No-Reflow Phenomenon After Acute Myocardial Infarction Is Associated With Reduced Clot Permeability and Susceptibility to Lysis

Jaroslaw Zalewski, Anetta Undas, Jacek Godlewski, Ewa Stepien, Krzysztof Zmudka

Objective—We assessed the relationship between fibrin clot properties and the no-reflow phenomenon after primary coronary intervention (PCI).

Methods and Results—Epicardial blood flow was assessed by TIMI scale and corrected TIMI frame count (cTFC), and perfusion by TIMI Myocardial Perfusion Grade (TMPG) after PCI during ST-segment elevation myocardial infarction (STEMI). Fibrin clot permeability (Kc) and susceptibility to lysis in assays using exogenous thrombin (t100%) and without thrombin (t50%) were determined in 30 no-reflow patients (TIMI ≤2) and in 31 controls (TIMI-3) after uneventful 6 to 14 months from PCI. Patients with TIMI ≤2 had lower Kc by 18% (P<0.0001) and prolonged fibrinolysis by 33% for t100% (P<0.0001) and by 45% for t50% (P<0.0001). cTFC was correlated with Kc (r = -0.56, P<0.0001), t100% (r = 0.49, P<0.0001), and t50% (r = 0.54, P<0.0001). Kc increased in a stepwise fashion with TIMI flow (P<0.0001) and TMPG (P<0.0001), whereas both fibrinolysis times decreased with TIMI flow (P<0.0001 for both) and TMPG (P<0.01 for both). Multiple regression models showed that only Kc, and fibrinogen were independent predictors of cTFC (P<0.05 for both), TIMI ≤2 flow (P<0.05 for both) and TMPG-0/1 (P<0.05 for both).

Conclusions—Survivors of myocardial infarction with a history of the no-reflow after PCI are characterized with more compact fibrin network and its resistance to lysis. (Arterioscler Thromb Vasc Biol. 2007;27:2258-2265.)

Key Words: myocardial infarction ■ primary coronary angioplasty ■ no-reflow phenomenon ■ fibrin clot ■ fibrinolysis

The efficiency of reperfusion therapy in acute myocardial infarction (MI) is limited by impaired microvascular reperfusion occurring after opening an infarct related artery (IRA).1–3 The absence of a complete myocardial perfusion occurring after opening an infarct related artery associated with a larger infarct size,8,9 lower left ventricular ejection fraction,10 increased mortality,5,11 and more frequent congestive heart failure attributable to LV remodeling.6,9 Main causes of this phenomenon are not fully understood and involve: microvascular embolization by the aggregates composed of platelets, erythrocytes, neutrophiles, and fragments of thrombus and ruptured plaque; endothelial dysfunction; vascular smooth muscle cell (VSMC) contraction, and surrounding tissue edema.12 Complex pathways of blood coagulation lead ultimately to the formation of a fibrin clot that is preceded by thrombin-mediated fibrinogen conversion to fibrin and fibrin cross-linking by activated factor XIII.13 The structure and function of the fibrin clot is affected by genetic and environmental factors, especially fibrinogen levels.14 Despite the data showing that fibrin thrombi may participate in microvascular obstruction4,12 and an early intervention in a closed artery with a large thrombus leads to distal embolization and tissue perfusion deterioration in some patients,7,15 an association between fibrin clot properties and the no-reflow phenomenon has not yet been studied. Therefore, we sought to evaluate the fibrin clot properties in patients with impaired epicardial and tissue reperfusion after primary coronary intervention (PCI) in myocardial infarction with persistent ST-segment elevation (STEMI) in ECG.

Methods

Patients

Sixty-one patients, 43 men, aged 62.7 ± 10.2 years, who underwent PCI for STEMI were enrolled in this case-control study. We studied 30 consecutive patients with a final TIMI ≤2 coronary blood flow after PCI (the no-reflow group) performed 6 to 14 (average 10.2 ± 2.5) months before the enrolment. Thirty-one

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patients with a final TIMI-3 flow immediately after PCI, matched for age, sex, risk factors, and concomitant treatment, served as controls. They were selected from 220 patients treated at the same time in whom full (TIMI-3) epicardial blood flow was restored. The inclusion criteria for performing PCI were a typical chest pain within 12 hours from the onset and ST segment elevation of ≥1 mm in ≥2 contiguous leads on standard ECG. The exclusion criteria were as follows: any acute illness, cancer, hepatic or renal dysfunction, previous coronary artery bypass surgery, history of venous thromboembolism or stroke, and current anticoagulant therapy. After PCI all patients were observed at the outpatient clinic. None of the patients developed recurrent MI between therapies. After PCI all patients were observed at the outpatient clinic.

