TAFI activity in coronary artery disease: A contribution to the current discussion on TAFI assays

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Dear Sir,

Thrombin activatable fibrinolysis inhibitor (TAFI), also known as plasma procarboxypeptidase B or procarboxypeptidase U, is involved in regulation of fibrinolysis. After activation by the thrombin-thrombomodulin complex, activated TAFIa cleaves lysine residues from partially degraded fibrin and thereby inhibits further up-regulation of fibrinolysis (1, 2). During the last years, a large number of studies investigated the role of TAFI as a possible risk factor for thrombotic disease. These studies were recently summarised in an excellent review article by Leurs and Hendriks (3). The outcome, however, is confusing. Several studies showed that high TAFI plasma levels are associated with increased risk for thrombosis, others found the opposite. Besides differences in population, risk factors, or definition of control groups, another reason for these contradictory results may be related to the different TAFI assay methods used. Some enzyme-linked immunosorbent assay (ELISA) methods are sensitive to certain polymorphisms of the TAFI gene, in particular the Thr325Ile polymorphism which can have a large impact on the immunoreactivity of the two TAFI isoforms (4, 5). In addition, depending on the immunogen, antibodies can have a different reactivity towards different forms of TAFI (free TAFI, TAFI bound to plasminogen, TAFIa, inactivated forms of TAFI and TAFIa) (3). Assays measuring TAFI activity are not affected by the above problems; however, they have certain disadvantages relating to thermal instability of TAFIa or to other latently active carboxypeptidases present in plasma (3). As a consequence, harmonisation and better standardisation of assay methods are required, and researchers are asked to exclusively use well characterised assays for clinical studies (3). Recently, Verdú et al. (6) used a novel commercial ELISA which is not sensitive to TAFI polymorphisms, and they showed that highly increased TAFI levels are a risk factor for venous thromboembolism.

Previously, we investigated TAFI antigen levels in patients with coronary artery disease (CAD) and controls who were angiographically free of CAD and we found certain associations of higher TAFI antigen levels with CAD (7). At that time we used a commercial ELISA which, as we know today, is influenced by the Thr325Ile polymorphism. For this reason, we now re-analysed the samples using a novel TAFI activity assay, Pefakit® TAFI by Pentapharm Ltd (Basel, Switzerland). This assay functions with a novel synthetic substrate for TAFIa, which shows no significant interference with other carboxypeptidases and reacts fast within minutes, thereby overcoming the relative instability of TAFIa.

We previously described the characteristics of the study population in detail (7). In summary, 496 consecutive subjects were recruited over a period of nine months and underwent coronary angiography because of suspected CAD, acute coronary syndrome (ACS), or for investigation of valvular defects or closure of a persisting foramen ovale. Based on the angiographic result, 362 subjects having stenoses of ≥20% in a major coronary artery or one of their branches were defined as CAD patients, and 134 subjects with normal angiogram were defined as controls. According to the commonly used definition of CAD, here termed as CADnorm, 338 subjects having stenoses of ≥50 were defined as CAD patients, and the remaining 158 subjects were defined as controls. There were 44 patients with ACS. All subjects gave informed consent according to a protocol approved by the local ethics committee.

Routine laboratory analyses included total cholesterol, triglycerides, high density lipoprotein cholesterol, glucose, thrombocyte count, leukocyte count, and fibrinogen. Cardiovascular risk factors were recorded including smoking, body mass index, hypertension, diabetes type 2, and family history for cardiovascular disease. During angiography, venous blood from the femoral vein and arterial intracoronary blood from the ostium of the left main or right coronary artery was collected as described previously (7). These samples were immediately processed and stored frozen at –80°C until measurement of TAFI activity.

We determined TAFI activity with the novel Pefakit® TAFI (Pentapharm Ltd). The principle of the method is the following: TAFI, present in the plasma sample, is activated by thrombin/thrombomodulin. TAFIa cleaves irreversibly a synthetic peptide substrate, producing a thiol derivative. This thiol derivative reacts with the colourless Ellman’s reagent splitting off the yellow-coloured 5-mercaptop-2-nitro-benzoic acid. The extinction at wavelength 405 nm measured at the end of the enzymatic reaction is directly proportional to the concentration of TAFI activated by thrombin/thrombomodulin.
Carboxypeptidase N (CPN), which is constitutively active in plasma, may also cleave the substrate. However, when the assay was performed without TAFI activation by thrombin/thrombomodulin, only 2–4% of activity, attributed to CPN, was detected. Since this was within the normal variation of the assay, CPN activity was neglected. Further interferences are not known. The assay is not affected by plasma factor deficiencies (FI, FII, FV, FX, FXIII, protein S, protein C, antithrombin III), plasma factor enrichment (antithrombin III, tissue factor pathway inhibitor, FXIII, fibrinogen), heparin plasma levels ≤8 U/ml, lupus anticoagulant antibodies, or haemolytic samples. Intra-assay variation was below 5%, and inter-assay variation was below 5% on the same day and below 7% between two days according to the manufacturer.

