Introduction

Acute myocardial infarction (AMI) is a major cause of morbidity and mortality worldwide. It is the result of partial or complete occlusion of the coronary arteries due to coronary thrombus formation, impairing myocardial blood supply. A hypercoagulable state, characterized by activation of the coagulation system, is detectable in patients with AMI [1–3]. Furthermore, within atherosclerotic lesions, coagulation factors, including contact factor components, are abundantly present [4]. This way, hypercoagulability in blood and in atherosclerotic lesions may have impact on the course of atherosclerosis as well as the risk of atherothrombotic complications.

The role of the contact activation system of coagulation in the development and progression of coronary artery disease (CAD) is still unclear. In animal studies, deficiency in coagulation factor XI (FXI) or FXII is associated with a decreased risk for arterial thrombosis, however, the results from clinical studies are not straightforward. Several studies found that high levels of FXI or activated FXI (FXIa) are associated with an increased risk of CAD [2,5,6], however, in studies focusing only on women this association is less clear [7–9]. Furthermore, FXI deficiency does not protect against AMI [10]. The association between FXII and CAD is complex, with a different association depending on whether zymogen or enzyme levels were measured. Low levels of FXII were found to be a risk factor for AMI, coronary heart disease (CHD) and all-cause mortality [5,11,12], high levels of FXIIa and low levels of FXIIa in complex with its main natural inhibitor C1-esterase inhibitor (FXIIa-C1INH) were associated with an increased risk of CHD [13–18]. However, other studies that measured FXIIa, FXIIa-C1INH or FXIIa-C1INH did not confirm an association with CHD [6,8,12,19,20]. In all these studies the levels of FXI or FXII were measured at one single time point. We set up a study to determine the activation of the contact system during the acute
phase as well as during follow-up in patients with a first AMI and used these data to determine whether contact activation could be used as a marker for the occurrence of a recurrent thrombotic event.

Materials and Methods

Study Design

The study design has been described previously [21]. Consecutive patients with a first AMI were included. Patients were included if they met the following inclusion criteria: chest pain lasting longer than 30 min but not exceeding 24 h, ST-segment elevation > 1 mm on electrocardiography and biochemical evidence of myocardial necrosis. Exclusion criteria were a history of AMI or stroke and present use of oral anticoagulants. Blood samples were drawn on admission and before administration of low-molecular-weight-heparin (LMWH) or any other intervention and repeated after 3 months and 6 months. To rule out the use of LMWH before blood sampling, anti-Xa levels were determined in all baseline samples. Only samples with undetectable anti-Xa (≤0.05 U ml⁻¹) were considered to be free of LMWH and only these samples were included in the analysis.

The clinical outcome was recorded 3 months, 6 months and 12 months after inclusion. The combined end point comprised cardiovascular death, recurrent MI, a second coronary intervention (PCI) or coronary artery bypass grafting (CABG) and ischemic stroke. The study protocol was approved by the Medical Ethics Review Committee of the Maastricht University Medical Center, the Netherlands. All patients gave written informed consent. Venous blood was collected in 10 mM EDTA containing 100 μg ml⁻¹ soybean trypsin inhibitor (STI) and 20 mM benzamidine for the measurement of enzyme inhibitory complexes and in 5.2% (w/v) citrated tubes for other measurements.

Assays

The levels of FXa, FXIIa and kallikrein in complex with C1-esterase inhibitor (C1INH) and FXa in complex with α₁-antitrypsin (FXa-C1INH, FXIIa-C1INH, kallikrein-C1INH and FXa-AT) were measured in plasma with enzyme-linked immunosorbent assays (ELISAs), as described previously [18]. The detection limits were 0.03 arbitrary units (AU) for all assays and values below the detection limit were set at 0.03 AU. The inter- and intra-assay coefficient variations (CVs) of these assays have been published [18].

