Associations between cardiovascular risk factors, hyper- and hypoagulability
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Chapter 7

Urinary prothrombin fragment 1+2 as a potential diagnostic tool for venous thrombosis and myocardial infarction

Josien van Es, Sara Biere-Rafi, Mohamed Ahdi, Joost CM Meijers, Pieter W Kamphuisen, Victor EA Gerdes

Submitted
ABSTRACT

Introduction Patients with venous-thromboembolism (VTE) and myocardial infarction (MI) have elevated prothrombin fragment 1+2 (F1+2) levels. In patients with postoperative VTE, urinary F1+2 (uF1+2) was higher than in individuals without VTE. We postulated that uF1+2 is elevated in patients with suspected venous or arterial thrombotic events and therefore performed this pilot study in patients with VTE and MI.

Methods In 20 patients with VTE, 20 with MI, and 25 age- and sex-matched healthy controls, F1+2 and D-dimer levels were measured in urine and plasma within 48 hours after diagnosis. In addition, in all subjects renal function was assessed.

Results Compared to controls, patients with VTE had higher levels of both plasma F1+2 (271 versus 160 pmol L\(^{-1}\) (p < 0.05)) and uF1+2 levels (38 versus 28 pmol L\(^{-1}\)), although the latter difference was not significant. Patients with acute MI had the same F1+2 levels as controls in both plasma and urine. Overall, plasma and uF1+2 levels were positively correlated. Differences in urinary F1+2 levels could not be attributed to differences in concentrations of creatinin or albumin in spot urine samples. Overall, D-dimer and F1+2 levels in urine were extremely low in all groups.

Conclusion Although urine F1+2 levels may be associated with postoperative venous thrombosis, we found no clear association in patients with acute VTE or MI.
**INTRODUCTION**

Venous and arterial thromboses are both vascular disorders, in which activated coagulation plays a key role. In the acute phase of both venous and arterial thrombosis, both D-dimer and F1+2 plasma levels are elevated, which reflects thrombin generation. [1-6] Due to its' small molecular size, F1+2 can reliably be measured in the urine by enzyme-linked immunosorbent assay (ELISA). [7,8] Recently, F1+2 levels were assessed in urine samples of patients, prior and three days after a total hip replacement.[9] Interestingly, patients with postoperative venous thromboembolism (VTE), consisting of deep venous thrombosis (DVT) or pulmonary embolism (PE), had higher levels of urinary F1+2 (uF1+2) than those without VTE, whereas low levels of uF1+2 were found in patients who had bleeding complications after surgery.[9] In patients with suspected VTE, the sensitivity of plasma F1+2 ranged widely from 100, 80 to 59% combined with specificity of 11, 51 and 77% respectively. [4,5,10,11] If a close correlation would exist between plasma and uF1+2 levels, uF1+2 could be used as a predictor of venous or arterial thrombosis in clinical or epidemiological studies, or as a test to rule out VTE. [11] The measurement of uF1+2 is a non-invasive and patient friendly manner to evaluate thrombin generation. It is therefore an attractive tool for the diagnostic strategy of VTE and myocardial infarction (MI). We investigated whether, similar to F1+2 in plasma, urinary F1+2 increases in acute VTE and MI patients, compared to healthy controls. We therefore performed a prospective pilot study in patients with documented venous thromboembolism, myocardial infarction and matched control subjects.

**METHODS**

Twenty consecutive both out- and hospitalized patients older than 18 years presenting with an objectively confirmed diagnosis of VTE or MI in the Academic Medical Centre or Slotervaart hospital in Amsterdam, the Netherlands, between August and December 2010 were included. The study protocol was approved by the Medical Ethics Review Committee, and all participants provided written informed consent. At presentation, medical history was obtained through a questionnaire including specific questions about the symptoms, known risk factors for arterial and venous thrombosis, and medication use. In addition body mass index was calculated, since extreme bodyweight is a risk factor for VTE and MI.[12,13] DVT of the legs was diagnosed with ultrasonography and PE was diagnosed with multi-slice CT scan. MI was diagnosed if either one of the following criteria were met: the presence of ECG changes, defined as ST-segment elevation or ST-segment depression or T-wave abnormalities, and/or based on biochemical marker evidence, defined as CKMB (mass,C) levels ≥ 15 ug L^{-1}, and/or Troponin I levels ≥ 0,04 ug L^{-1}.

Healthy controls were gender- and age-matched, 1:1, to the VTE and MI patients. Controls consisted of visitors of the AMC and Slotervaart hospital, and selection was based on the male/ female ratio and age (maximum difference of 5 years) of the cases. MI and VTE were the only
exclusion criteria. Since the age of MI and VTE patients was not completely similar, a total of 25 controls were included instead of 20.