Epicardial and Myocardial Reperfusion

The epicardial and tissue reperfusion efficiency was assessed by angiography and ECG, whereas myocardial injury was determined by elevated necrosis marker levels and LV function by transthoracic angiography and ECG, whereas myocardial injury was determined by plasma levels of elevated necrosis markers—creatine kinase (CK) and its MB isoenzyme (CKMB). Both were measured every 6 hours within the first 24 hours.

Myocardial Injury and LV Function

Myocardial injury was determined by plasma levels of elevated necrosis markers—creatine kinase (CK) and its MB isoenzyme (CKMB). Both were measured every 6 hours within the first 24 hours.

Table 1. Characteristics of Patients With and Without the No-Reflow Phenomenon

<table>
<thead>
<tr>
<th></th>
<th>Reflow (n=31)</th>
<th>No-Reflow (n=30)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.2±10.4</td>
<td>64.2±9.8</td>
<td>NS</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>23 (74.2)</td>
<td>20 (66.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Previous myocardial infarction, n (%)</td>
<td>7 (22.6)</td>
<td>4 (13.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Acute phase of myocardial infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time from onset of chest pain to opening of infarct related artery, h</td>
<td>5.0±3.1</td>
<td>5.1±3.0</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>48.3±10.0</td>
<td>44.9±8.2</td>
<td>NS</td>
</tr>
<tr>
<td>LAD as infarct related artery, n (%)</td>
<td>13 (41.9)</td>
<td>16 (53.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Multivessel coronary disease, n (%)</td>
<td>12 (38.7)</td>
<td>8 (26.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Abciximab administration, n (%)</td>
<td>8 (25.8)</td>
<td>10 (33.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Stent implantation, n (%)</td>
<td>29 (93.5)</td>
<td>28 (93.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time from STEMI to blood collection, months</td>
<td>9.7±2.4</td>
<td>10.7±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.6±1.2</td>
<td>4.5±1.0</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.7±1.0</td>
<td>2.6±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.1±0.3</td>
<td>1.2±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.7±1.0</td>
<td>1.7±1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.1±1.2</td>
<td>6.4±4.0</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>77.5±20.8</td>
<td>80.1±14.5</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.7±2.3</td>
<td>2.8±2.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are mean±SD, unless otherwise indicated.

LVEF indicates left ventricular ejection fraction; LAD, left anterior descending artery; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CRP, C-reactive protein; NS, not significant.

ECG

Standard 12-lead ECGs were obtained before angioplasty [B] and 30 minutes after opening of IRA [O30]. ST segment, which is a part of an ECG curve, was analyzed. Generally, the ST-segment elevation and depression of >1 mm may indicate myocardial infarct and ischemia, respectively. The sum of ST segment elevations and depressions in all leads (Σ ST) was measured 20 ms after the J point by a single investigator blinded to clinical and angiographic findings. The reduction of ST-segment elevation from baseline to 30 minutes after primary angioplasty (ST) was calculated according to the formula: ST = Σ STO30 / Σ STB. Moreover, the complete ST-segment elevation resolution (STR ≥70%), partial ST resolution (STR 30% to 70%), and no ST resolution (STR ≤30%) were assessed according to Schröder 3-component definition.17

