Plasma Soluble Fibrin Monomer Complex as a Marker of Coronary Thrombotic Events in Patients with Acute Myocardial Infarction

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Soluble fibrin monomer appears in the bloodstream during the extremely early stage of blood coagulation and generally forms a complex with fibrinogen, termed soluble fibrin monomer complex (FMC). Determination of FMC can provide information regarding the state of thrombotic diseases; thus it is important to investigate whether FMC serves as an early indicator of myocardial infarction (MI). We investigated hemostatic and fibrinolytic parameters including FMC to determine their capabilities for indicating thrombotic conditions in the coronary artery. Blood samples from 47 patients with acute MI were obtained within 48 hours (acute phase) and during 120 - 600 hours (recovery phase), respectively, after MI onset. Plasma FMC was significantly elevated in the acute phase, compared with that during the recovery phase and in healthy controls (p = 0.001), suggesting that its elevation indicates thrombotic events in the coronary artery of MI patients. D-dimer, a marker of thrombus formation accompanied with fibrinolysis, was increased in both phases in the patients. In addition, FMC and D-dimer were significantly increased within 24 hours after onset as compared to 24 - 48 hours (p = 0.003 and p = 0.011). Furthermore, cardiac troponin T, a marker of myocardial damage, was significantly higher after 24 hours than within the first 24 hours (p = 0.001). Receiver operating characteristic (ROC) analysis of FMC for early MI diagnosis indicates that FMC, rather than D-dimer, is a better marker within 24 hours of onset. Measuring plasma FMC may be useful for early diagnosis of MI recurrence and deciding primary treatment. — soluble fibrin monomer; myocardial infarction; D dimer; troponin T; CK-MB.

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pholipid syndrome. Various indicators of coagulation have been tested for evaluation of risk for ischemic heart disease (IHD) (Lowe et al. 1998; Dempfle et al. 1999; Kontny et al. 1999; Ottani and Galvani, 2001; Lowe et al. 2001; Saigo et al. 2001; Hetland et al. 2002), though no reliable indicator has been reported. Since most cases of MI occur due to thrombus-mediated obstruction of the coronary artery, it is of immense interest to determine whether FMC can serve as an early indicator of MI.

Recently, three novel immunoassays for SF and FMC were developed with specific monoclonal antibodies (Soe et al. 1996; Hamano et al. 2002; Suzuki et al. 2007). The monoclonal antibody IF-43 is directed against the neo-epitope present on the α-chain remnant corresponding to the Aα residues (52-78) in the E domain of the desAA fibrin monomer, which is prepared from a urea-solubilized human fibrin monomer. This monoclonal antibody recognizes thrombin-modified fibrinogen, as well as the desAA and desAABB fibrin monomers, which bind with fibrinogen or other D1 domain-containing plasmic fragments, such as the X-, Y-, and D-fragments, but not with intact fibrinogen or cross-linked fibrin degradation products (XDP) (Soe et al. 1996). Another monoclonal antibody, J2-23, recognizes a newly reported epitope [Aα(502-521)] on the C-terminal of the fibrinogen α-chain (Suzuki et al. 2007). J2-23 reacts with SF, but not with fibrinogen or plasmic fibrinogen-derived X-, Y-, and E-fragments of fibrinogen; thrombin-treated X-, Y-, and E-fragments; or plasmic cross-linked fibrin (DD, FDP) (Suzuki et al. 2007). The complexes detected with the monoclonal antibodies IF-43 and J2-23 are generally termed SF, while those detected with F405 are termed FMC. The monoclonal antibody F405 is directed against a desAA fibrin monomer-specific neo-epitope [Aα(17-23)] generated by thrombin-induced cleavage of FPA from fibrinogen (Hamano et al. 2002). F405 is also able to recognize the desAA and desAABB fibrin monomers, as well as the X-, Y-, and E-fragments of fibrin monomer, though not fibrinogen, fragments of fibrinogen, or XDP including D-dimer (DD) (Hamano et al. 2002). FMC is considered to be a useful marker for hypercoagulable states with accelerated fibrinolysis, because F405 also recognizes the degradation products of fibrin monomer by fibrinolytic activity and their complexes with fibrin(ogen) (Ieko et al. 2007).

