Original

**Thrombomodulin can Predict the Incidence of Second Events in Patients with Acute Myocardial Infarction**

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Abstract: Biomarkers of atherothrombosis can predict the risk of cardiovascular events. However, it is difficult to predict second adverse events using these biomarkers at the point in time when the first cardiovascular event occurs. Therefore, we evaluated atherothrombosis-related biomarkers to determine their associations with prognosis after percutaneous coronary intervention (PCI) in acute myocardial infarction (AMI) patients. A total of 309 AMI patients were enrolled in this study. The patients had undergone successful coronary interventions and the levels of various atherothrombosis-related biomarkers were assessed within the first postoperative hour. Biomarkers other than those assessed by routine biochemical tests were analyzed, including defined endothelial cell damage markers such as thrombomodulin (TM), inflammatory markers such as C-reactive protein (CRP), and coagulation and fibrinolysis system markers such as D-dimer, prothrombin fragment F1 + 2 (F1 + 2) and plasminogen activator inhibitor-1 (PAI-1). Major adverse cardiac events (MACEs) occurred in 98 patients during the follow-up period (872.6 ± 579.8 days). Multivariate analysis revealed that clinical parameters such as decreased levels of left ventricular ejection fraction and elevated levels of brain natriuretic peptide, hemoglobin A1c and TM were significantly associated with MACEs. The association between TM and MACEs was especially high (OR : 3.65, 95% CI ; 1.75-7.68). Neither dyslipidemia, hypertension, smoking, advanced age, a history of cardiac events nor the type of AMI were associated with MACEs. TM is independently associated with MACEs and may be predictive of second events following PCI in patients with AMI.

Key words: thrombomodulin, endothelial dysfunction, acute myocardial infarction, second event

Introduction

Coronary artery disease (CAD) is the leading cause of morbidity and mortality. Traditional risk factors for CAD such as hypertension, diabetes, dyslipidemia and smoking elicit endothelial...
dysfunction and ultimately the development of atherosclerosis\textsuperscript{1-3}. Although endothelial dysfunction is seen in the early phases of coronary artery atherosclerosis\textsuperscript{4}, atherosclerosis is caused by multiple interacting factors such as ongoing inflammatory responses and deficient fibrinolysis. Endothelial dysfunction, inflammatory responses and the coagulation and fibrinolysis systems are also associated with plaque formation, which leads to atherothrombosis\textsuperscript{5-7}. Hence, severe endothelial dysfunction can predict the risk of cardiovascular events and may play a role in the progression of CAD\textsuperscript{5,8-10}. Elevated inflammatory markers can also indicate a risk of atherosclerotic complications\textsuperscript{6}. The activation of the blood coagulation and fibrinolysis systems during the prothrombotic phase also predisposes patients to CAD\textsuperscript{7}. However, all of these biomarkers have been associated with first cardiac events only.

Percutaneous coronary intervention (PCI) with balloon angioplasty and coronary stenting can help prevent further cardiac events in patients with acute myocardial infarction (AMI). PCI modulates plaque formation, thereby inhibiting sudden coronary thrombosis caused by plaque rupture. However, coronary intervention with a catheter may be associated with arterial injury and the accompanying endothelial dysfunction. Less is known about endothelial dysfunction after PCI in patients with AMI, who are prone to second events. We conjecture that the atherothrombosis-related biomarkers of AMI are influenced not only by the culprit coronary lesion, but also by total systemic atherosclerosis, which includes the peripheral arteries.

This study evaluated the clinical significance of atherosclerosis-related biomarkers after PCI in AMI patients. We clarified their associations with prognosis and determined whether they could be used to predict second events.

**Methods**

**Patients**

The subjects were 367 consecutive patients admitted to our hospital with a diagnosis of AMI from November 2011 to November 2014. Patients consecutively admitted with chest pain or discomfort associated with ST-segment and/or T-wave changes on a standard electrocardiogram (ECG) within 24 hours of symptom onset were prospectively recruited. AMI was defined as acute myocardial infarction and included both ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI). Diagnosis of myocardial infarction was based on the following criteria\textsuperscript{11}: typical chest pain persisting for ≥ 30 min; ST-segment elevation ≥ 0.2 mV in two or more contiguous leads on a standard 12-lead ECG; and creatine kinase-MB ≥ twofold the upper limit of the normal range or troponin I > 0.1 ng/ml. STEMI patients had persistent ST-segment elevation and NSTEMI patients presented with ST-segment depression or T-wave changes.

