Plasma fibronectin levels and coronary artery disease

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Fibronectin is prominent in atherosclerotic lesions, contributes to lipoprotein uptake by phagocytic cells leading to foam cell formation, functions in cellular adhesion and cell-substrate anchoring and plays a role in the assembly and stabilization of platelet thrombi [1]. Because of its role in various cellular processes that could contribute to the development of atherosclerosis, we hypothesized that fibronectin might serve as a biomarker for the presence and/or severity of coronary artery disease (CAD).

We performed a single-center, case–control study of 187 non-consecutive patients between the ages of 35 and 85 years undergoing coronary angiography in a 15-month period. A lesion that obstructed >50% of the lumen in an epicardial coronary artery or a major branch as determined by quantitative coronary angiography was considered significant. Patients were assigned to one of four study groups: single-vessel CAD, which included patients with a significant stenosis in one of the three major coronary arteries or its major branches; double-vessel CAD; triple-vessel CAD (which included patients with significant left main disease); controls (10% stenosis). In order to increase the homogeneity of the control group, 11 patients with lesions >10% but 50% were excluded from this study. Fibronectin levels were determined in blood drawn at the time of angiography using an ELISA assay obtained from American Diagnostica, Inc. (Stamford, CT, USA). Fibronectin assays were conducted in duplicate and averages were used in this analysis. The intra-observer variability was <14%. Statistical analyses were performed using SAS Software version 9.1 (SAS Institute, Cary, NC, USA).

Significant CAD was present in 119 patients and, of these, 32 patients had single-vessel CAD, 34 patients had double-vessel CAD, and 53 patients had triple-vessel CAD or left main disease. In the patients with CAD, 39 were having an acute coronary syndrome at the time of coronary angiography whereas the remaining 80 patients had stable symptoms. The control group consisted of 57 patients.

The only significant demographic differences between the control group and patients with CAD were the increased use of statins and aspirin in patients with CAD and the slightly higher proportion of males and smokers in those with CAD (Table 1). There were no significant differences in demographics between groups of patients with single-vessel, double-vessel or triple-vessel CAD. Fibronectin levels were not normally distributed, but rather in a non-normal ‘skewed-right’ distribution. Skewed-right distributions have been reported with other plasma proteins, for example C-reactive protein and interleukin 6, in cohorts of patients with vascular disease [2,3]. No difference was found in plasma fibronectin levels among patients with and without CAD (Table 1, Fig. 1). Furthermore, we found no difference in fibronectin levels in groups of patients with different severity of CAD.

Demographics were similar between patients with stable CAD vs. patients with acute coronary syndromes with the exception of statin use (66% in stable CAD and 38% in acute coronary syndromes). The median (with 25th and 75th percentiles) was 478 (355–598) and 528 (346–753) μg mL\(^{-1}\) in patients with stable CAD and in those with an acute coronary syndrome, respectively. The odds ratio (95% CI) for stable CAD vs. acute coronary syndromes was 1.0000 (0.9989–1.0011).

Although it has been suggested that fibronectin may play a role in the development of atherosclerotic plaques and thrombus formation at the site of plaque rupture, we were unable to demonstrate any significant difference in plasma fibronectin levels based on the presence or severity of CAD as demonstrated by coronary angiography. This study would suggest that plasma fibronectin is not an accurate indicator for the presence of CAD and that its level is independent of the extent of atherosclerosis. Furthermore, despite its well-defined role in platelet thrombus formation [4], fibronectin was not elevated in patients with acute coronary syndromes.

Prior studies have reported conflicting results regarding a correlation between plasma fibronectin levels and the presence of CAD. Four separate smaller studies [5–8] reported that fibronectin levels were elevated in patients with angiographically proven CAD whereas the largest study, Zhang et al. [9] with 232 subjects, found that fibronectin levels were significantly lower in patients with CAD. In contrast, we found that fibronectin levels were similar in patients with and without CAD.

There are several potential explanations for these differing results. One is that all of the studies are small and thus subject to type I statistical errors. Another potential explanation is that...
most of the prior studies excluded patients on lipid-lowering therapy and/or patients with acute coronary syndromes, whereas neither of these were exclusion criteria for the present study. Other potential explanations include different ethnic populations (studies were performed in Turkey, Korea, China and the USA), and differences in time between coronary angiography and fibronectin determination (range from 0 to 30 days). Because fibronectin is an acute phase reactant [10], it is possible that levels are elevated at the time of coronary angiography and/or in other situations associated with physiologic stress.

Our study had several limitations. Firstly, the sample size in each category was small, but with each odds ratio approaching 1.0, it is unlikely that the results of a larger study would show any significant difference. Secondly, fibronectin has been reported to be elevated in acute coronary syndromes and this may have confounded finding any association in patients with stable symptoms. Thirdly, fibronectin levels were only determined at a single point in time.

In summary, the lack of consistent data regarding an association between fibronectin levels and CAD, and the lack of a dose response relationship in studies that have divided patients according to severity of disease – see Orem et al. [5], Song et al. [7] and the present study – cast doubt upon the use of fibronectin levels as a biomarker for CAD.