Angiographic Evaluation

Epicardial blood flow in IRA was evaluated by means of the thrombolysis in myocardial infarction (TIMI) scale16 and corrected TIMI frame count (cTFC).5 TIMI flow grades were defined as follows: 0, no antegrade flow beyond the point of occlusion; 1, contrast passes beyond the area of obstruction, but fails to opacify the entire coronary bed distal to the occlusion; 2, contrast passes beyond the area of obstruction and opacifies the coronary bed distal to the occlusion, but slower than in the non-infarct area; 3, antegrade flow into the bed distal to the obstruction occurs as promptly as in the non-infarct area.16 cTFC was defined as the number of cineframes required for contrast to reach a standard-ized distal coronary landmark in the culprit vessel.5 Myocardial perfusion was assessed by using the TIMI myocardial perfusion grades (TMPG).2 According to TMPG, grade 0 indicates the failure of the dye to enter the microvasculature, in grade 1 the dye slowly enters but fails to exit the microvasculature, in grade 2 the entry and the exit of the dye from the microvasculature is delayed in comparison to the noninfarct area and grade 3 presents the normal entry and exit of the dye from the microvasculature. Moreover, the angiograms were analyzed for the presence of the flow-limiting lesions in the non-IRA. A detailed evaluation was performed in 2 contralateral projections for each artery before and after angioplasty. Two experienced investigators reviewed each coronary angiogram in a blinded fashion. In case of controversy between the 2 investigators, a third one was sought and a consensus was reached.

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hours, and then after 36 and 48 hours. Maximum CK (CK\textsubscript{MAX}) and CKMB (CKMB\textsubscript{MAX}) were analyzed by latex nephelometry (Dade Behring).

Laboratory Investigations
Blood was drawn from an antecubital vein with minimal stasis. Lipid profiles, blood cell counts, platelets, glucose, creatinine, and D-dimer were assayed by routine laboratory techniques. Plasma samples (9:1 of 3.2% sodium citrate) were centrifuged within 20 minutes of collection, immediately frozen, and stored in aliquots at \(-80^\circ\text{C}\) until further use. Fibrinogen was determined using the Clauss method. High-sensitivity C-reactive protein (CRP) was measured by turbidity of this mixture was measured at 405 nm at 37°C in a Spectramax 340 kinetic microplate reader (Molecular Devices Corp). In this assay lysis time (t\textsubscript{LYS}, min) was defined as the time from the midpoint of the clear to maximum turbid transition, which characterizes clot formation, to the midpoint of the maximum turbid to clear transition, which represents clot lysis. The interassay and intraassay coefficients of variation were 6.0 and 5.6%, respectively.

Clot Permeability
Permeation properties of fibrin clots were investigated according to Mills et al. Briefly, 20 mmol/L calcium chloride and 1 U/mL human thrombin (Sigma) were added to citrated plasma. After incubation, tubes containing the clots were connected to a reservoir of a buffer (0.01 mol/L Tris, 0.1 mol/L NaCl, pH 7.5) and its volume permeation coefficient (K\textsubscript{s}), was calculated from the equation: K\textsubscript{s} = \frac{Q}{A \pi t}, where Q is the flow rate in time t, A is the cross-sectional area (in cm\(^2\)), \pi is the viscosity of liquid (in poise), and t is the length of a fibrin gel. The interassay variability of results was 7.9%.

Plasma Clot Lysis Assays
Fibrinolysis in the presence of recombinant tissue plasminogen activator (rt-PA, Boehringer Ingelheim) was evaluated as previously described. Briefly, 100 \(\mu\)L citrated plasma was mixed with 60 \(\mu\)L of HEPES buffer (0.025 mol/L HEPES, 0.137 mol/L NaCl, 3.5 mmol/L KCl, 3.5 mmol/L calcium chloride, 0.05% bovine serum albumin, pH 7.4; Sigma), containing 15 mmol/L calcium chloride, 10000-diluted recombinant human TF (Innovin, Dade Behring), 12 \(\mu\)mol/L phospholipid vesicles, prepared as previously described and 60 ng/mL rt-PA (Boehringer Ingelheim). Turbidity of this mixture was measured at 405 nm at 37°C in a Spectramax 340 kinetic microplate reader (Molecular Devices Corp). In this assay lysis time (t\textsubscript{LYS}, min) was defined as the time from the midpoint of the clear to maximum turbid transition, which characterizes clot formation, to the midpoint of the maximum turbid to clear transition, which represents clot lysis. The interassay and intraassay coefficients of variation were 6.0 and 5.6%, respectively.