The levels of FXIic and FXIIic were determined by one-stage aPTT-based clotting assays, performed on a Sysmex CA-7000 Automated Coagulation Analyzer with reagents obtained from Dade Behring (Liederbach, Germany) and calibrated to WHO standards. D-dimer measurements in platelet-poor plasma were performed using the Ddimer Plus test (Dade Behring Inc., Liederbach, Germany) according to the manufacturer’s instructions. Prothrombin fragment 1.2 (F1.2) was quantified by ELISA according to the manufacturer’s instructions (Dade Behring Inc.). Anti-Xa activity was determined using the Coamaatic Heparin test (Instrumentation Laboratory, Breda, the Netherlands).

Statistical Analysis

The data are expressed as median [interquartile range (IQR)] or as mean (standard deviation (SD)). Differences between two groups were analysed using the Mann–Whitney U test (levels of inhibitory complexes, D-dimer and F1.2) or the Student’s t-test (levels of FXIc and FXIIc), depending on distribution characteristics. Correlations between the enzyme inhibitory complexes were determined using Spearman’s rho correlation. The difference in the levels of the inhibitory complexes between the different time points was determined by the Friedman test, followed by the Dunn’s multiple comparison test. The association between dichotomized levels of enzyme inhibitory complexes and outcome was assessed using Pearson chi-square test, and expressed as corresponding odds ratios (ORs) and 95% confidence intervals (CIs). Results were viewed to be statistically significantly different at p < 0.05. Statistical analyses were performed using IBM SPSS Statistics 20 for Windows (Armonk, New York: IBM Corp.) and Prism for Windows 5.00 (GraphPad Software Inc., San Diego, CA, USA).

Results

Of the 135 patients included in this clinical study, plasma samples of 89 patients on admission were available for the measurement of enzyme inhibitory complexes. In total, 16 patients were excluded because anti-Xa levels were > 0.05 U ml⁻¹. Of 30 patients, the availability of plasma was not sufficient to perform analyses. The baseline characteristics of these 89 patients are represented in Table 1. Of them 14 had a recurrent cardiovascular event during the follow-up period of 1 year. The levels of the enzyme inhibitory complexes on admission did not differ between patients stratified for gender, smoking or the presence of hypertension, diabetes mellitus or hypercholesterolemia and did not correlate with age.

Enzyme-inhibitory Complexes

From 70 patients, complete sets of plasma samples from the three time points (on admission and at 3 months and 6 months after the acute event) were available to measure the levels of the enzyme inhibitory complexes. Fig. 1 shows the levels of the enzyme inhibitory complexes on admission and during the follow-up period. For most patients, the levels of FXIa-C1INH were highest on admission and declined during follow-up. There was a statistically significant reduction in FXIa-C1INH complex levels with 55.7% of the patients at 3 months and 70% at 6 months showing a decline in this inhibitor complex compared with levels on admission. The median level of FXIa-C1INH declined by 7.2% [IQR: -19.9% - 25.9%] and 9.5% [IQR: -7.1% - 22.1%] at 3 and 6 months, respectively. The levels of FXIa-AT, FXIIa-C1INH and kallikrein-C1INH did not change significantly over time.

Because of the wide distribution of the data we were interested to determine the correlation for each enzyme-inhibitor complex, comparing different time points. The levels of FXIa-C1INH on admission, correlated well with the levels at 3 months and at 6 months (Spearman’s rho: 0.84 and 0.88 p < 0.001, respectively). The same was true for the levels of FXIa-C1INH (Spearman’s rho: 0.78 and 0.67 p < 0.001, respectively) and FXIa-AT (Spearman’s rho: 0.78 and 0.77 p < 0.001, respectively). Since only few samples had levels above the detection limit for kallikrein-C1INH, we did not perform correlation analyses for this enzyme inhibitor complex. These high correlations indicate that patients with relatively high or low level of an enzyme inhibitor complex at one time point, will most likely remain relatively high or low at a later time point.