Patients underwent emergency coronary angiography and successful reperfusion therapy using primary PCI. Successful PCI was defined as achieving < 25% stenosis of the target vessel. Patients were excluded from this study if significant coronary stenosis (≥ 75%) was not detected by the coronary angiogram prior to PCI. If other significant coronary stenotic lesions were detected and deemed likely to induce AMI in the near future, other PCIs were performed during
Elevated TM and MASEs after PCI in AMI Patients

the same procedure. Exclusion criteria included out-of-hospital cardiac arrest and renal dysfunction on dialysis\textsuperscript{12}. This study was approved by the Ethics Committee of Showa University.

**Blood sampling**

Atherothrombosis-related biomarkers other than those assessed by routine biochemical tests (creatinine clearance, brain natriuretic peptide; BNP, hemoglobin A1c; HbA1c, high-density lipoprotein, and low-density lipoprotein) included endothelial cell-damage markers such as thrombomodulin (TM), inflammatory markers such as C-reactive protein (CRP), and markers of the coagulation and fibrinolysis systems such as D-dimer, prothrombin fragment F1 + 2 (F1 + 2) and plasminogen activator inhibitor-1 (PAI-1). PCI was performed with intravenous administration of heparin (3,000–10,000 units), aiming at an activated clotting time of ≥ 250 seconds. Serum samples were collected from the arterial sheath within one hour after reperfusion in tubes containing 3.2% sodium citrate. Samples were further subdivided into 2-ml volumes and dispensed into other collection tubes to measure D-dimer, TM, F1 + 2 and PAI-1. Plasma concentrations of TM and F1 + 2 were determined using an enzyme immunoassay sandwich method (BML, Inc., Tokyo, Japan). Plasma concentrations of D-dimer and PAI-1 were measured by the latex agglutination method (BML, Inc.).

**Study endpoints**

The blood sample data were assessed to analyze relationships with prognosis. The study endpoints were major adverse cardiac events (MACEs), which included death by any cause, recurrent myocardial infarction, unplanned repeat revascularization, surgical revascularization, fatal arrhythmia, admission for heart failure and stroke. Unplanned repeat revascularization was defined as a clinically driven repeat PCI or coronary artery bypass graft of the culprit or non-culprit vessel and included in-hospital events. Readmission for heart failure was defined as a worsening of heart failure symptoms requiring admission. Follow-up data on MACEs were collected from hospital data and other medical records.

**Statistical analysis**

Continuous variables were presented as the mean ± standard deviation (SD) and compared using the student’s \( t \) test or Wilcoxon rank sum test based on their distributions. Categorical variables were presented as numbers and percentages and compared using the chi-square test. Continuous variables were converted to categorical variables by calculating optimal cut-off levels from receiver operating characteristic (ROC) curves. Odds ratios (OR) and 95% confidence intervals (CI) were computed by logistic regression model analysis to clarify the impact of several potentially independent prognostic factors. The proportion of MACE-free patients was plotted using the Kaplan-Meier method, and significance determined using the log-rank test. Statistical analyses were performed using JMP software version 12.0 (SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as \( P < 0.05 \).
Results

Overall patient characteristics
Of the 367 patients who underwent PCI for AMI, 58 were excluded from this study: 36 due to cardiopulmonary arrest and 22 due to renal dysfunction on dialysis. The baseline characteristics of the remaining 309 patients are summarized in Table 1. All AMI patients underwent coronary angiography following angioplasty and/or stenting for culprit coronary lesions. The mean follow-up was \(872.6 \pm 579.8\) days (range: 2–1,930 days).

The AMI event was the first cardiac event in 252 (81.6\%) patients. The remaining 57 (18.4\%) patients had a history of coronary artery bypass graft or had undergone a previous PCI for myocardial infarction or angina pectoris. Table 1 presents a breakdown of the traditional coronary risk factors in all of the AMI patients: 235 (76.1\%) had hypertension, 135 (43.7\%) had diabetes, 202 (65.4\%) had dyslipidemia and 184 (59.5\%) were smokers. Creatinine clearance, BNP, CRP and HbA1c biomarkers were all slightly elevated. Among the coagulation markers, levels of the average D-dimer and F1\beta were slightly increased while the levels of PAI-1 and TM were within the normal range.

STEMI and NSTEMI patient characteristics
We divided the patients into STEMI and NSTEMI groups to evaluate pathological differences (Table 1). The patient characteristics clearly differed between the two groups. A higher proportion of NSTEMI patients (30.5\%) had a past history of old myocardial infarction than STEMI patients (11.5\%; \(P < 0.0001\)). The current cardiac event was the first experienced by most of the STEMI patients; therefore, a higher proportion of NSTEMI patients (37.1\%) had undergone previous PCI (47.4\%) or restenosis (22.1\%) than STEMI patients (13.2\%; \(P = 0.0002\)). Although there was no significant difference in the left ventricular ejection fraction (LVEF) between STEMI and NSTEMI patients, the BNP levels were lower in the STEMI patients (\(P < 0.0001\)). Among the inflammatory biomarkers, CRP levels were higher in the NSTEMI patients (\(P = 0.0093\)). Even though there were no differences in the levels of the coagulation markers D-dimer, F1+2 and PAI-1 between the two groups, the levels of TM were also significantly higher in the NSTEMI group (\(P = 0.0128\)).