In another assay, fibrinolysis in the presence of rt-PA, but without addition of exogenous thrombin, was monitored in a tissue factor (TF)-based assay as described. Briefly, 60 \(\mu\)L citrated plasma was mixed with 60 \(\mu\)L of HEPES buffer (0.025 mol/L HEPES, 0.137 mol/L NaCl, 3.5 mmol/L KCl, 3.5 mmol/L calcium chloride, 0.05% bovine serum albumin, pH 7.4; Sigma), containing 15 mmol/L calcium chloride, 10000-diluted recombinant human TF (Innovin, Dade Behring), 12 \(\mu\)mol/L phospholipid vesicles, prepared as previously described and 60 ng/mL rt-PA (Boehringer Ingelheim). Turbidity of this mixture was measured at 405 nm at 37°C in a Spectramax 340 kinetic microplate reader (Molecular Devices Corp). In this assay lysis time (t\textsubscript{LYS}, min) was defined as the time from the midpoint of the clear to maximum turbid transition, which characterizes clot formation, to the midpoint of the maximum turbid to clear transition, which represents clot lysis. The interassay and intraassay coefficients of variation were 6.0 and 5.6%, respectively.

Long-Term Follow-Up
Follow-up data of patients treated at the outpatient clinic included: recurrent MI, repeated coronary angioplasty, and heart failure requiring hospitalization. The cardiac function of the survivors was assessed according to the New York Heart Association (NYHA) and the Canadian Cardiovascular Society (CCS) functional scales.

Statistical Analysis
Statistical analyses were performed with a SPSS 12.01. A normal distribution of variables was tested by the Shapiro-Wilk statistic and the Bera-Jarque test. All continuous variables were expressed as mean\(\pm\)SD, or otherwise stated. Student t test was used to compare the differences between reflow and no-reflow groups. ANOVA and post-hoc analysis with Scheffe correction were used to compare the differences between K\textsubscript{s}, t\textsubscript{50\%}, and t\textsubscript{TF} in relation to STR, TIMI flow, and TIMI myocardial perfusion grade. Categorical variables were compared by \(\chi^2\) test or Fisher exact test. Linear regression analysis was performed to evaluate the relationship between K\textsubscript{s}, t\textsubscript{50\%}, and t\textsubscript{TF} and clinical, biochemical, and coronary angiography parameters. Adjusted to a small sample, the stepwise multiple logistic regression analysis was used to determine predictors of TIMI \(\leq2\) flow, TMPG-3, and TMPG-0/1, whereas the multiple linear regression analysis was used to
Table 2. Hemostatic Variables and Fibrin Clot Properties in Both Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reflow (n=31)</th>
<th>No-Reflow (n=30)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets, ×10^9/µL</td>
<td>237±189</td>
<td>241±193</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>4.0±1.0</td>
<td>4.2±1.0</td>
<td>NS</td>
</tr>
<tr>
<td>D-dimer, mg/L</td>
<td>302±91</td>
<td>345±77</td>
<td>NS</td>
</tr>
<tr>
<td>(K_s), 10^-9 cm²</td>
<td>9.9±0.7</td>
<td>8.4±0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(t_{50}%), min</td>
<td>7.7±1.1</td>
<td>10.3±1.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(t_{TF}), min</td>
<td>60±15</td>
<td>88±14</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are mean±SD. 
\(K_s\) indicates clot permeability; \(t_{50}\%), lysis time determined using the method of Williams et al.; \(t_{TF}\), lysis time determined using the method of von dem Borne et al.; NS, not significant.

determine predictors of corrected TIMI frame count. A probability value <0.05 was considered statistically significant.

**Results**

The characteristics of the patients studied are shown in Table 1. Both groups did not differ with regard to age, sex, risk factors (frequency of hypertension, diabetes mellitus, hyperlipidemia, current smoking), hemodynamic status on admission in acute phase of MI, concomitant treatment (frequency of aspirin, thienopiridines, statins, angiotensin converting enzyme inhibitor, \(\beta\)-blockers administration), time from the onset of symptoms to IRA opening, time from index event to blood collection, the percentage of patients in the Canadian Cardiovascular Society class ≥2 and the New York Heart Association class ≥2, lipid profile, and CRP level. Compared with patients with complete epicardial flow, the no-reflow patients had higher TMPG (2.45 versus 1.0; \(P=0.0001\)) and the New York Heart Association class \(P=0.03\), compared with the no-reflow group (Figure 1).

