The extrinsic coagulation pathway in coronary artery disease and endotoxemia
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The value of hemostatic markers in the triage of patients with chest pain presenting with a normal or non-diagnostic electrocardiogram

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Abstract

Background

The triage of patients presenting with chest pain to the emergency department (ED) is a challenge for physicians, especially in cases with an initial non-diagnostic electrocardiogram (ECG). As thrombus formation after coronary plaque disruption plays a major role in patients with an acute coronary syndrome (ACS), measurement of hemostatic markers could provide an important diagnostic tool, particularly in those ACS cases without evidence of myocardial injury.

Methods

We measured plasma levels of coagulation and fibrinolytic markers on admission in patients with chest pain presenting with a normal or non-diagnostic ECG to the ED within 6 hours after symptoms onset. Patients were divided in cardiac Troponin T (cTnT) negative (cTnT<0.06 μg/L, N=33) or positive (cTnT≥0.06 μg/L, N=65) ACS cases and control subjects (N=62) without a history of ACS and who presented with non-cardiac chest pain.

Results

There was no difference in the levels of prothrombin fragment F₁⁺₂, thrombin-antithrombin (TAT) complexes, soluble tissue factor (TF), and tissue factor pathway inhibitor (TFPI) activity between cTnT negative or positive ACS cases and controls. Elevated levels of plasminogen activator inhibitor (PAI) were observed in cTnT negative and positive ACS cases versus control subjects (72.3 and 57.8 vs 44.2 ng/ml, respectively), which was statistically significant for cTnT negative ACS cases compared to controls (p=0.014). The positive (PPV) and negative (NPV) predictive values to detect ACS cases by these markers varied between 68% and 75% and between 40% and 43%, respectively. To detect the subgroup with cTnT negative ACS, only TFPI and PAI showed diagnostic utility, however, the PPVs were low at 43% and 55%, respectively.

Conclusion

The measurement of F₁⁺₂, TAT complexes, soluble TF, TFPI activity, and PAI in plasma did not contribute to the triage of patients presenting with chest pain and a normal or non-diagnostic ECG.
Introduction
Of the patients presenting with chest pain at the emergency department (ED), the early diagnosis of acute coronary syndromes (ACS) is a major challenge for physicians. In addition to medical history and physical examination, the electrocardiogram (ECG) is critical for the initial evaluation of these patients. However, in 50%-80% of the cases, the ECG is normal or non-diagnostic at presentation, which makes the early differentiation from non-cardiac causes of chest pain difficult. Currently, biochemical cardiac markers have become an important diagnostic tool for ACS. Moreover, elevations in myocardial injury markers, such as cardiac Troponin T (cTnT) and I, are associated with early and late recurrent adverse cardiac events. Serial assessment of cardiac markers, including troponins, creatine kinase-MB isoenzyme and myoglobin, results in a high sensitivity to detect myocardial damage, but the absence of evidence of myocardial damage does not exclude ACS. Therefore, novel early markers of ACS are needed.

The main cause of ACS is atherosclerotic plaque disruption with superimposed arterial thrombus formation, and tissue factor (TF) induced thrombin generation plays a pivotal role in this process. Significantly higher levels of circulating TF, that contains procoagulant activity, free and total tissue factor pathway inhibitor-antigen (TFPI, the physiologic inhibitor of TF), and TFPI-activity, were found in patients with ACS compared to patients with stable angina and healthy control subjects. Conflicting results, however, have been reported about thrombin generation markers as prothrombin fragment F$_{1+2}$ and thrombin-antithrombin (TAT) complexes, which showed to be significantly increased, while others observed comparable or even reduced levels in patients with unstable angina or myocardial infarction compared with stable angina patients or healthy individuals. In all of these studies, patients with ACS presented with characteristic changes on the ECG, whereas the diagnostic value of hemostatic markers may be useful in the triage of patients presenting with a non-diagnostic ECG.