In the present study, we determined the plasma levels of FMC using the specific immunoassay and hemostatic and fibrinolytic parameters in acute MI patients to identify the parameters for the thrombotic condition of the coronary artery.

**Materials and Methods**

**Subjects**

Forty-seven Japanese patients (mean age 65.7 years, range 52 to 86; 33 males, 14 females) with acute MI who were admitted to the coronary care unit (CCU) of Sunagawa City Hospital between June 2002 and December 2003 were enrolled in this study. Each patient was confirmed by angiography to have total occlusion and significant coronary stenosis, and diagnosed with acute MI according to World Health Organization criteria (Report on the Joint International Society and Federation of Cardiology/World Health Organization Task Force and Standardization of Clinical Nomenclature. Circulation, 1979). None had a congenital thrombotic tendency or history of thrombosis due to other causes. Twenty-five patients were complicated with hypertension, 16 with hyperlipidemia, 9 with diabetes mellitus, and 6 with other conditions, as shown in Table 1. All patients were administered tissue-plasminogen activator (tPA) within 48 hours after diagnosis of MI, which was followed by treatment with heparin. Furthermore, within 24 hours of the onset of acute MI, 39 of the patients were treated with tPA according to the Guidelines for Management of patients with acute MI published in 2001 by the Ministry of Health, Labor and Welfare of Japan (http://minds.jcqhc.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male : Female</td>
<td>33 : 14</td>
</tr>
<tr>
<td>Age</td>
<td>65.7 ± 9.7 years (range 52 - 86)</td>
</tr>
<tr>
<td>Underlying diseases (including overlapping cases)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>25</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>16</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>9</td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
</tr>
</tbody>
</table>

| Samples from patients with AMI | |
| Both acute and recovery phase: | 24 patients (48 samples) |
| Acute phase only: | 21 patients (21 samples) |
| Recovery phase only: | 2 patients ( 2 samples) |

| Healthy subjects (control) | 50 |
| Male : Female | 27 : 23 |
| Age | 46.4 ± 11.3 years (range 24 - 63) |
Soluble Fibrin Monomer and Acute Myocardial Infarction

Although thrombolytic therapy in acute MI patients more than 24 hours after onset without the presence of chest pain is not recommended by the Guidelines, the 8 patients diagnosed more than 24 hours after onset were also treated with tPA, as they had continued chest pain and ECG findings demonstrated continued ST elevation. Seven patients aged above 75 years old were also treated with tPA, as they were weakly recommended for administration of thrombolytic agents by guidelines published in 2001. However, the current guidelines (Guidelines for management of patients with ST-elevation myocardial infarction) specifically note that thrombolytic therapy for acute MI patients is generally indicated for patients less than 75 years old with ST elevation in ECG and less than 12 hours after onset (Circ., J., 2008). Two patients died of heart rupture. As a control group, 50 healthy subjects aged 24 to 63 years old (mean age 46.4 years) were also studied. The study protocol was approved by the Research Ethics Committee of Health Sciences University of Hokkaido. Written informed consent for all procedures was obtained from all patients and control subjects prior to beginning the study.

Blood sampling

Blood sampling to obtain acute phase samples was performed within 48 hours (mean 7.2 hours) after the onset of MI (at diagnosis), just prior to the beginning of thrombolytic therapy. Blood samples during the recovery phase were drawn between 120 and 600 hours (mean 297.6 hours) after the onset of MI. Entry into the recovery phase was confirmed using laboratory examinations of peripheral blood and ECG findings. As a result, paired samples from both acute and recovery phases were obtained from 24 patients. In 21 of the remaining patients, including 2 death cases, recovery phase samples were not obtained for various reasons, thus recovery phase only samples were obtained from 2 patients. Citrated blood samples were centrifuged at 3000 rpm for 30 minutes at 4°C and the supernatants were filtered using a cellulose acetate filter (DISMIC-25cs, ADVANTEC, Tokyo, Japan) to remove residual platelets. Serum samples were obtained from non-citrated blood by centrifugation at 3000 rpm for 30 minutes, followed by incubation for 30 minutes at room temperature. All plasma and serum samples were stored at -80°C until analysis.