Associations between clinical data and MACEs
A total of 98 patients experienced MACEs: there were 24 deaths due to cardiovascular events (\(n = 13\)), malignant tumors (\(n = 4\)), infection (\(n = 6\)) and one from unknown reasons (141.7 \pm 214.4 days after AMI); four myocardial infarctions (158.3 \pm 144.1 days after AMI); 29 unplanned repeat revascularizations for stable angina (356.0 \pm 328.1 days after AMI); one surgical revascularization (94.0 day after AMI); two fatal arrhythmias (269.5 \pm 181.7 days after AMI); 24 cases of heart failure (201.3 \pm 197.2 days after AMI); and 14 strokes (177.4 \pm 327.9 days after AMI). The MACEs occurred within one year of the AMI in most of the patients (227.6 \pm 272.6 days after AMI).
The characteristics of patients who experienced MACEs and those who did not are shown in Table 2. Patients who experienced MACEs were generally older and more likely to have suffered from diabetes, atrial fibrillation or had a history of OMI, PCI, heart failure and stroke.
There were no differences in body mass index or the proportions of males versus females, smoker status, hypertension and dyslipidemia between the two groups. Those in the MACEs...
group had a lower prevalence of LVEF. Among the serum biomarkers, BNP was higher and creatinine clearance was lower in the MACEs group ($P < 0.001$). Low-density lipoprotein and HbA1c were also associated with MACEs ($P = 0.0069$ and $P = 0.0109$, respectively). Although there were no differences in the levels of D-dimer, F1 + 2 and PAI-1 between the STEMI and NSTEMI groups, the levels of these biomarkers were significantly higher in the MACEs group (D-dimer: $P < 0.0001$, F1 + 2: $P = 0.0003$, PAI-1: $P = 0.0017$). A high TM value was also strongly associated with MACEs ($P < 0.0001$).

**Multivariate analysis**

To identify an independent predictor for MACEs, we performed a multivariate analysis using logistic regression on each value that was significantly associated with MACEs in the univariate analysis described above. Optimal cutoff values were determined from the ROC curve. The results of the multivariate analysis are presented in Table 3. We identified diabetes, low LVEF, increased HbA1c and TM as predictors of MACEs. The optimal cut-off values obtained from the ROC curve of LVEF, BNP, Hba1c, and TM in AMI patients were 48%, 116.3 pg/ml, 6.1%.

<table>
<thead>
<tr>
<th>Table 3. Univariate and multivariate logistic regression analysis for biomarkers of MACEs</th>
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<td><strong>Univariate logistic regression</strong></td>
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<td>Age $\geq 68$ y.o.</td>
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<td>Diabetes</td>
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<td>OMI</td>
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<td>Previous PCI</td>
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<td>Atrial fibrillation</td>
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<tr>
<td>LVEF $\leq 48$%</td>
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<tr>
<td>In-stent restenosis</td>
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<tr>
<td>BNP $\geq 116.3$ pg/ml</td>
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<td>CRP $\geq 0.51$ mg/dl</td>
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<td>Ccr $\leq 66.5$ ml/min</td>
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<td>LDL $\leq 121$ mg/dl</td>
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<td>HbA1c $\geq 6.1$%</td>
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<td>D-dimer $\geq 0.99$ µg/ml</td>
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<td>F1 + 2 $\geq 322$ pmol/l</td>
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<td>TM $\geq 3.5$ FU/ml</td>
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<td>PAI-1 $\geq 32$ ng/ml</td>
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OMI, old myocardial infarction; PCI, percutaneous coronary intervention; LVEF, left ventricular ejection fraction; BNP, brain natriuretic peptide; CRP, C-reactive protein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; F1 + 2, prothrombin fragment F1 + 2; TM, thrombomodulin; PAI-1, plasminogen activator inhibitor-1.
and 3.7 FU/ml, respectively. The following biomarker levels were significantly associated with MACEs: LVEF ≤ 48% (OR: 2.66, 95% CI: 1.39–5.18); BNP ≥ 116.3 pg/ml (OR: 3.06, 95% CI: 1.60–5.99); HbA1c ≥ 6.1% (OR: 4.00, 95% CI: 1.65–10.09); and TM ≥ 3.5 FU/ml (OR: 3.65, 95% CI: 1.75–7.68), and medical history of diabetes was significantly associated with MACEs (OR: 2.81, 95% CI: 1.15–7.19).