The purpose of this study was to evaluate the diagnostic value of coagulation markers (i.e. markers of thrombin generation (F$_{1+2}$ and TAT complexes), soluble TF, and TFPI activity), and a fibrinolytic marker (plasminogen activator inhibitor (PAI)) for the early identification of ACS in patients presenting to the ED with chest pain and a normal or non-diagnostic ECG.
Methods
Patients
Study patients were selected from a consecutive, observational cohort of patients who were admitted to the ED of one large teaching hospital and two academic hospitals. All patients suffered from typical chest pain suggestive of myocardial ischemia that started within 6 hours of presentation and without electrocardiographic signs, at presentation, that are typical of acute myocardial infarction or ischemia. Exclusion criteria were severe skeletal muscle damage or trauma, cardiac resuscitation, known or suspected thromboembolic disease, current use of oral anticoagulation, and infectious disease or signs of inflammation. All patients were observed at the ED for ≤24 hours before discharge or hospital admission. The study population of the present study consisted of cases with an ACS and control subjects. The cases were divided into two groups: group 1, which included cases with recurrent chest pain, a negative cTnT (defined as a peak cTnT < 0.06 μg/L in two serial measurements), and dynamic ST-segment or T-wave changes on the ECG during observation; and group 2, which included cases with recurrent chest pain and a positive cTnT (defined as a peak cTnT ≥ 0.06 μg/L in two serial measurements). The control subjects were patients without a history of cardiovascular disease, who were considered to have chest pain of non-cardiac origin, and who were event free during subsequent 6 months of follow-up. The study protocol was approved by the institutional review boards, and all patients gave informed consent.

Blood sampling and assays
On admission to the ED, venous blood samples were taken from each subject, and collected in citrate containing tubes for the measurements of hemostatic markers, and in another tube without additional substrates for the measurement of cTnT. At 12 hours after the onset of symptoms, an additional blood sample was collected from all study patients for the second measurement of cTnT. All blood samples were centrifuged for 15 minutes at 1500g immediately after collection. Plasma was stored at -70 °C until assayed. F$_{1-2}$ and cTnT levels were assessed using commercial enzyme-linked immunosorbent assays (ELISAs, Dade Behring, Marburg, Germany and Roche Diagnostics, Almere, the Netherlands, respectively). Plasma levels of TAT complexes and PAI were also quantified by ELISA as previously described. Soluble TF concentrations were determined by ELISA using specific monoclonal antibodies raised against recombinant TF (Central Laboratory of the Netherlands Red Cross Blood Transfusion, Amsterdam,
Triage of patients with chest pain and a non-diagnostic ECG using hemostatic markers

The Netherlands). TFPI activity was measured on a Behring Coagulation System (Dade Behring, Marburg, Germany) according to a method described by Sandset et al.28

**Statistical analysis**

Descriptive statistics included mean values and standard deviation (SD), or median values and interquartile ranges (IQR), for outcomes with or without a normal distribution, respectively. Inter-group comparisons between the control group and each ACS case group were tested for all study measurements. Comparison of baseline characteristics was by χ² analysis for categorical variables and either the unpaired Student’s t test or Mann-Whitney U test for continuous variables where appropriate. The comparisons of the hemostatic markers were performed by Kruskall-Wallis H tests and where there was a statistically significant result, subsequent comparisons between the control and both case groups were made using a Mann-Whitney U test. Receiver operator characteristic (ROC) curves were constructed for the sensitivities and specificities of the hemostatic markers for the detection of ACS (i.e. cTnT negative or positive ACS). Additional ROC curves were constructed to specifically detect cTnT positive ACS or cTnT negative ACS. The positive (PPVs) and negative predictive values (NPVs) were calculated from the section of the ROC curve with the optimal balance between sensitivity and specificity. Statistical significance was defined as p<0.05.