Recurrent Events

Table 2 shows the differences in the levels of FXIc, FXIIc, D-dimer, F1.2 and the enzyme inhibitory complexes on admission between patients that developed a recurrent event during follow-up and those that did not. The levels of D-dimer and F1.2 were higher in the
patients that had a recurrent event during follow-up. The levels of the other factors did not differ between the groups. Fig. 2 shows the distribution of FXIa-C1INH, FXIa-AT, FXIIa-C1INH and kallikrein-C1INH among patients that had an event during follow-up and those that did not. There was no significant difference between the groups. To get more insight into the predictive value of FXIa-C1INH, FXIa-AT and FXIIa-C1INH, we also determined the odds ratios (OR) for a recurrent event after dichotomizing the data to low levels (< median of the total group) versus high levels (≥ median of the total group) for FXIa-C1INH and FXIa-AT and low levels (below detection limit) and high levels (above detection limit) for FXIIa-C1INH. The OR for high compared to low levels of these enzyme inhibitory complexes were: 0.49 (95% CI: 0.15 – 1.6), 1.85 (95% CI: 0.57 – 6.04) and 0.99 (95% CI: 0.30 – 3.25) for FXIa-C1INH, FXIa-AT and FXIIa-C1INH, respectively. Only one sample had kallikrein-C1INH levels above the detection limit, therefore no OR was determined.

Discussion

Although experimental studies show an involvement of the contact system of coagulation in arterial thrombosis, atherosclerosis and ischemic stroke, very little is known about the role of the contact activation system in humans. One problem in the human studies is inconsistency in data in relation to outcomes, due to heterogeneity in patient populations as well as in assays that were performed. Another issue is that in previous clinical studies in all cases contact activation indices were determined at one point in time, potentially missing patterns of changes in time that may distinguish acute phase responses from constitutive activity levels.

In this study, we investigated the activation of the contact system of coagulation during the acute phase, and 3 and 6 months after an AMI. Furthermore, we explored whether activation of the contact system during the acute phase could be used to predict clinical outcome.

Table 2

Levels of FXIc, FXIIc, D-dimer, F1.2, FXIa-C1INH, FXIa-AT, FXIIa-C1INH and kallikrein-C1INH of acute myocardial infarction patients stratified for the occurrence of a recurrent event during a follow-up period of 1 year.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Recurrent event</th>
<th>No recurrent event</th>
<th>p-value (recurrent compared to no recurrent event)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXIc (U dl⁻¹)</td>
<td>119 (17)</td>
<td>117 (25)</td>
<td>119 (15)</td>
<td>0.68</td>
</tr>
<tr>
<td>FXIIc (U dl⁻¹)</td>
<td>101 (21)</td>
<td>101 (15)</td>
<td>101 (22)</td>
<td>0.99</td>
</tr>
<tr>
<td>F1.2 (nmol ml⁻¹)</td>
<td>227 [174 – 319]</td>
<td>314 [207 – 666]</td>
<td>213 [170 – 299]</td>
<td>0.03</td>
</tr>
<tr>
<td>FXIa-C1INH (AU)</td>
<td>0.22 [0.17 – 0.35]</td>
<td>0.20 [0.17 – 0.30]</td>
<td>0.22 [0.17 – 0.41]</td>
<td>0.47</td>
</tr>
<tr>
<td>FXIa-AT (AU)</td>
<td>0.26 [0.23 – 0.31]</td>
<td>0.29 [0.25 – 0.35]</td>
<td>0.26 [0.23 – 0.30]</td>
<td>0.17</td>
</tr>
<tr>
<td>FXIIa-C1INH (AU)</td>
<td>0.03 [0.03 – 0.05]</td>
<td>0.03 [0.03 – 0.06]</td>
<td>0.03 [0.03 – 0.05]</td>
<td>0.92</td>
</tr>
<tr>
<td>Kallikrein-C1INH (AU)</td>
<td>0.03 [0.03 – 0.03]</td>
<td>0.03 [0.03 – 0.03]</td>
<td>0.03 [0.03 – 0.03]</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Levels of FXIc and FXIIc are expressed as mean (standard deviation). The other levels are expressed as median [interquartile range]. A.U: arbitrary units, C1INH: C1-esterase inhibitor.
after a first AMI. Activation of the contact system was measured as the levels of the activated enzymes in complex with their natural inhibitors: FXIa, FXIIa and kallikrein in complex with C1 esterase inhibitor (C1INH) and FXIa in complex with AT. We ensured that samples did not contain any anticoagulant by careful selection at inclusion as well as by screening samples by anti-Xa activity for spurious LMWH presence (data not shown) [21].

