Platelets play a crucial role in the pathogenesis of acute coronary syndrome (ACS) and of acute ischemic stroke. Activated platelets adhere to the vessel wall at the site of ruptured plaque, and initiate arterial thrombus formation, which leads to ischemia or infarction.1–3 Increasing evidence suggests that markers of platelet activation can be used to identify disease activity4,5 and the degree of tissue necrosis following acute myocardial infarction (AMI) or acute ischemic stroke6,7.

In the initial stage of AMI, circulating platelets exposed to subendothelial collagen are activated and secrete several thrombotic and pro-inflammatory molecules.2,8 Soluble CD40 ligand (sCD40L), an important pro-inflammatory mediator9,10 secreted by activated platelets,10 directly participates in thrombus formation during the acute phase of AMI.11 In platelets, CD40L is rapidly translocated to the platelet surface after stimulation and is upregulated in fresh thrombus.10 The CD40L expressed on the surface of activated platelets is then enzymatically cleaved within a period of minutes to hours, subsequently generating sCD40L that is released into the circulation.12 Although it may also be shed from stimulated lymphocytes, it is estimated that more than 95% of circulating sCD40L is derived from platelets.12,13

Some recent studies have demonstrated that the infarct-related artery (IRA) usually has high-burden thrombus formation (HBTF)14,15 and lipid pool-like contents.16 Additionally, an association between HBTF and the no-reflow phenomenon during primary percutaneous coronary intervention (PCI), with or without adjunctive tirofiban therapy, has been reported.14,17 Moreover, a link between the angio-

**Background** This study tested the hypothesis that in the acute phase of myocardial infarction (MI), the circulating level of soluble CD40 ligand (sCD40L), an index of platelet activation, is predictive of angiographic morphologic features that indicate high-burden thrombus formation (HBTF) in the infarct-related artery (IRA).

**Methods and Results** This prospective study included 162 consecutive patients: 64 with HBTF and 98 with low-burden thrombus formation (LBTF). All patients had a Killip’s classification ≤3 ST-segment elevation acute myocardial infarction (AMI) of onset <12 h who were undergoing primary percutaneous coronary intervention (PCI). Blood samples for measurement of the circulating levels of sCD4L and high-sensitivity C-reactive protein (hs-CRP) and white blood cell (WBC) count were collected before PCI. The circulating levels of sCD40L and hs-CRP, and the WBC count were also evaluated in 20 normal control subjects. Blood was aspirated by export suction catheter from the intracoronary artery (ICA) in 49 HBTF patients. The WBC count, and the circulating levels of hs-CRP and sCD40L were significantly higher in the HBTF and LBTF groups than in the normal control subjects (all p<0.005). Additionally, the circulating levels of sCD40L and the WBC count were substantially higher in the HBTF than in the LBTF patients (all p<0.001). Furthermore, in HBTF patients the ICA had a significantly higher sCD40L level and WBC count compared with the values for the systemic circulation (all p<0.001). Multiple statistical analyses identified increased circulating level of sCD40L as the most independent predictor of HBTF in the IRA (p<0.0001).

**Conclusions** The sCD40L level is the most independent predictor of angiographic morphologic features that indicate HBTF in the acute phase of MI. (Circ J 2007; 71: 1857–1861)

**Key Words:** Acute myocardial infarction; Angiography; High-burden thrombus formation; Morphology; Soluble CD40 ligand
graphic morphologic features that indicate HBTF and the no-reflow or slow flow phenomenon has been demonstrated.\textsuperscript{15} Interestingly, although CD40L has been shown to directly participate in thrombus formation during the acute phase of AMI,\textsuperscript{13} the association between an increased circulating level of sCD40L and increased risk of HBT in IRA is currently unclear. Therefore, this study tested the hypothesis that in the acute phase of myocardial infarction (MI), the circulating level of sCD40L, as an index of platelet activation, may be predictive of the angiographic morphologic features of HBTF of the IRA of patients with ST-segment elevation (SE) AMI.

