluble fibrin (Fig. 1). When a steady-state concentration is surpassed, the fibrin polymers will further polymerize to form an insoluble gel cross-linked by factor XIII (25).

Soluble fibrin polymers, including TpP, have been evaluated as diagnostic markers, performing well in some studies (24,26,27). The prognostic performance of several species of soluble fibrin in patients with ACS has been mixed when tested using several different immunologic and nonimmunologic assays (28–31). Early assays provided qualitative or semiquantitative results with low sensitivity and specificity (25). Immunoassays offer more specific, quantitative assessments of soluble fibrin. We used a monoclonal antibody assay specifically targeted at neoepitopes exposed during the creation of desAABB fibrin polymers, and found TpP to be a good predictor of future cardiovascular events across the spectrum of ACS.

In our study, the association between greater levels of TpP and death or ischemic complications was consistent regardless of the time from symptom onset to biomarker sampling or index diagnosis. In terms of anticoagulant received during the hospitalization, we found that heparin use was associated with lower TpP levels, data that are in accord with a result from a previous small study, wherein 9 subjects were found to have significantly lower TpP concentrations after a bolus of heparin (32). Additionally,
heparin use was less common in patients with UA, who were found to have greater TpP concentrations. Yet, we observed that there was no association between TpP and achieved PTT in patients on unfractionated heparin, and the correlation between TpP levels and the duration of heparin therapy was weak. Importantly, there was no evidence of heterogeneity in the relationship of TpP and the use of unfractionated, low molecular weight, or no heparin when evaluating outcomes in this study. Moreover, in the multivariable model that adjusted for heparin use, TpP continued to be associated with the composite end point.

The results of multivariable modeling also suggested that TpP offered prognostic information independent of other clinical predictors, including traditional cardiovascular risk factors and index diagnosis, in patients presenting with ACS. After adjusting for baseline characteristics and clinical features on presentation, we found that patients with TpP levels greater than the median had a 43% increased risk for death or ischemic complications. Reflecting its unique pathophysiological axis, TpP levels were only weakly correlated with established biomarkers, including TnI, hsCRP, and BNP. Moreover, after further adjusting for these biomarkers in a multivariable model, the relationship between TpP and long-term cardiovascular outcomes remained significant.

Elevated levels of TpP in the post-ACS setting, especially in subjects with later sample acquisition, may reflect enhanced systemic activation of the coagulation system, beyond what occurs with regard to local, intracoronary fibrin deposition. The concept of the “vulnerable cardiovascular patient” with thrombogenic blood was highlighted by an expert panel report, suggesting that transient alterations in the coagulation and fibrinolytic system are likely to be important factors contributing to acute cardiac events (33). Measuring markers such as TpP or D-dimer may be one way of quantifying an individual's degree of thrombogenicity during and after an ACS. Such biomarkers hold the promise of identifying patients who would benefit from a longer duration or greater intensity of antithrombotic therapy. To that end, potent intravenous and oral Xa inhibitors are under evaluation for both the acute and long-term treatment of ACS (34–36). Markers of activated coagulation, such as TpP, may someday prove useful in identifying which patients would be most likely to derive the greatest benefit from these potent antithrombotic strategies.

Study limitations. Several limitations to this analysis should be recognized. First, although our analysis suggests that the TpP levels assessed during the hospitalization for ACS carry independent prognostic information, the study was not designed to assess the value of measuring TpP immediately at presentation or to compare with other biomarkers obtained at this time point. However, within the subset of patients who had TpP samples drawn within 24 h of symptom onset, the relationship between increased TpP levels and risk of the composite end point was still directionally consistent.

Second, since we measured troponin in these samples, more sensitive troponin assays have been developed; determining the utility of TpP in the context of these assays will be important. However, troponin and TpP are assessing different pathobiologic processes, and we found the correlation between the markers to be low in this study. Third, this analysis was conducted within an orbofiban treatment arm of the OPUS–TIMI 16 trial, and it is possible that the study medication influenced levels of TpP. However, all subjects in this analysis were in the same treatment arm. Additionally, because the clinical outcomes in this arm did not differ significantly from those in the placebo arm, the results should be applicable to the broader population of post-ACS patients. Nonetheless, as the landscape of antiplatelet and anticoagulant treatments continues to change, TpP will need to be evaluated in the setting of different background therapies, administered during an ACS event, and in long-term follow-up.

Fourth, we performed extensive multivariable adjustments, including baseline characteristics, features on presentation, treatment during the acute hospitalization, and other biomarkers; however, we did not have and therefore could not adjust for discharge medications. Fifth, TpP reference ranges in this analysis were generated from healthy volunteers. The determination of TpP ranges in other populations, including hospitalized patients without ACS, will be useful. Sixth, in our analysis, we predominantly use a cutoff point based on the median value. Future work will be needed to determine the optimal cutoff point based on timing of sample acquisition. Finally, the value of assessing serial samples and the comparison of TpP with other markers of coagulation was not evaluated in this study. Future studies are needed to address these areas.

Conclusions

Thrombus precursor protein is used to measure the penultimate product in the formation of fibrin and, in this study, greater levels of this marker were associated with an increased risk of death or ischemic complications. After adjustment for baseline characteristics, features on presentation, and other biomarkers, a TpP level greater than the median remained a significant predictor of worse cardiovascular outcomes. These data suggest that a marker of active coagulation, such as TpP, can add independent and incremental prognostic information in patients presenting across the spectrum of ACS and that TpP, in conjunction with other markers of hemostasis, warrants additional evaluation in future studies.

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