**Results**

**Baseline characteristics**

A total of 160 patients were included in this study: 62 controls and 98 cases divided in two groups (group 1: 33 cases with cTnT negative ACS, and group 2: 65 cases with cTnT positive ACS). Baseline characteristics are summarized in Table 1. Compared to controls who did not have a history of cardiovascular disease, both case groups had more often a history of myocardial infarction, coronary angioplasty and coronary bypass grafting, which was statistically significant for all three variables except for the number of previous coronary bypass grafting in the cTnT positive ACS case group. Both case groups also showed a trend to a higher number of patients with risk factors for coronary atherosclerosis, compared to the control group. However, this was only statistically significant for a history of hypertension. The median duration of chest pain at presentation to the ED was approximately 2 hours for all three groups.
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Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (N=62)</th>
<th>cTnT negative ACS (cTnT&lt;0.06; N=33)</th>
<th>cTnT positive ACS (cTnT≥0.06; N=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y); mean ± SD</td>
<td>60 ± 10</td>
<td>60 ± 9</td>
<td>63 ± 12</td>
</tr>
<tr>
<td>Male gender: N (% )</td>
<td>45 (73)</td>
<td>25 (76)</td>
<td>45 (69)</td>
</tr>
<tr>
<td>Previous MI: N (% )</td>
<td>0 (0)</td>
<td>7 (21) †</td>
<td>12 (19) †</td>
</tr>
<tr>
<td>Previous PCI: N (% )</td>
<td>0 (0)</td>
<td>10 (30) †</td>
<td>6 (9) †</td>
</tr>
<tr>
<td>Previous CABG: N (% )</td>
<td>0 (0)</td>
<td>4 (12) †</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Hypertension: N (%)</td>
<td>15 (24)</td>
<td>15 (46) †</td>
<td>30 (46) †</td>
</tr>
<tr>
<td>Hypercholesterolemia: N (%)</td>
<td>14 (23)</td>
<td>13 (39)</td>
<td>19 (29)</td>
</tr>
<tr>
<td>Diabetes: N (%)</td>
<td>5 (8)</td>
<td>7 (21)</td>
<td>11 (17)</td>
</tr>
<tr>
<td>Current smoking: N (%)</td>
<td>19 (31)</td>
<td>9 (27)</td>
<td>18 (28)</td>
</tr>
<tr>
<td>Family history: N (%)</td>
<td>21 (34)</td>
<td>16 (49)</td>
<td>33 (51)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L): mean ± SD</td>
<td>5.6 ± 1.1</td>
<td>5.2 ± 0.8</td>
<td>5.7 ± 1.1</td>
</tr>
<tr>
<td>Duration of chest pain (min): median (IQR)</td>
<td>123 (88-210)</td>
<td>120 (74-180)</td>
<td>125 (75-240)</td>
</tr>
</tbody>
</table>

†p value vs controls <0.05. cTnT=cardiac troponin T (μg/L). ACS=acute coronary syndrome. SD=standard deviation. MI=myocardial infarction. PCI=percutaneous coronary intervention. CABG=coronary artery bypass grafting. IQR=interquartile range.

Plasma levels of hemostatic markers

$F_{1+2}$ and TAT complexes

Plasma levels of $F_{1+2}$ and TAT complexes were slightly higher in cTnT positive ACS cases compared to controls (Figure 1, median (IQR) levels of $F_{1+2}$: 0.9 (0.7-1.3) vs 0.8 (0.6-1.1) nmol/L; median (IQR) levels of TAT: 2.3 (1.0-7.6) vs 1.0 (<1.0-4.8) ng/ml). Plasma levels in the cTnT negative ACS case group were equal to those found in the control group.

![Figure 1. Median (+ 75th percentile) plasma levels of prothrombin fragment $F_{1+2}$ (Panel A) and thrombin-antithrombin (TAT) complexes (Panel B) of controls and cTnT negative and positive ACS cases at presentation to the emergency department. cTnT=cardiac Troponin T (μg/L). ACS=acute coronary syndrome.](image-url)
Soluble TF and TFPI activity

There was no difference in TF plasma levels and TFPI activity between cases and controls (Figure 2). Among the three study groups, median soluble TF range was from 43.5 to 49.2 pg/ml, whereas TFPI activity range was between 110.5 and 120.0 U/dl.