We performed statistical analysis using the SPSS for Windows Statistical Package, version 13.0 (SPSS Inc., Chicago, IL, USA). According to the distribution of the variables, parametric or non-parametric statistical methods were used, i.e. Pearson’s correlation coefficient and unpaired t-test, or Spearman’s correlation coefficient and Mann-Whitney test.

TAFI activity highly correlated with the previously measured TAFI antigen levels. In venous samples, the correlation coefficient was 0.539 (p<0.001), and in intracoronary samples 0.545 (p<0.001).

We observed statistically significant correlations of TAFI activity with the following cardiovascular risk factors: In the control group, TAFI activity correlated with total cholesterol (0.270, p=0.002), leukocyte count (0.269, p=0.002), and fibrinogen (0.340, p<0.001). In CAD patients, TAFI activity correlated with body mass index (0.134, p=0.011), total cholesterol (0.225, p<0.001), triglycerides (0.139, p=0.009), and fibrinogen (0.239, p<0.001). We observed no associations of TAFI activity with age, gender, smoking, hypertension, diabetes, or family history of cardiovascular disease.

Table 1 shows that TAFI activity was higher in CAD patients than in controls. This association was not significant when CAD was defined as presence of stenoses of ≥20%. When we compared TAFI activity between controls and patients with 1-, 2-, or 3-vessel disease, we found no significant differences in the whole study population. However, in a subgroup consisting of patients suspected for the first time of having stable CAD (no acute MI, no previous cardiac interventions), there was a trend towards increasing TAFI activity with increasing number of affected vessels, and we found significant differences between controls and patients with 3-vessel disease (venous samples: 113.0 vs. 122.0%, p=0.017; intracoronary samples: 100.5 vs. 107.7%, p=0.042). We found no correlations of TAFI activity with the total number of atherosclerotic plaques. TAFI activity was not different in patients with ACS compared to CAD patients without ACS or compared to the whole study population.

We performed the first clinical study on TAFI activity in CAD using the novel Pefakit® TAFI assay assay. The strengths of our study are controls in whom CAD was clearly excluded by angiography, angiographically well-characterised patients, and intracoronary samples. A limitation of course is the retrospective study design. The Pefakit® TAFI activity assay uses a novel synthetic substrate and allows fast and easy handling. Due to its assay principle, this assay is unlikely to be sensitive to the TAFI genotypes and isoforms which affect TAFI antigen measurement by certain ELISAs. Theoretically, this activity assay could be affected by genetic polymorphisms influencing TAFI activity, but such polymorphisms are not known so far.

In this study, we observed associations of TAFI activity with both cardiovascular risk factors and CAD. The results are in accordance with our previous results on TAFI antigen levels (7). We therefore support our previous conclusion, that increased TAFI activity and/or antigen levels may contribute to CAD.

In addition, we conclude that the Pefakit® TAFI activity assay is a good alternative to replace ELISA methods in clinical studies.

References

Table 1: Venous and intracoronary TAFI activity in CAD patients and controls. CAD patients have stenoses of ≥50% in a major coronary artery or one of their branches (CADNorm definition). The subgroup consists of patients suspected for the first time of having stable CAD (no acute MI, no previous cardiac interventions). TAFI activity, expressed in percentage of normal plasma, is shown as mean (95% confidence interval).

<table>
<thead>
<tr>
<th>Group</th>
<th>Venous TAFI activity</th>
<th>P-value</th>
<th>Intracoronary TAFI activity</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Whole group (n=496)</td>
<td></td>
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<tr>
<td>CAD patients (n=338)</td>
<td>118.4 (115.5–121.3)</td>
<td>0.041</td>
<td>104.1 (101.4–106.9)</td>
<td>0.139</td>
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<td>Controls (n=158)</td>
<td>112.3 (108.5–116.2)</td>
<td>100.6 (97.0–104.2)</td>
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<tr>
<td>Subgroup (n=328)</td>
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<tr>
<td>CAD patients (n=173)</td>
<td>119.7 (115.7–123.7)</td>
<td>0.040</td>
<td>105.8 (101.9–109.5)</td>
<td>0.048</td>
</tr>
<tr>
<td>Controls (n=155)</td>
<td>112.1 (108.2–115.9)</td>
<td>100.5 (96.9–104.2)</td>
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