**Sample storage and laboratory analysis**

Within 48 hours after diagnosis, blood samples for F1+2 and D-dimer were drawn and collected in tubes containing 0.109 mol L⁻¹ trisodium citrate. Within one hour after collection, platelet-poor plasma was obtained by two times centrifugation for 15 min at 1500g and 15°C. The plasma was stored in 2-mL cryovials containing 0.5 mL of plasma at -80°C. Simultaneously, spot urine samples were collected and plasma and urine F1+2 levels were determined using a commercially available ELISA (Enzygnost, Siemens healthcare Diagnostics, Marburg, Germany), D-dimer levels were determined with a particle-enhanced immunoturbidimetric assay (Innovance D-Dimer, Siemens Healthcare Diagnostics, Marburg, Germany).

To be able to adjust for the concentration of creatinin and albumin in the spot urine samples and micro-albuminuria, we analyzed microalbumin and creatinin using immunoturbidimetry and spectrofotometry respectively (both P800, Roche diagnostics).

**Statistical analysis**

Results are presented as mean ± standard deviation or median with inter-quartile range (IQR), depending on the observed distribution. The primary objective of this study was to assess the relationship between uF1+2 levels and VTE or MI, which was expressed as odds ratios (ORs) with 95% confidence intervals. Plasma- and urinary F1+2 levels of the controls were non normally distributed, we used the Mann-Whitney U test to compare cases and controls. All statistical analyses were performed in SPSS version 15.0 (SPSS Inc. Chicago, IL, USA).

**RESULTS**

The baseline characteristics of the three study groups are shown in Table 1. Of the patients who were diagnosed with VTE, eight patients had PE and 12 had DVT. Mean age and gender distribution were comparable as the groups were matched by these variables. Also ethnicity, BMI, smoking habits, and urine creatinine and albumin levels were not different.

A significant correlation was found indeed between the F1+2 levels in the plasma and urine (regression coefficient 0.463, p < 0.001). As shown in Table 2, compared to controls, patients with VTE had higher levels of both plasma F1+2 (271 versus 160 pmol L⁻¹, p < 0.05) and plasma D-dimer (5.12 versus 0.38 mg L⁻¹ fibrinogen equivalent units (FEU), p < 0.01). UF1+2 levels in VTE patients were low, but seemed to be higher compared to uF1+2 levels in controls, but this difference did not reach statistically significance (p = 0.11). In MI patients, plasma F1+2 and D-dimer levels were comparable to the control group (p= 0.87 and p= 0.49, respectively) (Table 2). Also uF1+2 levels did not differ between MI patients and control persons. In all subjects, urine levels of D-dimer were not detectable.
To account for concentration of the spot urine samples we calculated uF1+2 / urine creatinine ratios. This yielded similar results (Table 2). Subsequently, we adjusted for (micro) albuminuria by dividing uF1+2 by microalbumin / creatinine ratio. These assessments showed similar results between the three different groups (table 2).

**Table 1.** Baseline characteristics of the two study groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=25)</th>
<th>VTE (n=20)</th>
<th>MI (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years (mean ± SD)</strong></td>
<td>56 ± 18</td>
<td>56 ± 18</td>
<td>59 ± 18</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female n, (%)</td>
<td>9 (36%)</td>
<td>9 (45%)</td>
<td>7 (32%)</td>
</tr>
<tr>
<td>Male, n, (%)</td>
<td>16 (64%)</td>
<td>11 (55%)</td>
<td>13 (59%)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian n, (%)</td>
<td>19 (76%)</td>
<td>17 (85%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>Non-Caucasian n, (%)</td>
<td>6 (24%)</td>
<td>3 (15%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[median (IQR)]</td>
<td>24 (22-29)</td>
<td>28 (26-32)</td>
<td>27 (24-30)</td>
</tr>
<tr>
<td>Smoker, n, (%)</td>
<td>7 (29%)</td>
<td>7 (37%)</td>
<td>5 (23%)</td>
</tr>
</tbody>
</table>

BMI = body mass index, IQR = inter-quartile range, MI = myocardial infarction, SD = standard deviation, VTE = venous thromboembolism