![Graph A](image1.png)  
**Figure 2.** Median (+ 75th percentile) plasma levels of tissue factor (TF, Panel A) and tissue factor pathway inhibitor (TFPI) activity (Panel B) of controls and cTnT negative and positive ACS cases at presentation to the emergency department. cTnT=cardiac Troponin T (µg/L). ACS=acute coronary syndrome.

PAI

As shown in Figure 3, significantly higher PAI plasma levels were observed in the cTnT negative ACS cases compared to controls (median (IQR) levels: 72.3 (44.9-131.7) vs 44.2 (30.2-83.1) ng/ml, p=0.014). The cTnT positive ACS cases also showed higher PAI levels compared to controls, however, it did not reach statistical significance (median (IQR) levels: 57.8 (35.5-107.2) vs 44.2 (30.2-83.1) ng/ml, p=0.1).

![Graph B](image2.png)  
**Figure 3.** Median (+ 75th percentile) plasma levels of plasminogen activator inhibitor (PAI) of controls and cTnT negative and positive ACS cases at presentation to the emergency department. cTnT=cardiac Troponin T (µg/L). ACS=acute coronary syndrome.
Diagnostic utility of hemostatic markers

The diagnostic utility of the hemostatic markers to detect patients suffering form ACS or to identify a subgroup of cTnT positive ACS cases is shown in Table 2. Soluble TF did not have any diagnostic value as the area under the ROC curves was below 0.5 (area under the curves: 0.435 and 0.452 for detecting ACS at large and the subgroup with cTnT positive ACS, respectively). The cut-off values presented in Table 2 correspond to the points of the ROC curves showing the best balance between sensitivity and specificity. Except for PAI antigen, each marker had the same optimal cutpoint for detecting ACS at large or the subgroup with cTnT positive ACS. The proportions of study patients with plasma levels above the optimal cutpoint varied between 23% and 27% for the different markers.

As shown in Table 2, the PPVs and NPVs to identify cases with an ACS were comparable for all 4 markers varying between 68% and 75%, and between 40% and 43%, respectively. To detect cases with a cTnT positive ACS, F1+2 demonstrated a better accuracy compared to the other markers, however, its PPV and NPV were only 62% and 66%, respectively.

After exclusion of cTnT positive ACS cases, only TFPI and PAI demonstrated diagnostic value to detect cTnT negative ACS (area under the ROC curves (95% confidence interval): 0.57 (0.44-0.70) and 0.65 (0.54-0.77), respectively). At cut-off values of TFPI>140 U/dl and of PAI>110 ng/ml, the sensitivity, specificity, PPV,