**Clot Permeability**

Significantly lower permeability coefficient, indicating pores of a smaller size in the fibrin network, was found in the no-reflow patients compared with subjects with the TIMI-3 flow (Table 2).

As shown in Table 3, \(K_s\) was negatively correlated with fibrinogen level, cTFC, enzymatic injury, reduction of ST-segment elevation, D-dimer level, and CRP (r from −0.24 to −0.6, \(P<0.05\)). Permeability showed positive association with LV ejection fraction. There was no correlation between \(K_s\) and time from pain onset to the opening of IRA or age.

Clot permeability increased in a stepwise fashion with TIMI flow grades (\(P<0.0001\)) and the TIMI myocardial perfusion grades (\(P<0.0001\); Figure 2). There was a significant, though much weaker, relationship between \(K_s\) and STR (\(P=0.03\)).

**Clot Lysis**

In the no-reflow group \(t_{50}\%) and \(t_{TF}\) were significantly longer, indicating reduced clot susceptibility to lysis, compared with the TIMI-3 flow patients (Table 2).

Lysis time, \(t_{TF}\), was positively correlated with cTFC, fibrinogen, enzymatic injury, and reduction of ST-segment elevation (r from 0.43 to 0.54, \(P<0.05\)) and negatively correlated with LV ejection fraction (Table 3). Similarly, \(t_{50}\%\) was correlated with the same variables, however these associations were weaker than those found for \(t_{TF}\) (r from 0.36 to 0.51, \(P<0.05\); Table 3). Lysis time, \(t_{50}\%), decreased in a stepwise fashion with higher TIMI flow grades (\(P<0.0001\)) and the increase of TIMI myocardial perfusion grades (\(P=0.0018\); Figure 2). In post-hoc analysis \(t_{50}\%\) did not differ between TIMI-0/1 and −2 patients and those with TMPG-2 and −3. There was no relationship between \(t_{50}\%\) and STR. \(t_{TF}\) decreased gradually with increasing TIMI flow grades (\(P=0.0001\)) and TIMI myocardial perfusion grades (\(P=0.0004\); Figure 2). In post-hoc analysis, \(t_{TF}\) did not differ between TIMI-0/1 and −2 patients and those with the final TMPG-2 and −3.
Independent Predictors of Impaired Reperfusion

The linear regression analysis for cTFC and stepwise logistic regression analysis for TIMI flow and TIMI myocardial perfusion grade are shown in Table 4. Before the inclusion to the multiple analysis model, independent variables including Ks, t50%, and tTF were associated with TIMI flow ($P < 0.001$ for all), TMPG-3 ($P = 0.009$, $P = 0.004$, $P = 0.036$; respectively), and TMPG-0/1 ($P < 0.001$, $P = 0.001$, $P = 0.001$; respectively). There were strong correlations among the independent variables ($r = -0.85$ for Ks, and t50%, $r = -0.88$ for Ks and tTF, $r = 0.89$ for t50% and tTF, $P < 0.001$ for all). Finally, Ks and fibrinogen level were independent predictors of cTFC ($P < 0.05$ for both) and TIMI flow ($P < 0.05$ for both, $R^2 = 0.88$). Moreover, tTF was an independent predictor of complete (TMPG-3) reperfusion (OR 0.94, 95% CI 0.9 to 1.0, $P < 0.05$). Fibrinogen level and Ks were independent predictors of the lack (TMPG-0/1) of myocardial perfusion ($R^2 = 0.37$, $P < 0.05$).

Discussion

The current study is the first to demonstrate significant associations between unfavorable fibrin clot properties and the no-reflow phenomenon observed in STEMI patients after primary angioplasty. We have found that in patients with impaired both epicardial and myocardial reperfusion fibrin clots are composed of more dense fibrin networks, which are more resistant to lysis compared with those in individuals with a complete reperfusion. This finding suggests that altered clot properties may characterize patients with the no-reflow phenomenon and might help identify subjects at increased risk of such a complication.

