Procoagulant and proinflammatory activity in acute coronary syndromes

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Abstract

Objectives: Both the hemostatic and inflammatory system are thought to play a role in the pathogenesis of acute coronary syndromes. However, their respective contribution and interrelationship remain unclear, therefore, we studied the relationship between activation of the coagulation system and proinflammatory activity in ischemic coronary syndromes.

Methods: Thrombin–antithrombin III (TAT), prothrombin fragments F 1+2, fibrinopeptide A (FPA), interleukin-6 (IL-6) and interleukin-8 (IL-8) were measured in 50 patients with unstable angina (UA), 60 patients with acute myocardial infarction (AMI) and in 50 patients with stable angina (SA).

Results: FPA levels were significantly higher in patients with UA and AMI than in patients with SA (p = 0.0015 and p < 0.0001), and were higher in patients with AMI than UA (p = 0.0013). Plasma IL-6 concentrations were significantly higher in patients with UA and AMI than in patients with SA (p = 0.0020 and p < 0.001), and again were higher in AMI than UA (p = 0.001). Interestingly, FPA or IL-6 elevations on admission were found in different patients. In contrast, TAT, F 1+2 and IL-8 levels were not different between the three groups.

Conclusions: IL-6 and FPA were shown to be independent predictive markers with equal discriminative power to distinguish stable (SA) from unstable (UA + AMI) patients. Moreover, hemostatic and inflammatory markers can be elevated independently in the acute phase of ischemic coronary syndromes.

Keywords: Acute coronary syndromes; Coronary disease; Cytokines; Coagulation

1. Introduction

Partial or complete thrombotic occlusion of a coronary artery, secondary to rupture of an atherosclerotic plaque, is the key event in the development of acute ischemic coronary syndromes [1]. In various studies, evidence of activation of the hemostatic system has been demonstrated in the acute phase of coronary artery disease [2–4]. Thus, elevated levels of thrombin–antithrombin III (TAT), prothrombin fragments F 1+2 and fibrinopeptide A (FPA) were measured both in patients with unstable angina (UA) and acute myocardial infarction (AMI) [2,3,5,6]. Moreover, a persistent hypercoagulable state has been demonstrated in patients with UA or AMI long after clinical stabilization [2].

More recently, an inflammatory component has also been suggested to play a role in the progression to acute coronary syndromes [7–10]. Activated macrophages and T-lymphocytes have been demonstrated at the site of plaque disruption [11] and, in a substantial proportion of patients with unstable angina (UA), elevated plasma levels of C-reactive protein (CRP) [12] and interleukin-6 (IL-6) [7] have been found. In addition, interleukin-8 (IL-8) has been suggested to be a sensitive marker of unstable coronary artery disease [13].

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Both IL-6 and IL-8 are multifunctional cytokines with proinflammatory as well as procoagulant properties. Thus, in chimpanzees, the protective effect of anti-IL-6 antibodies in endotoxin-induced coagulation has been demonstrated [14]. Moreover, IL-8 has been suggested as a key participant in the cross-talk between the coagulation and cytokine cascades [15]. By linking the inflammatory and hemostatic systems, IL-6 and IL-8 may play a pivotal role in the pathophysiology of acute coronary syndromes [7,8].

The aim of this study was to investigate the relationship between coagulation and inflammation in the acute phase of ischemic coronary syndromes. Therefore, we measured hemostatic and inflammatory parameters in patients with UA or AMI on hospital admission. Patients with stable angina (SA) served as controls. Plasma levels of TAT, F$_{1+2}$ and FPA were measured as markers of thrombin generation (TAT, F$_{1+2}$) and thrombin activity (FPA), respectively. Plasma IL-6 and IL-8 concentrations were measured as proinflammatory markers.

2. Methods

2.1. Patients

Consecutive patients with characteristic chest pain within the last 12 h before presentation at the emergency room were eligible for the study. Unstable angina was defined by the presence of at least one episode of ischemic pain of less than 30 min duration, accompanied by diagnostic ST-segment shift or T-wave changes, but without enzymatic evidence of acute myocardial infarction (CK-MB levels less than twice the upper limit of normal or 8 U/l). We aimed to include the patients with “severely” unstable angina (Braunwald class IIIB), with ST-segment or T-wave changes on the electrocardiogram that reacted to administration of sublingual nitrates or calcium antagonists. Acute myocardial infarction was defined by a combination of ischemic chest pain lasting for more than 30 min, characteristic ECG changes and a typical rise and fall of CK-MB levels with a peak of more than twice the upper limit of normal. Exclusion criteria were heparin administration or fibrinolytic therapy before blood sampling, known or suspected thrombotic disease, major surgery in the past three months, evidence of acute or chronic infectious disease or signs of an inflammatory disorder, oral anticoagulants or anti-inflammatory medication, with the exception of aspirin. Patients with chronic stable angina from the outpatient clinic, characterized by exercise-induced ischemic chest pain and coronary artery disease documented by coronary angiography, without changes in symptoms within the previous or following three months, served as controls. The study was approved by the institutional review board of the Academic Medical Center and, before participation, informed consent was obtained from all patients. The investigation conformed with the principles outlined in the Declaration of Helsinki.