<table>
<thead>
<tr>
<th>Cutpoint hemostatic marker</th>
<th>Detecting ACS at large</th>
<th>Area under the ROC curve</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1+2 &gt; 1.1 nmol/L</td>
<td>0.53 (0.44-0.63)</td>
<td>31 (22-40)</td>
<td>80 (70-90)</td>
<td>71 (57-85)</td>
<td>42 (33-51)</td>
<td></td>
</tr>
<tr>
<td>TAT complexes &gt; 6.0 ng/ml</td>
<td>0.52 (0.42-0.61)</td>
<td>27 (18-36)</td>
<td>80 (70-90)</td>
<td>68 (53-83)</td>
<td>40 (31-49)</td>
<td></td>
</tr>
<tr>
<td>TFPI activity &gt; 140 U/dl</td>
<td>0.57 (0.48-0.66)</td>
<td>29 (20-38)</td>
<td>79 (69-89)</td>
<td>68 (54-82)</td>
<td>42 (33-51)</td>
<td></td>
</tr>
<tr>
<td>PAI &gt; 110 ng/ml</td>
<td>0.61 (0.52-0.70)</td>
<td>28 (19-37)</td>
<td>85 (76-94)</td>
<td>75 (61-89)</td>
<td>43 (34-52)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cutpoint hemostatic marker</th>
<th>Detecting cTnT positive ACS</th>
<th>Area under the ROC curve</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1+2 &gt; 1.1 nmol/L</td>
<td>0.62 (0.53-0.71)</td>
<td>40 (28-52)</td>
<td>83 (75-91)</td>
<td>62 (47-77)</td>
<td>66 (57-75)</td>
<td></td>
</tr>
<tr>
<td>TAT complexes &gt; 6.0 ng/ml</td>
<td>0.59 (0.50-0.68)</td>
<td>29 (18-40)</td>
<td>79 (71-87)</td>
<td>50 (34-66)</td>
<td>61 (52-70)</td>
<td></td>
</tr>
<tr>
<td>TFPI activity &gt; 140 U/dl</td>
<td>0.55 (0.46-0.64)</td>
<td>28 (17-39)</td>
<td>76 (67-85)</td>
<td>44 (29-59)</td>
<td>60 (51-69)</td>
<td></td>
</tr>
<tr>
<td>PAI &gt; 95 ng/ml</td>
<td>0.54 (0.45-0.63)</td>
<td>29 (18-40)</td>
<td>76 (67-85)</td>
<td>45 (30-60)</td>
<td>61 (52-70)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as percentages (95% confidence interval). ACS=acute coronary syndrome. cTnT=cardiac Troponin T. ROC=receiver operator characteristic. Sens=sensitivity. Spec=specificity. PPV=positive predictive value. NPV=negative predictive value. F1+2=prothrombin fragment 1-2. TAT=thrombin-antithrombin. TFPI=tissue factor pathway inhibitor. PAI=plasminogen activator inhibitor.
and NPV (with respective 95% confidence intervals) to identify cTnT negative ACS was for TFPI: 31% (15%-47%), 79% (69%-89%), 43% (23%-63%), and 69% (58%-80%), respectively, and for PAI: 34% (18%-50%), 85% (76%-94%), 55% (33%-77%), and 72% (62%-82%), respectively.

Combining the results of markers of thrombin generation, TFPI and PAI, did not improve the diagnostic accuracies.

Discussion

In the present study, we measured coagulation markers (thrombin generation markers ($F_{1+2}$ and TAT complexes), soluble TF, and TFPI activity) and a fibrinolytic marker (PAI) in patients admitted to the ED with chest pain and an initially normal or non-diagnostic ECG, within 6 hours after symptom onset. Except for PAI antigen, there were no significant differences in the plasma levels of these markers between cases with a cTnT negative or positive ACS and control subjects.

A large study of Pope et al. showed that 5.4% of patients with a non-diagnostic ECG who were discharged from the ED were ultimately found to have an ACS\textsuperscript{29}. Serial measurement of specific markers of myocardial necrosis such as creatine kinase-MB isoenzymes and troponins are used as a diagnostic tool. However, an ACS cannot be excluded when these markers are not increased\textsuperscript{8}. Although TF mediated thrombus formation after coronary plaque disruption plays a significant role in the initiation of ACS\textsuperscript{12}, systemic measurement of hemostatic markers did not demonstrate additional diagnostic value to the early triage of our study patients with chest pain.