Methods

Patient Population and Inclusion Criteria

Chang Gung Memorial Hospital, all patients with ST-SE AMI of <12h duration are considered eligible for primary PCI. The angiographic morphologic features of the IRA that indicate HBTF were described in detail in our previous study.\textsuperscript{15} Briefly, HBTF is defined as: (1) an angiographic thrombus with its greatest linear dimension greater than 3-fold the reference lumen diameter (RLD), (2) a cut-off pattern (ie, lesion morphology with an abrupt cut-off without tapering before the occlusion), (3) the presence of accumulated thrombus (>5 mm of linear dimension) proximal to the occlusion, (4) the presence of floating thrombus proximal to the occlusion, (5) persistent dye stasis distal to the occlusion, (6) RLD of the IRA ≥4.0 mm. The transradial approach has been the designed protocol for AMI in our hospital since 2001 and, except for patients in cardiogenic shock, the PercuSurge GuardWire\textsuperscript{TM} device (Medtronic AVE) is used when there are the angiographic morphologic features that indicate HBTF. The detailed procedure is described in our previous study.\textsuperscript{18}

To avoid other variables that could influence the circulating levels of high-sensitivity C-reactive protein (hs-CRP) and sCD40L, and the white blood cells (WBC) count; this study excluded patients with a history of recent surgery or trauma within the preceding 2 months, renal insufficiency (creatinine >1.5 mg/dl), malignancy or liver cirrhosis, febrile disorders, acute or chronic inflammatory disease on study entry or history of recent infection, and those with AMI onset >12h. Patients were also excluded if antiplatelet agents had been used for more than 3 days before AMI. Finally, we also excluded cardiogenic shock patients because they were not suitable candidates for the PercuSurge utilization in our study protocol. Therefore, between November 2002 and April 2004, a total of 162 patients, including 64 with HBTF and 98 with low-burden thrombus formation (LBTF), constituted the study patient population. Additionally, 20 age- and gender-matched healthy volunteers were enrolled as controls from the healthy-examination department in our hospital. Informed consent was given by each study subject and the Institutional Review Committee on Human Research approved the study protocol.

Definitions and Data Collection

AMI was defined as typical chest pain lasting for more than 30 min with ST-SE >1 mm in 2 or more consecutive precordial or inferior leads. Procedural success was defined as successful primary PCI with residual stenosis of the index lesions <20% after stent deployment and <50% after balloon angioplasty and a final Thrombolysis In Myocardial Infarction (TIMI) flow of grade 3 in the IRA. Multivessel disease was defined as stenoses >50% in ≥2 major epicardial coronary arteries.

Detailed in-hospital and follow-up data, including age, sex, coronary risk factors, serial MB fraction of creatine kinase (CK-MB) levels, WBC count, creatinine level, Killip score on admission, severity of congestive heart failure (CHF), angiographic findings and number of diseased vessels were collected prospectively and entered into a computerized database.

Procedure and Protocol

A transradial artery approach using a 6Fr arterial sheath is routinely used for treatment of AMI in our hospital unless the Allen’s test for both hands is positive. A 6Fr Kimny guiding catheter (Boston Scientific, Scimed Inc, Maple Grove, MN, USA) is used for diagnosis and primary PCI. In the present study, when coronary diagnostic results demonstrated the angiographic morphologic features of HBTF, which met the criteria for use of PercuSurge device therapy,\textsuperscript{18} the 6Fr arterial sheath was replaced with a 7Fr arterial sheath, and a 7Fr standard interventional guiding catheter was used for PCI.

Thrombectomy with an export suction catheter (ESC) was performed after the PercuSurge distal protection balloon was inflated prior to coronary angioplasty. Blood samples for intracoronary artery (ICA) levels of hs-CRP and sCD40L, and platelet and WBC counts were obtained from beyond the occluded lesion, using the ESC after distal protection balloon inflation. To avoid the possibility of dilution of these inflammatory mediators, 2–3 ml of blood from inside the ESC were first removed, followed by collection of 6–8 ml of blood from ICA. Within the study period, abciximab was not available, so at the beginning of this study a loading dose of tirofiban (20 µg/kg of body weight) was administered to patients upon presentation in the emergency room, followed by a maintenance infusion of 0.15 µg·kg⁻¹·min⁻¹ for 18–24h. However, tirofiban therapy was subsequently ceased because it was found not to provide any additional benefit to AMI patients who underwent primary PCI.\textsuperscript{17,18} Therefore, only 39 (11.4%) of the study patients received tirofiban therapy.