2.2. Blood sampling and assays

On admission, before any medication was administered, venous blood samples were collected for CK-MB, TAT, F$_{1+2}$, FPA, IL-6 and IL-8 measurements. In order to avoid puncture artifacts, blood was collected by atraumatic venepuncture into tubes containing either trisodium citrate dihydrate (3.2%) or an anticoagulant mixture from Byk-Sangtec, for measurements of F$_{1+2}$, TAT and FPA, respectively. After centrifugation (3000×g at room temperature for 30 min), the plasma was frozen on dry ice and stored at −80°C, within 90 min of blood sampling. TAT complexes and prothrombin fragments F$_{1+2}$ were measured using an enzyme immunoassay (ELISA) (Behring, Enzygnost, Germany) and FPA levels were determined with a double antibody radioimmunoassay (RIA) (Byk-Sangtec, Germany). IL-6 and IL-8 were measured using a commercially available ELISA [Central Laboratory of the Dutch Red Cross Blood Transfusion Service (CLB), Amsterdam, Netherlands].

2.3. Statistical analysis

The Mann-Whitney U test was used to evaluate differences between SA, UA and AMI groups. Receiver operating characteristic (ROC) curves were fitted from the continuously distributed data for IL-6 and FPA with maximum likelihood estimation, using the computer program CLABROC (by C.E. Metz). The areas under the fitted ROC curves were calculated, to estimate the overall discriminative capacity of the variables. To examine the discriminative capacity of IL-6, FPA and the combination of IL-6 and FPA, logistic regression was used. Disease was defined as the presence of either UA or AMI. Three models were estimated predicting the probability of disease, one for IL-6, one for FPA, and one for the combination of IL-6 and FPA. Disease was defined as the presence of either UA or AMI. Three models were estimated predicting the probability of disease, one for IL-6, one for FPA, and one for the combination of IL-6 and FPA. The log-likelihood of the models was compared using a chi-square test. A ROC curve for the combination of IL-6 and FPA was calculated using the predicted values of the logistic regression model. The area under the curve (AUC) of the three ROC curves were compared using the method described by Hanley and McNeil [16]. This method reduces the standard error of differences in area, correcting for the correlation of test results. A probability of $p<0.05$ was considered to be statistically significant.

3. Results

3.1. Patients

Procoagulant and proinflammatory activity was mea-
Table 1
Characteristics of patients

<table>
<thead>
<tr>
<th></th>
<th>SA (n=50)</th>
<th>UA (n=50)</th>
<th>AMI (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64±11</td>
<td>64±12</td>
<td>64±13</td>
</tr>
<tr>
<td>Males (%)</td>
<td>44 (73)</td>
<td>31 (62)</td>
<td>32 (64)</td>
</tr>
<tr>
<td>History of CAD(^a):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– UA</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>– AMI</td>
<td>11 (22)</td>
<td>10 (20)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>– CABG(^b)</td>
<td>21 (42)</td>
<td>11 (22)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Risk factors:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Cholesterol (mmol/l)</td>
<td>5.75±1.20</td>
<td>5.80±0.95</td>
<td>5.81±0.99</td>
</tr>
<tr>
<td>– Family history</td>
<td>26 (52)</td>
<td>25 (50)</td>
<td>26 (43)</td>
</tr>
<tr>
<td>– Diabetes</td>
<td>8 (16)</td>
<td>7 (14)</td>
<td>8 (13)</td>
</tr>
<tr>
<td>– Hypertension</td>
<td>18 (36)</td>
<td>25 (50)</td>
<td>23 (38)</td>
</tr>
<tr>
<td>– Smoking</td>
<td>22 (44)</td>
<td>13 (26)</td>
<td>25 (42)</td>
</tr>
<tr>
<td>Medication:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Oral nitrates</td>
<td>16 (32)</td>
<td>17 (34)</td>
<td>11 (18)</td>
</tr>
<tr>
<td>– Ca antagonists</td>
<td>29 (58)</td>
<td>17 (34)</td>
<td>14 (23)</td>
</tr>
<tr>
<td>– β-blockers</td>
<td>25 (50)</td>
<td>27 (54)</td>
<td>14 (23)</td>
</tr>
<tr>
<td>– Aspirin</td>
<td>36 (72)</td>
<td>23 (46)</td>
<td>14 (23)</td>
</tr>
<tr>
<td>Time from onset to admission (min)</td>
<td>287±210</td>
<td>286±211</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) CAD, coronary artery disease;
\(^b\) CABG, coronary artery bypass grafting.