Laboratory measurements

For hemostatic parameters, we measured the plasma levels of FMC using a latex immunoturbidimetry assay with an Auto LIA-FM (Roche Diagnostics K.K., Tokyo, Japan), while thrombin-antithrombin complex (TAT) was measured with an ELISA kit (TAT Test Kokusai F; International Reagent Corp., Kobe, Japan). As for fibrinolytic markers, the plasma levels of D-dimer and plasmin-α2-plasmin inhibitor complex (PPI) were measured with an ELISA method using DD Test Kokusai F (International Reagent Corp.) and PIC Test Kokusai F (International Reagent Corp.) kits, respectively. Serum creatine kinase (CK) levels were determined using a Dri-Chem Slide kit (Fujif; Kanagawa, Japan). Serum levels of TnT and CK-MB were determined using Elecsys Troponin T (TnT) STAT (Roche Diagnostics) and Elecsys CK-MB STAT (Roche Diagnostics) kits, respectively.

Statistics

Statistical analysis was performed using a paired t-test to compare the results between the acute and recovery phases. Comparisons between the control and each phase were performed using an unpaired Student’s t-test or Welch’s t-test, as well as between the results within 24 hours after the onset of MI and those after 24 hours. P-values below 0.05 were considered to be statistically significant. Correlations between FMC and the other parameters in the samples taken within 24 hours of onset were analyzed using Pearson’s correlation coefficient. The usefulness of FMC level for early diagnosis of MI was examined using a receiver operating characteristic (ROC) analysis with StatMate III (ATMS, Tokyo, Japan).

Results

Comparisons between acute and recovery phases

In the patients, CK and CK-MB in the acute phase were significantly higher than in the recovery phase, as well as compared to the healthy controls (Fig. 1), while no significant difference was seen between the control and recovery phase values for those enzymes. In addition, TnT was higher in both the acute and recovery phases in the patients than in the controls (Fig. 1). Likewise, FMC in the acute phase was 6.43 ± 3.99 µg/ml, which was significantly higher than that in the recovery phase (2.38 ± 2.00 µg/ml) (p = 0.001), while both those values were significantly elevated as compared with the controls (1.41 ± 0.70 µg/ml) (Fig. 2). TAT and D-dimer were not different between the acute and recovery phases. In addition, TAT was higher in the recovery phase of the patients than in the control group, while D-dimer in the recovery phase was also elevated in comparison with the control group, whereas that level was significantly lower than the acute phase level. PPI in the acute phase was also higher than that in the recovery phase (p = 0.045), with a small increase of PPI in the recovery phase in comparison with the control group observed. By the end of the experiment, all parameters were increased in both phases as compared with those in the controls.

Comparison between within and after 24 hours from onset

We investigated the same parameters in 37 samples taken within 24 hours after onset and evaluated whether they indicated the onset of coronary thrombosis, then those results were compared with those from 8 samples taken between 24 and 48 hours after onset. FMC within 24 hours was 14.81 ± 25.87 µg/ml, which was significantly elevated as compared with that from 24 to 48 hours (1.15 ± 0.84 µg/ml; p = 0.003), and the level from 24 to 48 hours was similar to that in the controls. D-dimer and PPI within 24 hours were also significantly higher than after 24 hours (p = 0.011 and 0.001, respectively), as those levels returned to normal ranges from 24 to 48 hours. There was no significant difference between the two groups in regard to TAT levels (Table 2). In contrast, CK, CK-MB, and TnT within 24 hours were significantly lower than after 24 hours. Furthermore, within 24 hours from the onset of MI, FMC was correlated with TAT (r = 0.940, p < 0.001), but not with TnT (r = 0.022), CK-MB (r = 0.087), CK (r = 0.108), D-dimer (r = 0.110), or PPI (r = 0.285). Furthermore, ROC analysis of FMC for early diagnosis of MI showed that FMC was superior to D-dimer and CK-MB within 24 hours.
Discussion

Coronary thrombosis is an important determinant in regard to the prognosis of patients with acute MI. However, it is difficult to identify those at high risk for coronary thrombosis progression, partly because there are no clinically meaningful laboratory tests available for its detection. Enzymes derived from the myocardial muscle, such as CK and CK-MB, are generally used as markers of MI. However, they are thought to directly reflect damage in the myocardial muscle and not coronary thrombosis. Considering that early diagnosis and treatment are important for the prognosis of patients with acute MI, markers indicating obstructive events in the coronary artery would be more useful than those showing myocardial muscle damage.