Clopidogrel (300 mg preoperative loading dose, then 75 mg/day) was given for at least 4 weeks to patients who underwent primary stenting. Aspirin (orally 100 mg/day) was given to each patient indefinitely.

Analysis of Blood Samples

Blood samples were obtained from the radial artery after radial sheath insertion in the cardiac catheterization room, and by venous puncture from control the subjects. WBC count, biochemical parameters and electrolytes were determined by standard laboratory methods. hs-CRP was measured by immunonephelometry using the BN\textsuperscript{TM} system (Dade Behring Inc, Newark, DE, USA). The lower detection limit is <0.15 mg/L. The intra-individual variability of the serum hs-CRP level was assessed in both study patients and normal control subjects whose respective mean intra-assay coefficients of variance were 2.81% and 2.77%. The concentration of sCD40L in plasma was determined in duplicate using a standard enzyme-linked immunosorbent assay (ELISA) and a commercial kit (R&D Systems; Minneapolis, MN, USA) according to the manufacturer’s instructions. Intra-individual variability of sCD40L levels was assessed in the study patients and control subjects and in our laboratory, the mean intra-assay coefficients of variance were <7%.
Table 1  Baseline Characteristics of Study Patients and Normal Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>HBTF group (n=64)</th>
<th>LBTFT group (n=98)</th>
<th>Normal control (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.6±10.1</td>
<td>60.2±10.9</td>
<td>60.6±10.3</td>
<td>0.978</td>
</tr>
<tr>
<td>Male gender</td>
<td>85.9% (55)</td>
<td>86.7% (85)</td>
<td>80.0% (16)</td>
<td>0.885</td>
</tr>
<tr>
<td>Current smoking</td>
<td>46.9% (30)</td>
<td>48.0% (47)</td>
<td>–</td>
<td>0.884</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>34.4% (22)</td>
<td>30.6% (30)</td>
<td>–</td>
<td>0.616</td>
</tr>
<tr>
<td>Hypertension</td>
<td>46.9% (30)</td>
<td>55.7% (54)</td>
<td>–</td>
<td>0.274</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>43.8% (28)</td>
<td>45.9% (45)</td>
<td>–</td>
<td>0.786</td>
</tr>
<tr>
<td>Previous MI</td>
<td>9.4% (6)</td>
<td>8.2% (8)</td>
<td>–</td>
<td>0.788</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.08±0.21</td>
<td>1.09±0.22</td>
<td>10.1±0.21</td>
<td>0.532</td>
</tr>
<tr>
<td>WBC count (&lt;10×10^9/ml)</td>
<td>11.5±2.9^a</td>
<td>9.1±2.1^b</td>
<td>5.7±1.1^c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet counts (&lt;10×10^12/ml)</td>
<td>20.6±5.5</td>
<td>21.1±4.7</td>
<td>22.0±3.8</td>
<td>0.243</td>
</tr>
<tr>
<td>sCD40L (pg/ml)</td>
<td>1.774±525.5^a</td>
<td>1.150±542.7^b</td>
<td>393.9±117.8^c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>4.1±4.1^a</td>
<td>4.0±5.1^a</td>
<td>1.4±1.3^b</td>
<td>0.001</td>
</tr>
<tr>
<td>Anterior wall MI</td>
<td>51.6% (33)</td>
<td>56.1% (35)</td>
<td>–</td>
<td>0.569</td>
</tr>
<tr>
<td>Pre-PCI TIMI flow (≥1)</td>
<td>9.4% (6)</td>
<td>35.7% (35)</td>
<td>–</td>
<td>0.0002</td>
</tr>
<tr>
<td>Multivessel disease</td>
<td>50.0% (32)</td>
<td>50.0% (49)</td>
<td>–</td>
<td>1.0</td>
</tr>
<tr>
<td>Tirofiban therapy</td>
<td>9.4% (6)</td>
<td>11.2% (11)</td>
<td>–</td>
<td>0.613</td>
</tr>
<tr>
<td>Reperfusion time (min)</td>
<td>283.0±125.8</td>
<td>286.3±115.7</td>
<td>–</td>
<td>0.627</td>
</tr>
<tr>
<td>Stenting*</td>
<td>90.6% (58)</td>
<td>88.8% (87)</td>
<td>–</td>
<td>0.707</td>
</tr>
<tr>
<td>PercuSurge GuardWire</td>
<td>100% (64)</td>
<td>6.1% (6)</td>
<td>–</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-PCI TIMI-3 flow</td>
<td>96.8% (62)</td>
<td>88.8% (87)</td>
<td>–</td>
<td>0.964</td>
</tr>
<tr>
<td>Advanced CHF</td>
<td>17.2% (11)</td>
<td>18.4% (18)</td>
<td>–</td>
<td>0.848</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>4.7% (3)</td>
<td>4.1% (4)</td>
<td>–</td>
<td>0.909</td>
</tr>
</tbody>
</table>