Next, we clarified the relationship between these biomarkers and the event-free period of patients based on cutoff values as shown in Figure 1. Event-free survival was significantly worse for patients when TM was >3.5 FU/ml ($P<0.0001$; Figure 1A). Patients with lower TM levels seemed to suffer from fewer subsequent events. Increased TM levels were associated with shortened event-free intervals. Similar results were identified with BNP ≥ 116.3 pg/ml, HbA1c ≥ 6.1% and LVEF ≥ 45% (Figures 1B–D).

![Fig. 1. Kaplan-Meier survival plots demonstrating how levels of (A) thrombomodulin (TM), (B) left ventricular ejection fraction (LVEF), (C) hemoglobin A1c (HbA1c) and (D) brain natriuretic peptide (BNP) changed in patients during follow-up. Each biomarker was divided into two groups based on an optimal cutoff value. The Kaplan-Meier analysis shows the cumulative proportion of patients without cardiovascular events for each marker. The incidence of cardiovascular events was significantly higher in patients with decreased levels of LVEF and elevated levels of TM, HbA1c, and BNP.](image-url)
In the present study, we analyzed biomarkers of CAD to assess the risk of a second event following PCI in AMI patients. Our study demonstrates that elevated BNP, HbA1c and TM levels, and decreased LVEF levels are associated with MACEs after reperfusion by PCI in AMI patients. This is the first study to identify an association between elevated TM levels and the incidence of MACEs in AMI patients, suggesting that TM can predict the risk of MACEs in patients with impaired fibrinolytic activity and platelet activation by PCI.

Patients with CAD have an increased risk of suffering from fatal coronary events, including sudden death. As this is a multifactorial disease, several confounding factors can independently affect the incidence of atherothrombosis. For example, low cardiac function and poorly controlled diabetes can cause adverse events in patients with CAD\(^{13-15}\), and are also confounding risk factors of systemic atherosclerotic complications, low cardiac function and poorly controlled diabetes.

Endothelial dysfunction is thought to be a predictor of prognosis in CAD patients. Suwaidi \textit{et al}\(^{5}\) found that coronary endothelial dysfunction induced by vasoconstrictive responses to the vasodilator acetylcholine is associated with cardiac events in patients with mild coronary disease, and may therefore play a role in the progression of CAD. Schachinger \textit{et al}\(^{9}\) showed that coronary endothelial dysfunction could predict the progression of long-term atherosclerotic disease and the risk of cardiac events. They proposed that endothelial function may be a useful tool for diagnosing and predicting the prognosis of CAD. These reports indicate that endothelial dysfunction of the coronary artery seen even in the early phases of atherosclerosis can predict adverse clinical events in patients with CAD. Other reports have also identified an association between endothelial dysfunction and a poor prognosis in CAD patients\(^{10}\). Targonski \textit{et al}\(^{16}\) showed that the presence of coronary endothelial dysfunction in patients without CAD is independently associated with an increased risk of cerebrovascular events. They suggested that detection of this early stage of atherosclerosis may help to identify patients who would benefit from aggressive preventive strategies. Their findings support our study, given that systemic arterial diseases, not just diseases of the coronary artery, may encourage the progression to MACEs by elevating TM. The importance of endothelial function in cases with recurrent CAD has also been reported\(^{17-19}\), with Patti \textit{et al}\(^{17}\) prospectively speculating that impaired flow-mediated dilation could independently predict the occurrence of in-stent restenosis in patients undergoing PCI.

We found that serum TM was a powerful predictor of MACEs after PCI in patients with all types of AMI encountered. TM, a transmembrane glycoprotein expressed on the surface of endothelial cells, affects both thromboresistance and anti-inflammatory function\(^{20}\). Therefore, TM is a sensitive marker of endothelial function. As the microvessel wall is injured and destroyed, TM is degraded by proteases in the endothelial cells and released into the blood\(^{21,22}\). Hypercoagulability and hyperfibrinolysis may be correlated with MACEs after PCI in AMI patients. Elevated levels of BNP and HbA1c and decreased levels of LVEF were also sensitive predictors of MACEs in our multivariate analysis. As mentioned earlier, low cardiac function and poorly
controlled diabetes can cause adverse events in patients with CAD. Coronary risk factors such as hyperlipidemia are also causal factors of AMI.

Most of the associations that have been identified between risk factors for CAD and adverse events are identified at the primary prevention stage. However, we identified an indication for secondary prevention, demonstrating that all risk factors, including the traditional coronary risk factors, can be confounded in real-world clinical practice. Our results suggest that this has clinical significance for patients who have experienced severe cardiac events. Specifically, we found that TM may be a useful predictive factor of second events in AMI patients who have undergone PCI.

Conflict of interest disclosure

We have no conflicts of interest to disclose.

References


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