Acknowledgements

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

References


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Table 1 Demographic characteristics and plasma fibronectin levels as a function of extent of coronary artery disease (CAD). Age is expressed in mean ± SD. Fibronectin is expressed as median with 25th and 75th percentiles. All other values are n (%)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>All CAD</th>
<th>Single-vessel CAD</th>
<th>Double- vessel CAD</th>
<th>Triple-vessel CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>57</td>
<td>119</td>
<td>32</td>
<td>34</td>
<td>53</td>
</tr>
<tr>
<td>Age</td>
<td>56 ± 11</td>
<td>61 ± 11</td>
<td>60 ± 10</td>
<td>60 ± 10</td>
<td>61 ± 11</td>
</tr>
<tr>
<td>Body mass index &gt; 25 m kg⁻²</td>
<td>41 (72%)</td>
<td>78 (66%)</td>
<td>23 (72%)</td>
<td>21 (62%)</td>
<td>34 (64%)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>13 (23%)</td>
<td>46 (39%)*</td>
<td>13 (41%)</td>
<td>17 (50%)</td>
<td>16 (30%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10 (18%)</td>
<td>28 (24%)</td>
<td>5 (16%)</td>
<td>6 (18%)</td>
<td>17 (32%)</td>
</tr>
<tr>
<td>African American</td>
<td>15 (26%)</td>
<td>24 (20%)</td>
<td>8 (25%)</td>
<td>6 (18%)</td>
<td>10 (19%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>40 (70%)</td>
<td>94 (79%)</td>
<td>24 (75%)</td>
<td>28 (82%)</td>
<td>42 (79%)</td>
</tr>
<tr>
<td>Male</td>
<td>23 (40%)</td>
<td>73 (61%)*</td>
<td>17 (53%)</td>
<td>23 (68%)</td>
<td>20 (38%)</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>20 (35%)</td>
<td>68 (57%)*</td>
<td>17 (54%)</td>
<td>17 (50%)</td>
<td>34 (64%)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>27 (47%)</td>
<td>88 (74%)*</td>
<td>23 (72%)</td>
<td>25 (74%)</td>
<td>40 (75%)</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>1.0</td>
<td>0.9998 (0.9989–1.9997)</td>
<td>1.0003 (0.9992–1.0014)</td>
<td>0.9996 (0.9982–1.0010)</td>
<td>0.9994 (0.9982–1.0006)</td>
</tr>
</tbody>
</table>

*<0.05 vs. control.

Fig. 1. Plasma fibronectin levels in patients with and without coronary artery disease (CAD). Plasma fibronectin levels for each group with median values, 25th–75th percentiles and 10th–95th percentiles.
Hyperbilirubinemia interferes with ADAMTS-13 activity measurement by FRETS-VWF73 assay: diagnostic relevance in patients suffering from acute thrombotic microangiopathies

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Emerging new treatment strategies (e.g. rituximab) for patients suffering from acute thrombocytopenic purpura (TTP) with severe autoantibody-mediated ADAMTS-13 deficiency lead to an increasing demand for rapid and accurate ADAMTS-13 activity determination in any patient presenting with acute thrombotic microangiopathy (TMA). Until recently, only laborious and time-consuming ADAMTS-13 activity assays were available in specialized laboratories. It is thus not unexpected that the popularity of the FRETS-VWF73 assay [1], which is a rapid, easy to perform and robust [2–4] method for the determination of ADAMTS-13 activity, is steadily growing.

Lately, however, we observed atypical kinetic curves with lower starting fluorescence units and reduced reaction rates (fluorescence/time) in hyperbilirubinemic plasmas of patients referred for diagnostic work-up. In four characteristic plasma samples containing 136, 163, 445 and 600 μmol L⁻¹ of bilirubin (normal range 3–26 μmol L⁻¹), we found markedly lower ADAMTS-13 activity values measured by a slightly modified FRETS-VWF73 assay [1,3] than by the quantitative immunoblotting assay [5] (37 vs. 75%, 43 vs. 80%, 22 vs. 75%, and 8 vs. 60%, respectively), suggesting interference of bilirubin with the FRETS-VWF73 assay rather than an inhibitory effect on ADAMTS-13 enzymatic activity.

To address this phenomenon further, we examined the effect of varying bilirubin concentrations on ADAMTS-13 activity determined by the FRETS-VWF73 assay. A normal human plasma pool (NHP, bilirubin content 2 μmol L⁻¹) and hyperbilirubinemic patient’s plasma containing 600 μmol L⁻¹ bilirubin were heat-inactivated for 30 min at 56°C to abolish inherent ADAMTS-13 activity. Subsequently, heat-inactivated NHP and patient’s plasma were mixed in a total volume of 20 μL to achieve bilirubin concentrations of 2, 77, 152, 226, 301, 451 and 600 μmol L⁻¹. These samples were then mixed 1:1 (v:v) with either untreated NHP, or with a 1:1 (v:v) mixture of NHP and heat-inactivated NHP, resulting in final ADAMTS-13 activities of 50% and 25%, respectively. Final bilirubin concentrations were 2, 39, 77, 114, 152, 226 and 301 μmol L⁻¹. Analogous samples with the same final ADAMTS-13 activities were obtained by dilution of a synthetic bilirubin solution with heat-inactivated NHP in a total volume of 20 μL followed again by a 1:1 (v:v) mixture with untreated NHP resulting in final bilirubin concentrations of 2, 39, 77, 114, 152, 226, 301 and additionally 451 μmol L⁻¹. The synthetic bilirubin (Sigma-Aldrich, St. Louis, MO, USA) was freshly dissolved in 0.1 N sodium hydroxide to a concentration of 1 mmol L⁻¹, brought to pH 7.9 by the addition of HCl and strictly protected from light. The solvent solution, 0.1 N sodium hydroxide–HCl, pH 7.9, was carried along as a negative control.

ADAMTS-13 activity was determined by the FRETS-VWF73 assay [1] with minor modifications as described [3]. Fluorescence was recorded every 5 min for an extended period of 42 cycles for the better visibility of curve particularities. However, the reaction rate was calculated as described by linear regression analysis of fluorescence over time from 5 to 60 min (cycles 2–13) to circumvent possible distortion of results by substrate exhaustion. In addition, all samples were analyzed...