**Table 2.** F1+2 and D-dimer results of the different patients groups and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>VTE</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma F1+2 (pmol • L⁻¹)</strong></td>
<td>160 (120-254)</td>
<td>271 * (201-541)</td>
<td>157 (115-268)</td>
</tr>
<tr>
<td><strong>Urine F1+2 (pmol • L⁻¹)</strong></td>
<td>28 (15-46)</td>
<td>38 (23-71)</td>
<td>25 (16-80)</td>
</tr>
<tr>
<td><strong>Plasma D-Dimer (mg • L⁻¹ FEU)</strong></td>
<td>0.38 (0.19-0.65)</td>
<td>5.12 ** (1.81-8.45)</td>
<td>0.47 (0.28-0.60)</td>
</tr>
<tr>
<td><strong>Urine D-Dimer (mg • L⁻¹ /L FEU)</strong></td>
<td>Undetectably low</td>
<td>Undetectably low</td>
<td>Undetectably low</td>
</tr>
<tr>
<td><strong>Urine creatinin (mmol • L⁻¹)</strong></td>
<td>10.5 (7.6)</td>
<td>11.5 (5.8)</td>
<td>12.1 (9.2)</td>
</tr>
<tr>
<td><strong>Urine microalbumin (mg • L⁻¹)</strong></td>
<td>11 (4-22)</td>
<td>11 (8-18)</td>
<td>14 (2-52)</td>
</tr>
<tr>
<td><strong>u albumin/u creatinin (mg • L⁻¹ /mmol • L⁻¹)</strong></td>
<td>0.57 (0.31-1.22)</td>
<td>0.85 * (0.65-1.88)</td>
<td>0.83 (0.55-2.95)</td>
</tr>
<tr>
<td><strong>u F1+2 / u creatinin (pmol • L⁻¹ /mmol • L⁻¹)</strong></td>
<td>3.1 (2.0-4.2)</td>
<td>3.9 (2.5-7.9)</td>
<td>3.0 (2.0-6.2)</td>
</tr>
<tr>
<td><strong>(u F1+2) x (u creatinin / microalbumin) (pmol) x (mmol • L⁻¹ / mg • L⁻¹)</strong></td>
<td>48.3 (23.3-90.9)</td>
<td>42.2 (22.0-122.5)</td>
<td>28.4 (10.6-54.0)</td>
</tr>
</tbody>
</table>

*p < 0.05 **p < 0.01

FEU = fibrinogen equivalent units, IQR = inter quartile range, MI = myocardial infarction, SD = standard deviation, U = urine, VTE = venous thromboembolism
DISCUSSION

This pilot study confirms that in patients with acute VTE, plasma levels of F1+2 are elevated, but we now show that also F1+2 levels in the urine are increased, but to a lesser extent than in plasma. Furthermore, there was a significant correlation between the F1+2 levels in the urine and plasma. F1+2 levels in both urine and plasma were similar between patients with acute MI and healthy controls.

Several studies have shown that plasma F1+2 levels are increased in patients with MI and VTE. [1,2,10,14,15] A prospective study previously showed that elevated plasma F1+2 levels in patients with cancer also predicted the occurrence of VTE, with a hazard ratio of 2.0 (95% CI, 1.2-3.6). [6] Moreover, also urine F1+2 levels seemed to predict postoperative VTE after orthopedic surgery. [9,16]

In this study, uF1+2 levels in preoperative patients were slightly lower compared to our controls (median 19 versus 28 pmol/L). The group of patients who developed a VTE three days post surgery, had a median uF1+2 level of 127.3 (IQR 19-1200 pmol L⁻¹) which was higher than our VTE patients (38 pmol L⁻¹ (IQR 23-71 pmol L⁻¹). [9] This difference might be due to the orthopedic procedure.

To our knowledge, this is the first study that measured F1+2 levels in urine and plasma in VTE and MI patients compared to healthy controls. These results show that F1+2 levels of the VTE patients are marginally increased in the urine. This was, however, not significantly different compared to healthy controls, probably due to the small size of the study. Although this study was not designed to assess the diagnostic value of uF1+2, the very small differences between the groups together with the very low levels will limit an important role for uF1+2 in the diagnosis of VTE.

Patients with MI had similar plasma and urinary F1+2 levels compared with the controls. Also no differences were found for D-dimer levels. These findings are in contrast to previous studies [2,14,15,17], and might be due to the use of intravenous heparin in the patients with acute MI in the first 48 hours, in contrast to the VTE patients, who were included before anti-coagulants were administered. This might have lowered plasma D-dimer and F1+2 levels, and consequently also the urine levels. However, in a prospective study no clear effect of unfractionated heparin on plasma F1+2 was observed. [18,19]

Results might also been influenced by the concentration of the spot urine samples and microalbuminuria. We, however, assessed differences in renal function by evaluating the levels of microalbuminuria and urine creatinine levels, and the results were similar in all groups.

This study was designed as a small pilot study to investigate whether F1+2 levels differ between patients with VTE, MI and healthy controls. Consequently, we had a small study sample, and therefore, the non-significant p-values might be due to a lack of power.

In summary, although uF1+2 levels may be associated with postoperative venous thrombosis, we found no clear association in patients with acute VTE or MI and therefore the role of uF1+2 in the diagnosis of thrombotic disease or as predictor in clinical or epidemiological studies, seems limited.
REFERENCES