Data are mean±SD or % (no.) of patients.
HBTF, high-burden thrombus formation; LBTFT, low-burden thrombus formation; MI, myocardial infarction; WBC, white blood cell; sCD40L, soluble CD40 ligand; hs-CRP, high-sensitivity C-reactive protein; PCI, percutaneous coronary intervention; TIMI, Thrombolysis In Myocardial Infarction; CHF, congestive heart failure.

*All stents were bare metal. †Means with different letters (a, b, c) indicate significant difference (at 0.05 level) by Wilcoxon rank-sum test and Bonferroni’s correction.

Table 2  Comparison of the Levels of Inflammatory Biomarkers and Platelet Count in the Systemic Circulation and Intracoronary Artery in HBTF Group

<table>
<thead>
<tr>
<th></th>
<th>Systemic circulation</th>
<th>Intracoronary artery</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count (&lt;10×10^9/ml)</td>
<td>11.5±2.9</td>
<td>14.2±3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sCD40L (pg/ml)</td>
<td>1.774±525.5</td>
<td>2.387±433.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count (&lt;10×10^12/ml)</td>
<td>20.8±5.7</td>
<td>21.8±5.7</td>
<td>0.314</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>4.1±4.1</td>
<td>3.9±4.7</td>
<td>0.735</td>
</tr>
</tbody>
</table>

Data are mean±SD. Abbreviations see in Table 1.

Statistical Analysis
Categorical variables were compared using chi-square test or Fisher’s exact test. Continuous variables were analyzed by Wilcoxon test or Kruskal-Wallis test for nonparametric data among 3 groups, followed by a multiple comparison procedure with the Wilcoxon rank-sum test and Bonferroni’s correction. Statistical analysis was performed using SAS statistical software for Windows version 8.2 (SAS institute, Cary, NC, USA). A probability value <0.05 was considered statistically significant.

Results
The baseline relevant data are shown in Table 1. There were no significant differences in terms of age, sex, creatinine level and platelet count among the patients with HBTF, patients with LBTFT or normal control subjects. The risk factors for coronary artery disease and the incidence of anterior wall MI did not differ between patients with HBTF and LBTFT. However, the WBC count and the circulating levels of hs-CRP and sCD40L were significantly higher in the HBTF and LBTFT groups than in the normal control subjects. The circulating levels of hs-CRP did not differ between patients with HBTF and those with LBTFT. However, the circulating level of sCD40L and the WBC count were substantially higher in patients with HBTF than in patients with LBTFT.

Angiographic findings demonstrated that the incidence of multivessel disease, tirofiban therapy and stent implantation did not differ between patients with HBTF and those with LBTFT. Additionally, the reperfusion time, advanced CHF (defined as ≥3 on the New York Heart Association Functional Classification) during hospitalization and the 30-day mortality rate also did not differ between these 2 groups of patients. However, the pre-PCI TIMI flow ≥1 grade was significantly lower in HBTF patients than in LBTFT patients. Additionally, the incidence of using the PercuSurge GuardWire device was significantly higher in HBTF patients than in LBTFT patients. A final TIMI-3 flow of the IRA was observed more frequently in the HBTF patients than in LBTFT patients, but did not reach the statistical significance (p=0.064).

Table 2 shows the comparison of the levels of inflammatory biomarkers and platelet counts in the systemic circulation and ICA in the HBTF group. There were no significant differences in terms of hs-CRP level and platelet count between systemic circulation and ICA; however, the sCD40L
level and the WBC count were significantly higher in the ICA than in the systemic circulation.

