Adjustments in the diagnostic work-up, treatment and prognosis of pulmonary embolism
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Urinary prothrombin fragment 1+2 in patients with venous thrombosis and myocardial infarction

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ABSTRACT

Background: 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. To explore the relationship between plasma and uF1+2 we performed a pilot study in patients with thrombotic events and healthy controls.

Methods: In 40 patients with VTE or 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: Plasma and uF1+2 levels were positively correlated. 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), the latter, however, was not statistically significant. Patients with acute MI had similar F1+2 levels as controls in both plasma and urine. Differences in urinary F1+2 levels could not be attributed to differences in concentrations of creatinin or albumin in spot urine samples.

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

In the acute phase of venous and arterial thrombosis, D-dimer and F1+2 plasma levels are both elevated, which reflects thrombin generation (1;2). F1+2 can be measured in the urine by enzyme-linked immuno sorbent assay (ELISA) (3). Recently, F1+2 levels were assessed in urine samples of patients, prior and 3 days after a total hip replacement (4). Interestingly, increased urinary F1+2 (uF1+2) level predicted postoperative venous thromboembolism (VTE) after total hip replacement, whereas low levels of uF1+2 were found in patients who had bleeding complications after surgery (4). uF1+2 may be an interesting marker for thrombin generation in epidemiological studies, in which citrated plasma, necessary for plasma F1+2 or D-dimer measurement, is often not stored. The concentration of plasma F1+2 is increased in patients with acute VTE (4;5). The test is less sensitive and specific compared to the D-dimer assays, which precludes clinical use. To our knowledge it is not fully known how F1+2 undergoes renal clearance [3,4].

To explore the relationship between plasma and uF1+2 we performed a pilot study in patients with thrombotic events and healthy controls.

Forty consecutive patients > 18 years presenting with an objectively confirmed diagnosis of VTE or MI and 25 controls in the Academic Medical Centre and 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. DVT was diagnosed with compression ultrasonography and PE was diagnosed with multi-slice CT-scan. MI was diagnosed when 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\(^{-1}\). Controls were gender- and age-matched, 1:1, to the VTE and MI patients and consisted of visitors of the AMC and Slotervaart hospital. 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 of the controls. Since the age of MI and VTE patients was not completely similar, a total of 25 controls were included instead of 20.

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 1h after collection, platelet-poor plasma was obtained by twice 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).

Results are presented as mean ± standard deviation or median with inter-quartile range (IQR), depending on the observed distribution. All statistical analyses were performed in SPSS version 16.0 (SPSS Inc. Chicago, IL, USA).

**RESULTS**

Mean age was 65 years (SD 18) and 38% was female. Both 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 between the groups. Of the patients who were diagnosed with VTE, 8 patients had PE and 12 patients had DVT.

A correlation was found indeed between the F1+2 levels in the plasma and urine (regression coefficient 0.463, $r^2 = 0.214$, $p < 0.001$). 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), $p < 0.01$). Urinary F1+2 Levels in VTE patients were comparable to uF1+2 levels in controls. 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 1). 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 1). Subsequently, we adjusted for (micro) albuminuria by dividing uF1+2 by microalbumin / creatinine ratio. These assessments showed similar results in the 3 groups (Table 1).
Table 1: F1+2 and D-dimer results of the different patients groups and controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>VTE n=20</th>
<th>MI N=20</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma F1+2 (pmol L⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (IQR)</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></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (IQR)</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></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (IQR)</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>0.63 (0.35-2.95)</td>
</tr>
<tr>
<td>median (IQR)</td>
<td></td>
<td></td>
<td></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>median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(u F1+2) x (u creatinin / microalbumin) (pmol) x (nmol 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 shows that plasma levels of F1+2 are elevated in patients with acute VTE and not in MI patients, but uF1+2 levels in VTE and MI patients were similar to the uF1+2 levels in controls. This is in contrast with the uF1+2 levels predicting postoperative VTE after orthopaedic surgery (4,5). The group of patients who developed VTE 3 days after surgery, had a higher median uF1+2 level of 127.3 (IQR 19-1200 pmol L⁻¹) than VTE patients in this analysis (4). This difference might be due to the orthopaedic procedure, after which there is also a large wound area.

The limitations of our study merit some considerations. There was a difference in time to inclusion between the MI and VTE patients. Patients with acute MI were first referred to an intervention centre for the required procedure. Upon return within 48
hours patients were enrolled, whereas patients with VTE were included straight after
the diagnosis. Given that the T½ of F1+2 is approximately 90 minutes, this difference
in time to inclusion might have played a role in the results (6). Consequently, the
use of intravenous or subcutaneous heparin in patients with MI in the first 48
hours administered could have lowered the plasma and urinary D-dimer and F1+2
levels. However, in prospective studies no clear effect of unfractionated heparin on plasma F1+2
was observed (7). Results might also have been influenced by the concentration of the
spot urine samples and micro albuminuria. When adjusted for urinary micro-albumin
and creatinine levels, however, similar results were found. This study was designed as
a small pilot study to investigate whether there would be robust differences of urinary
F1+2 levels between patients with VTE, MI and healthy controls. Consequently, we had
a small study sample. Last, to our knowledge, the pre-analytical phase for measurement
of F1+2 in urine samples has not been thoroughly investigated, and F1+2 determination
may have been influenced by other proteins or proteases present in urine.

Although uF1+2 levels are associated with postoperative VTE, we found no
clear associations with acute VTE or MI and therefore a role of uF1+2 as predictor in
epidemiological studies seems limited.

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