We found that the levels of FXIa-C1INH were elevated during the acute event in patients with a first AMI compared to the steady state situation at 3 and 6 months after the AMI. We did not observe this effect for FXIa-AT. Upon activation, C1INH is the main inhibitor of FXIa, but, the half-life of the FXIa-AT complex in vivo is longer than the half-life of FXIa-C1INH: 349 min and 104 min respectively [22]. Because of this, FXIa-AT reflects chronic activation of FXI whereas FXIa-C1INH better reflects the acute phase response [22]. This is illustrated in a previous study, where in 20 patients with either an AMI (blood sampling 7 to 10 days after the event) or unstable angina, the levels of FXIa-AT were significantly elevated but the levels of FXIa-C1INH were comparable to controls [22]. In contrast, Minnema and colleagues showed that patients with an AMI had higher levels of FXIa-C1INH during the acute attack compared to patients with stable angina pectoris. In these patients, the levels of FXIa-AT were not elevated during the acute attack [2]. In contrast to the present study, they did not obtain follow up blood samples, such that the comparison could only be made between those with AMI and patients with either unstable or stable angina [2].

The levels of FXIa-C1INH were not elevated during the acute attack, suggesting that in these patients FXI activation was probably caused by an increase in thrombin generation rather than by FXII activation. Indeed, as reported previously, thrombin generation capacity was increased during AMI. The measurement of the endogenous thrombin potential (ETP) in these patients, showed that the ETP-values were highest during the acute attack and were diminished 3 and 6 months after the event [21].

We observed that higher levels of D-dimer and F1.2 on admission were associated with a recurrent cardiovascular event in the year following the first AMI in this study. This is in agreement with earlier findings. The level of the activation peptide of prothrombin, F1.2, was found to be elevated in patients with ACS and was associated with cardiac mortality and long-term outcome in patients with ACS [23–25]. Elevated levels of D-dimer are observed in patients with ACS [25] and are a predictor for coronary heart disease [26]. Furthermore, elevated levels of D-dimer are predictive of a recurrent coronary events in post infarction patients and in patients with ischemic-type chest pain [27,28]. In our study the levels of the inhibitory complexes on admission were not predictive for the occurrence of a recurrent event in the year after the first AMI. High levels of FXIa-C1INH, FXIa-AT, FXIa-C1INH and kallikrein-C1INH were not a risk factor for the occurrence of a recurrent cardiovascular event during the follow-up period. Possibly, the study was underpowered to demonstrate the predictive value for the enzyme-inhibitory complexes, however, the OR’s were in no way indicative of any risk association. Similarly, in a larger study in patients with chest pain, the levels of FXIa-C1INH, FXIa-AT, FXIa-C1INH and kallikrein-C1INH were also not predictive for clinical outcomes 2 years after admission. This was the case both for patients with TnT-levels ≤ 0.05 ng ml⁻¹ and TnT-levels > 0.05 ng ml⁻¹ on admission [20].

We observed a high correlation between the levels of the enzyme inhibitory complexes on admission and those during follow-up. This indicates that in patients with relatively high levels of enzyme inhibitory complexes, these levels stay relatively high. However, they did not predict a recurrent cardiovascular event in the year after a first AMI. This highlights the fact that in spite of a marked interindividual variation in constitutive levels of contact activation, the link between individual levels of these products and arterial vascular disease remains to be explored.

In conclusion, we observed that during an AMI the levels of FXIa-C1INH were elevated compared to the steady state 3 and 6 months after the AMI. In the absence of evidence of acute FXII activation, the temporary increase may primarily result from thrombin generation and feedback FXI activation. The main contribution of FXII in this regard may be in starting and strengthening (arterial) clot formation [29]. The clinical significance of contact activation in the context of coronary artery disease remains to be demonstrated.

**Conflicts of Interest**

None declared.
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References