Multiple stepwise logistic regression analysis of the variables shown in Table 1 from 162 study patients demonstrated that the circulating level of sCD40L, together with the WBC count, was independently predictive of HBTF (Table 3), whereas pre-PCI TIMI flow ≥1 was strongly and independently predictive of freedom from HBTF.

Discussion

The results of this study have several striking clinical implications. First, the level of sCD40L was markedly increased in the acute phase of AMI and was substantially higher in the patients than in the normal control subjects. Second, the circulating level of sCD40L and the WBC count were significantly higher in the IRA of patients with HBTF than in that of patients with LBTF. Third, the sCD40L level and the WBC count were remarkably higher in the ICA than in the systemic circulation of HBTF patients. Finally, the circulating level of sCD40L and the WBC count were independently predictive of HBTF in the IRA, whereas pre-PCI TIMI flow ≥1 was the only predictor of LBTF. Therefore, these findings further support the finding from a recent study\(^{19}\) that inflammatory cells, including polymorphonuclear and mononuclear cells, and activated platelets are frequently obtained from human coronary thrombi following AMI.

An important finding in the present study is that sCD40L was markedly increased in the acute phase of AMI and was substantially higher in patients than in normal control subjects. A link between increased CD40L expression and plaque instability has been demonstrated by a previous study\(^{20}\) Additionally, the circulating level of sCD40L is frequently found to be increased in ACS patients\(^{8–11,20}\) Our finding is further supported by a previous study that demonstrated that CD40L is cleaved from activated platelets within a period of minutes to hours, subsequently generating circulating CD40L\(^{12}\) Additionally, our finding of a markedly increased level of sCD40L following AMI is also consistent with the findings of previous studies\(^{8–11,20}\) and reinforces the finding of another previous study that the CD40-CD40L system participates in plaque vulnerability and rupture\(^{20}\)

The most important finding of this study is that the circulating level of sCD40L was the most independent predictor of the angiographic morphologic features that indicate HBTF. Additionally, we also found that patients with HBTF in the IRA had a significantly less incidence of pre-PCI TIMI flow ≥1. Furthermore, a pre-PCI TIMI flow ≥1 was found to be strongly and independently predictive of LBTF in this study. Therefore, our findings extend those of a previous study\(^{11}\) of the direct participation of sCD40L in coronary arterial thrombus formation during the acute phase of AMI. Moreover, our finding of a higher level of sCD40L in the ICA than in the systemic circulation of patients with HBTF further supports the concept that sCD40L plays a crucial role in HBTF in the IRA. Accordingly, our findings could, at least in part, further explain why previous studies demonstrated that pre-PCI TIMI flow >1 was strongly predictive of a final normal coronary blood flow following primary PCI\(^{15,21}\)

Previous studies have demonstrated that an increased WBC count in patients with AMI is strongly associated with reduced epicardial blood flow, reduced myocardial reperfus-

\section*{Study Limitations}

First, we previously found that pre-intervention thrombectomy with a PercuSurge distal protection balloon can improve the final angiographic results of primary PCI.\(^{18}\) However, despite the frequent use of thrombectomy in patients with HBTF, in this study only a tendency toward a statistically significant higher final TIMI-3 flow was found in the patients with HBTF compared with those with LBTF in the IRA, which could be related to the small patient sample size, but can also be explained by the contributive effect of LBTF, which has been found to be strongly predictive of freedom from slow-flow or no-reflow phenomenon.\(^{22}\) Second, this study did not provide additional information with respect to the impact of the circulating level of sCD40L on the long-term outcomes of the patients. A recent study has already reported that increased sCD40L levels are associated with a poor outcome 90 days after PCI.\(^{23}\) Finally, thrombectomy was not routinely used in the patients with LBTF in the IRA, so this study did not provide additional information in terms of any differences in WBC counts and sCD40L level between the systemic circulation and that of the ICA in the LBTF group.

\section*{Conclusion}

The circulating level of sCD40L, together with the WBC count, was independently predictive of the angiographic morphologic features that indicate HBTF in the acute phase of AMI. Accordingly, the results of this study raise the need for a prospective study to evaluate whether combination therapy with antiplatelet/antiinflammatory agents can provide an additional benefit for patients with AMI and HBTF in the IRA who are undergoing primary PCI.

\section*{References}