Our results do not confirm earlier studies showing significantly increased plasma concentrations of TF, TFPI, $F_{1+2}$ and TAT-complexes on admission, in patients with unstable angina or acute myocardial infarction compared to patients with stable angina\textsuperscript{13-15,26,21}. However, in these studies, patients with ACS were included if the ECG demonstrated characteristic changes of the ST-segments or T-waves and were likely at higher risk. It has been demonstrated that among patients with unstable angina, those with reversible ST-segment changes have significantly higher levels of thrombin activity, as measured by fibrinopeptide A levels, compared to those without these changes, and ST changes correlated significantly with the presence of angiographic thrombus\textsuperscript{30,31}. However, this could not be confirmed by Becker et al., who reported no differences in thrombin activity and thrombin generation between
patients with and without ECG changes\textsuperscript{32}. In addition, others failed to show significant differences in plasma levels of $F_{1-2}$ and TAT complexes between patients with acute myocardial infarction, unstable angina and stable angina, despite the characteristic ECG changes presented by patients with an ACS\textsuperscript{22,23}. Thus, symptomatic myocardial ischemia may not primarily be related to acute thrombin generation, but is possibly the result of a combination with platelet aggregation, transient increased vasomotor tone and transient increased myocardial oxygen demand\textsuperscript{33}. Alternatively, local thrombin generation in the coronary arteries may be insufficiently reflected by systemic plasma levels.

We observed elevated plasma levels of PAI antigen in both ACS case groups compared to controls, but this was only significant in cases with a cTnT negative ACS. An important source of PAI during atherosclerotic thrombus formation may be the secretion by endothelial cells and activated platelets\textsuperscript{34}. However, increased PAI plasma levels are also associated with common risk factors for coronary artery disease such as hypertension and particularly diabetes\textsuperscript{34}. These two factors were more prevalent in both case groups, in particular in the cTnT negative ACS case group, and this contributed to the enhanced PAI levels in these groups compared to controls.

Patients suffering from cTnT positive ACS previously demonstrated a significantly increased coagulation activation compared to patients with cTnT negative unstable angina\textsuperscript{35,36}. However, we could not confirm this in our study population. We observed no differences in plasma levels of the hemostatic markers between cases with cTnT positive and negative ACS. Despite evidence of myocardial necrosis, the extent of coagulation activation may be limited in cases with a cTnT positive ACS, and whose ECG does not show characteristic changes of myocardial ischemia. This was reflected by the poor diagnostic performance of the hemostatic markers to identify these cases. Prothrombin fragment $F_{1-2}$ showed the best diagnostic utility, but the PPV and NPV were only 62\% and 66\%, respectively. Since these patients can be identified after serial measurement of cardiac serum markers, it remains a difficult task to detect patients suffering from an ACS, without evidence of myocardial damage and presenting with a non-diagnostic ECG. In the present study, after excluding the cTnT positive ACS cases from the analyses, only TFPI and PAI demonstrated diagnostic value to detect cTnT negative ACS cases, but with low PPVs of 43\% and 55\%, respectively. Based on these markers, as much as 66\%-69\% of the patients with cTnT negative ACS would still not have been detected. Thus in our study population
of low risk chest pain patients, the utility of hemostatic markers showed limited diagnostic value to identify patients with either a cTnT positive or negative ACS.

**Study limitations**
As shown in Figure 1 and 3, the assays measuring TAT complexes and PAI antigen demonstrated marked variability in each group. We could not identify clinical characteristics that appeared to be responsible to these wide ranges, including the sampling times for PAI, since significantly higher PAI levels have been found in the morning than in the evening both in control subjects and patients with ACS. However, we cannot exclude the possibility that other factors such as platelet activation during venipuncture have resulted in enhanced PAI levels. In addition, as patients with unstable angina have been shown to develop frequent bursts of coagulation activation, the short plasma half-life of TAT and PAI of 5 and 8-10 minutes, respectively, compared to that of $F_{1-2}$ (90 minutes), may also have contributed to the variability in both ACS case groups.

**Conclusions**
The measurement in plasma of thrombin generation markers ($F_{1-2}$ and TAT complexes), soluble TF, TFPI activity, and PAI antigen, did not contribute to the triage of patients presenting with chest pain and a normal or non-diagnostic ECG in the ED. Although plaque disruption with thrombus formation is the most common underlying pathogenic mechanism during an ACS, in this patient population in whom there is a need for additional markers of ACS, this does not lead to elevation of systemic plasma levels that are clinically useful for the triage of low risk chest pain patients.

**Acknowledgements**
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**References**