No significant difference was observed between the three groups for both TAT and F\(_{1+2}\) values, although median TAT levels were slightly higher in patients with AMI than in patients with SA or UA (Table 2). In contrast, a statistically significant difference was seen in FPA levels between SA and UA (p=0.0015), between UA and AMI (p=0.0013), and between SA and AMI (p<0.0001) (Fig. 1). Due to assay error, FPA values from two patients of the SA group and one patient from the UA group were excluded. In addition, one patient with SA had an unexplained high plasma FPA level of 61.3 μg/l, with levels of TAT and F\(_{1+2}\) of 4.8 μg/l and 1.02 nmol/l, respectively.

3.3. Proinflammatory activity

Plasma IL-6 concentrations were significantly different between SA and UA (p=0.002), SA and AMI (p<0.001) and between UA and AMI (p=0.001), as depicted in Fig. 2. Median levels were 1.62 pg/ml (range 0.44–6.4 pg/ml) in patients with SA, 2.35 pg/ml (0.76–10.41 pg/ml) in patients with UA and 4.65 pg/ml (0.57–17.57 pg/ml) in patients with AMI.

Plasma IL-8 levels were not different between patients with SA and UA (Table 2).

3.4. Hemostatic and inflammatory parameters as markers of acute coronary syndromes

Neither TAT, F\(_{1+2}\) nor IL-8 levels distinguished patients...
with acute ischemic episodes (UA and AMI) from control patients (SA). In contrast, as can be seen in the ROC curves of Fig. 3a, both IL-6 and FPA had equal discriminative capacity.

The logistic regression model for the combination of IL-6 and FPA resulted in $\beta_1 = 0.68$, $\beta_2 = 0.034$, and a constant of $-1.31$ ($-2 \log\text{-likelihood} = 148.1$). The combined model differed significantly from the two models of the independent tests (IL-6, $p < 0.0001$; FPA, $p = 0.019$).

In Fig. 3b, the ROC curves for IL-6, FPA and the combination of IL-6 and FPA, calculated according to the logistic regression model, are depicted. The areas under the ROC curves for IL-6 and FPA were 0.79 and 0.75, respectively. For the combination of IL-6 and FPA, the AUC was 0.82. The areas under the three ROC curves were not statistically different, although a trend towards a larger area for the combination of the two markers was seen (IL-6 vs. combination, $p = 0.10$; FPA vs. combination, $p = 0.13$). In Fig. 4, the independent predictive value of IL-6 and FPA is visualized by the presence of two
subpopulations of patients. Thus, patients with elevated plasma levels of FPA and normal IL-6 levels, and patients with increased IL-6 concentrations and normal FPA values were observed. The mean time from onset of symptoms until hospital admission was not different between patients with elevated levels of FPA (290 min) and patients with elevated IL-6 levels (300 min) ($p=0.34$).

4. Discussion

Both the hemostatic and inflammatory system appear to be involved in the acute phase of ischemic coronary syndromes. The individual contributions and interrelationships between hemostatic changes and inflammatory components in the pathophysiology of acute coronary syndromes, however, are at present unknown. In this study, we investigated the possible relationship between the pro-inflammatory cytokines (IL-6 and IL-8) and coagulation system activity (TAT, F$_{1+2}$, FPA) in patients with unstable angina and acute myocardial infarction, compared with a control population consisting of patients with stable angina. To this end, we obtained carefully timed blood samples within 12 h after the onset of symptoms. In addition, patients with UA had diagnostic ST-segment or T-wave changes on admission, as a sign of ongoing instability. We demonstrated a significant difference in levels of FPA and IL-6 in patients with UA and AMI compared to patients with SA, and we showed with ROC analysis that FPA and IL-6 had similar discriminatory capacities for distinguishing stable (SA) from unstable (UA+AMI) patients. However, FPA and IL-6 did not show simultaneous elevation, but were elevated in different patients. TAT, F$_{1+2}$ and IL-8 were not different between the three groups.

Of the coagulation markers measured in this study, only FPA levels were significantly different between the three groups, with median values of 6.2 µg/l in patients with UA and 10.9 µg/l in patients suffering from an AMI, compared to 3.8 µg/l in patients with SA. The ability of FPA to discriminate between patients with stable and unstable angina has been investigated previously. Whereas some investigators reported a significant difference in FPA levels between stable and unstable disease [5,6], others could not confirm these findings [17].