FMC has shown promise as a provider of information
Soluble Fibrin Monomer and Acute Myocardial Infarction

regarding fibrin formation immediately prior to the formation of thrombus. In the present study, we first compared levels of the selected markers in the acute phase to those in the recovery phase, and found that CK, CK-MB, FMC, and PPI levels were significantly higher in the acute phase. Furthermore, ROC analysis revealed FMC as superior for early diagnosis of MI within 24 hours of onset, as compared to D-dimer and CK-MB. Hence, we propose FMC as a useful marker for early diagnosis of acute MI. In addition, we observed a small but statistically significant increase of FMC in the recovery phase in comparison with the control group, which suggests that a hypercoagulable state might still exist in the recovery phase.

Coagulation and fibrinolytic parameters in patients with thrombosis are easily changed after a thrombotic event, while those parameters in patients with AMI seem to change

**Table 2. Thrombotic-fibrinolytic parameters within and after 24 hours from the onset of myocardial infarction.**

<table>
<thead>
<tr>
<th>Parameters [Reference value]</th>
<th>Healthy controls (&lt;i&gt;n&lt;/i&gt; = 50)</th>
<th>Acute myocardial infarction</th>
<th>&lt;i&gt;p&lt;/i&gt;-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within 24 hours (&lt;i&gt;n&lt;/i&gt; = 37)</td>
<td>From 24 to 48 hours (&lt;i&gt;n&lt;/i&gt; = 8)</td>
<td></td>
</tr>
<tr>
<td>CK (IU/L) [32.0 - 197.0]</td>
<td>115.0 ± 85.0</td>
<td>443.4 ± 791.5</td>
<td>1453.0 ± 1040.9</td>
</tr>
<tr>
<td>CK-MB (ng/ml) [0 - 5.0]</td>
<td>1.8 ± 1.3</td>
<td>41.8 ± 105.9</td>
<td>158.5 ± 157.4</td>
</tr>
<tr>
<td>TnT (ng/ml) [0 - 0.10]</td>
<td>0.06 ± 0.05</td>
<td>0.50 ± 1.18</td>
<td>3.65 ± 1.38</td>
</tr>
<tr>
<td>FMC (µg/ml) [0 - 4.49]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.41 ± 0.70</td>
<td>14.81 ± 25.87</td>
<td>1.15 ± 0.84</td>
</tr>
<tr>
<td>TAT (ng/ml) [0 - 3.75]</td>
<td>1.02 ± 0.78</td>
<td>6.15 ± 13.65</td>
<td>5.05 ± 6.95</td>
</tr>
<tr>
<td>D-dimer (µg/ml) [0 - 1.00]</td>
<td>0.26 ± 0.22</td>
<td>4.44 ± 9.31</td>
<td>0.32 ± 0.09</td>
</tr>
<tr>
<td>PPI (µg/ml) [0 - 0.80]</td>
<td>0.40 ± 0.13</td>
<td>4.81 ± 7.12</td>
<td>0.59 ± 0.21</td>
</tr>
</tbody>
</table>

CK, creatine kinase; TnT, troponin T; FMC, fibrin monomer complex; TAT, thrombin-antithrombin complex; PPI, plasmin-α2-plasmin inhibitor complex.

All data are presented as means ± standard deviation (S.D.). <sup>a</sup>Data were analyzed using an unpaired Student’s <i>t</i>-test or Welch’s <i>t</i>-test (within 24 hours vs. 24-48 hours). <sup>b</sup>Reference values for FMC used in our assays were previously obtained from 108 healthy individuals (Naito et al., 2002).