There are reports suggesting a role of platelet-fibrin thrombus in the no-reflow phenomenon. It has been shown that fragmented thrombus and ruptured plaque released from a culprit lesion during thrombolysis or primary angioplasty led to the embolization and peripheral microvascular obstruction which can be detected in above 15% of patients. Distal embolization showed associations with reduced myocardial reperfusion, more extensive myocardial damage, and worse clinical outcome including higher long-term mortality.7,15 Our results provide a plausible explanation for these observations by showing that certain unfavorable clot features predispose to perfusion impairment after PCI most likely through distal embolization by thrombi resistant to lysis.
Table 4. The multiple Regression Models With cTFC, TIMI and TMPG as the Dependent Variables

<table>
<thead>
<tr>
<th>A. Dependent Variable</th>
<th>Independent Variable</th>
<th>Contribution of Variance, %</th>
<th>P Value</th>
<th>Coefficient</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTFC value Final model</td>
<td></td>
<td>28.2</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ks</td>
<td></td>
<td>6.9</td>
<td>&lt;0.05</td>
<td>-11.9</td>
<td>-21.9</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
<td>5.9</td>
<td>&lt;0.05</td>
<td>-5.8</td>
<td>-11.0</td>
</tr>
<tr>
<td>tTF</td>
<td></td>
<td>3.1</td>
<td>NS</td>
<td>0.4</td>
<td>-0.1</td>
</tr>
<tr>
<td>t50%</td>
<td></td>
<td>1.8</td>
<td>NS</td>
<td>-4.1</td>
<td>-10.8</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>0.7</td>
<td>NS</td>
<td>2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Time of ischemia</td>
<td></td>
<td>0.4</td>
<td>NS</td>
<td>1.1</td>
<td>0.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Dependent Variable</th>
<th>Independent Variable</th>
<th>P Value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMI 2</td>
<td>Ks</td>
<td>0.022</td>
<td>1.3</td>
<td>88.1</td>
</tr>
<tr>
<td>TIMI 2</td>
<td>Fibrinogen</td>
<td>0.029</td>
<td>1.3</td>
<td>17.4</td>
</tr>
<tr>
<td>TIMI 2</td>
<td>tTF</td>
<td>0.04</td>
<td>1.9</td>
<td>4.0</td>
</tr>
<tr>
<td>TIMI 2</td>
<td>t50%</td>
<td>0.023</td>
<td>1.3</td>
<td>18.9</td>
</tr>
<tr>
<td>TMPG-0/1</td>
<td>Ks</td>
<td>0.022</td>
<td>1.3</td>
<td>88.1</td>
</tr>
<tr>
<td>TMPG-0/1</td>
<td>Fibrinogen</td>
<td>0.029</td>
<td>1.3</td>
<td>17.4</td>
</tr>
<tr>
<td>TMPG-0/1</td>
<td>tTF</td>
<td>0.04</td>
<td>1.9</td>
<td>4.0</td>
</tr>
<tr>
<td>TMPG-0/1</td>
<td>t50%</td>
<td>0.023</td>
<td>1.3</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1, 2, and 3. A, The multiple linear regression model with cTFC after PCI as a dependent variable; B, The logistic regression model with TIMI and TMPG as the dependent variables. TMPG indicates TIMI myocardial perfusion grade; OR, odds ratio; CI, confidential intervals.

To analyze clot structure, we used in the current study a plasma-based method, namely clot permeation in a pressure-driven system successfully applied by several groups. Given correlation between permeability and velocity of fibrinolysis, we used 2 methods to assess this association. Because the method introduced by Williams et al requires addition to a plasma sample human thrombin and rt-PA at relatively high concentrations, another model of fibrinolysis in which clot is formed on phospholipid vesicles and TF without exogenous thrombin was also used. A close association between results obtained using both lysis assays suggests that amount of thrombin converting fibrinogen to fibrin in the presence of rt-PA does not markedly affect clot properties in our clinical setting. However, we observed a stronger association between tTF and reperfusion parameters such as cTFC and reduction of ST-segment elevation as well as plasma fibrinogen and D-dimer as compared with the values for t50%. It might be speculated that a TF-based assay is a more appropriate approach to evaluate the interaction between lysis and reperfusion. Interestingly, the relationship fibrin structure/function and the no-reflow was detected several months after the index event. Therefore, it cannot be attributed to acute changes circulatory blood such as increased fibrinogen and CRP, which alter unfavorably clot structure that indicates the presence of persistent structural features in individual patients.