We could not demonstrate a significant difference in plasma levels of TAT complexes and F$_{1+2}$ between SA, UA and AMI. These results confirm the results of an earlier study by Jude et al. [18], who also failed to demonstrate significant differences in F$_{1+2}$ values between UA and AMI compared with SA, but are in contrast with those of Merlini et al. [2] who reported significantly elevated levels of both F$_{1+2}$ and FPA on admission in patients with UA and myocardial infarction. In our study, patients were eligible if they presented within 12 h after the onset of symptoms. In contrast, Merlini et al. [2] included patients within 6 h after the onset of symptoms. The shorter interval between the onset of chest pain and hospital admission may account for the finding of significantly elevated levels of F$_{1+2}$ by these authors, compared to our study, although the mean interval between the
onset of symptoms and blood sampling was less than 6 h (286 min) in our study as well. Discrepant values of FPA, F_{1+2} and TAT have been observed in earlier studies in patients with cardiovascular disease. In contrast with the original concept of the “pre-thrombotic state”, which suggested that upstream markers such as F_{1+2} or TAT would be elevated at an earlier stage than downstream markers such as FPA in patients at risk for thrombosis, more recent studies indicate that FPA is a marker that is elevated at least as early as the upstream activation indices.

In addition to hemostatic parameters, we measured the extent of simultaneous inflammatory activity in an attempt to associate inflammation and coagulation activation in acute coronary syndromes, conditions which have been shown to be closely linked in human and animal models of inflammation [19–21]. IL-6 levels were significantly higher in patients with the acute phase of coronary artery disease than in patients with SA. This is in accordance with a study by Biasucci et al. [7], who demonstrated elevated levels of IL-6 in a substantial proportion of patients with UA. In contrast to the report by Kanda et al. [13], in our study, IL-8 levels were not significantly different between stable and unstable coronary artery disease. The lack of a difference in plasma levels of IL-8 may be due to binding of IL-8 to the chemokine receptor on the erythrocyte, as we have recently shown in patients with AMI [22]. Unfortunately, measuring erythrocyte-bound IL-8 was not possible in the present study.

Significant differences in FPA and IL-6 levels between UA and AMI patients were found. The pathogeneses of UA and AMI are thought to be similar, with the rupture of an unstable plaque and subsequent thrombus formation, and the two conditions may represent different ends of the spectrum. However, the severity of atherosclerosis or plaque burden, area at risk or ischemic burden and size of the thrombus and, possibly, the subsequent elevations of FPA or IL-6 were not assessed in our study. These require immediate coronary angiography on admission, which was not performed.

The present study does not provide evidence of a close correlation between cytokine release and coagulation activity in acute coronary syndromes. As is shown in Fig. 4, most patients with UA or AMI had either elevated FPA levels or elevated levels of IL-6. The absence of a simultaneous elevation of IL-6 and FPA levels confirms a recent study, in which it was shown that, in patients with unstable angina, activation of the coagulation system occurs independently of an inflammatory reaction [4]. Moreover, Biasucci et al. [3] demonstrated that, in patients with unstable angina, episodes of thrombin production were not necessarily associated with ischemic episodes and, conversely, some ischemic episodes were not associated with evidence of thrombin generation.

Because of the much shorter half-life of FPA (3–5 min) [6] compared to IL-6 (4.2 h) [23], on admission, a previous activation of the coagulation system may have become undetectable, whereas IL-6 levels were still elevated. The time from onset of symptoms until hospital admission, however, was not different between patients with elevated FPA levels and patients with elevated IL-6 levels and, therefore, cannot explain these findings. In addition, considering the rapid clearance of FPA from the plasma, increased FPA levels on admission may be the result of an ongoing activation of the hemostatic system and repeated ischemic episodes. Low IL-6 levels in these patients may indicate that those ischemic episodes did not induce a measurable inflammatory response. One may speculate that episodes of unstable coronary artery disease are initiated either by activation of the coagulation system or by inflammatory activity. However, a dissociation in time between cytokine release and coagulation activation, or individual differences in IL-6 responsiveness, cannot be excluded.

Interestingly, ROC analysis showed that the inflammatory marker IL-6 and the hemostatic marker FPA had equivalent discriminative power for the detection of ischemic coronary syndromes. In addition, in a logistic regression model, it was demonstrated that IL-6 and FPA had independent predictive value. Moreover, a slightly higher diagnostic efficiency was achieved by combining IL-6 and FPA levels.

In conclusion, our study shows that plasma levels of TAT, F_{1+2} and IL-8 are not different between patients with stable and patients with unstable coronary artery disease. In contrast, IL-6 and FPA levels are significantly higher in patients with UA and AMI, than in patients with SA. In addition, within a population of patients with unstable coronary artery disease, hemostatic and inflammatory markers can be elevated independently.

References

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