**Fig. 3. ROC analysis for early diagnosis of MI within 24 hours of onset.**
Closed circles: FMC, open circles: D-dimer, closed triangles: CK-MB.
over time after the onset of MI. Although we intended to obtain samples from the patients at the same time point, it was not possible because their therapy regimens took precedence over factors related to our study. Therefore, we compared the levels of the markers within 24 hours after the onset of MI to those from 24 to 48 hours after onset, with FMC shown to be useful for diagnosis of AMI within 24 hours. On the other hand, FMC was found to be less useful for diagnosis after 24 hours, as the levels from 24 to 48 hours were within a normal range. We concluded that TnT and CK-MB are superior to FMC for detecting AMI more than 24 hours after onset, as well as in the recovery phase. TnT levels in the patients were slightly elevated in the first 24 hours, as compared with that in the controls, and then elevated further from 24 to 48 hours. Based on our observed elevation of TnT during the recovery phase, we considered that myocardial breakdown in MI patients persists in the recovery phase. TnT is one of the best markers of MI, as it indicates myocardial damage. Furthermore, Giannitsis et al. (2000) reported that TnT in patients with unstable angina was correlated with the plasma levels of hemostatic markers, especially FMC. In the present study, FMC was elevated only within 24 hours of onset and was well correlated with TAT in that time period, but not with TnT, which can likely be explained by the fact that TAT and FMC reflect thrombin formation, whereas TnT does not. In addition, our findings indicate that markers of thrombin generation or fibrin formation may be more useful for diagnosis of MI in the early phase (within 24 hours after onset) than markers of myocardial breakdown. However, the present results are limited by the low number of samples used for testing.

D-dimer and PPI in the acute phase were significantly higher than those in the recovery phase, suggesting intracoronary thrombosis. Lowe et al. (1998) reported that D-dimer has a strong and independent association with the incidence of IHD, and later concluded that it reflects activation of blood coagulation (Lowe et al. 2001). However, elevated D-dimer and PPI levels within 24 hours might be related to secondary fibrinolysis with fibrin formation, and those markers can be affected by administration of thrombolytic agents (Mombelli et al. 1984). Therefore, D-dimer and PPI levels may not be helpful for diagnosis of recurrent MI, though they can be useful for detection of intracoronary thrombosis at the first occurrence of MI. In addition, when patients with MI receive insufficient fibrinolytic therapy and relapse into MI, D-dimer and PPI levels may not reflect the recurrence of intracoronary thrombosis. D-dimer elevation in patients with AMI has been reported following percutaneous coronary intervention (PCI) and thrombolytic therapy (Steppich et al. 2005; Brügger-Andersen et al. 2007). In the study of Brügger-Andersen et al. (2007), SF was reported to be elevated, though their results may be unreliable, because they were obtained with an older kit, Enzymun FM. In the present study, we determined FMC levels with the monoclonal antibody F-405, which can recognize not only soluble fibrin, but also the products of soluble fibrin degradation caused by fibrinolytic activity. In addition, detection of FMC with F-405 provides accurate information related to fibrin formation, because the antibody is able to recognize as little as 1 molecular unit of fibrin monomer degradation products derived from 1 molecular unit of fibrin monomer caused by fibrinolytic activity (Ieko et al. 2007).

FMC levels are not directly affected by therapy with heparin or thrombolytic agents, and they seem to be the best marker for indicating thrombotic events in the coronary artery and may also contribute to an accurate diagnosis of MI. If coronary thrombosis can be detected with a marker prior to the development of myocardial damage, MI occurrence can be prevented by early therapy with thrombolytic agents. In addition, the present results suggest that FMC is useful for deciding primary treatment, such as fibrinolytic therapy or PCI. In a study that utilized the same FMC assay as in the present study, Dempfle et al. (2003) suggested that it would be also useful for monitoring fibrin formation in acute MI patients for deciding primary treatment of fibrinolytic therapy or PCI. Our results support that speculation.

In conclusion, FMC is a useful marker of coronary thrombosis immediately prior to the development of myocardial cell damage. We propose that plasma FMC measurement should be considered for patients with acute MI.

**Acknowledgments**

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**References**