At present, there is no uniform criterion to define the no-reflow precisely. In our study the efficacy of reperfusion during STEMI was assessed both in the epicardium and myocardium. The difference in all the clot variables studied was observed in patients with TIMI-3 and 2 flow, but not in those with TIMI-0/1 and 2. Moreover, we showed that there was a significant correlation between cTFC, which is a quantitative parameter of reperfusion epicardial flow and lysis times and clot permeability. The lower clot permeability and the longer lysis time, the more pronounced epicardial blood flow impairment. A larger prospective study is needed to validate these intriguing observations.

The patients with impaired myocardial perfusion have still poor prognosis despite complete epicardial TIMI-3 flow. Thus, tissue-level reperfusion was evaluated by means of ST-segment elevation resolution and by angiography-determined grades of TIMI myocardial perfusion. A marked and early resolution of ST-segment elevation is a sensitive and specific marker of a successful microvascular and tissue-level perfusion and is associated with a smaller infarct size, better recovery of left ventricular function, and a lower mortality rate. Among the studied clot variables, tTF displayed the strongest association with the degree of ST-segment resolution in our study. Nevertheless, ST-segment resolution showed the weakest correlation with fibrin clot properties as compared with other indicators of reperfusion efficacy. In contrast, TMP grades showed strong associations with the clot properties. The fibrin network of the patients with complete or partial (TMPG-2/3) perfusion was characterized by higher permeability and susceptibility to lysis compared with the patients without reperfusion (TMPG-0/1).

In our study clot permeability and fibrinogen concentration but not lysis times independently affect the occurrence of both TIMI 2 flow and the increased value of corrected TFC. Interestingly, the same factors predicted the lack of tissue reperfusion expressed as TMPG-0/1.

In addition, it should be stressed that unfavorably altered clot properties in the no-reflow patients were apparent despite chronic aspirin administration. Aspirin was shown to increase clot permeability and to promote fibrin clot lysis. However, in stable survivors of MI who displayed the no-reflow
during the acute phase of STEMI these effects of aspirin were likely to be abolished by other potent modulators, eg, enhanced thrombin formation.

Although genetic factors have been shown to influence plasma concentrations of hemostatic proteins,25,26 growing evidence indicates that the sum of genetic and environmental influences affects fibrin structure and function.14,22 We have shown that fibrin clots of stable patients evaluated after myocardial infarction who experienced no-reflow during the acute phase of STEMI, are composed of more compact fibrin network which is more resistant to lysis in comparison to results in the patient group with a proper flow. Both compared groups, well matched for age, sex, risk factors, and concomitant treatment, differed only in terms of the final TIMI flow after primary angioplasty and the magnitude of enzymatic injury. Thus, our findings support the hypothesis that fibrin clot properties, determined rather by genetic factors, might predispose to the no-reflow.

Limitations
Our study has some limitations. First, the number of the patients studied was limited. However, we meticulously matched the no-reflow patients with those with a proper flow for demographic and clinical features that might confound the interpretation of our results. Second, our analysis was based on a determination of each variable at a single time point in stable conditions, not in an acute phase of myocardial infarction. Third, angiographic assessment of TMPG was subjective. To make an objective myocardial perfusion evaluation, at least 2 experienced investigators reviewed each coronary angiogram in a blinded fashion. In the case of a lack of agreement between the 2 investigators, a third one was sought, and the conclusions were drawn. Finally, clot analysis using scanning electron microscopy, which can provide additional structural information, was not performed.

Conclusions
In conclusion, our findings suggest that the altered fibrin clot structure associated with attenuated fibrinolysis can be detected in patients with a history of impaired reperfusion. Reduced clot permeability and fibrinolysis after primary angioplasty may be a novel prothrombotic mechanism, which might contribute to the pathogenesis of the no-reflow phenomenon. A prospective study in patients, including clot analysis using plasma obtained before primary angioplasty will be useful to validate our observations and determine their clinical implications for the management of subjects at risk for the no-reflow phenomenon.

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References


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