Soluble CD36 in Plasma Is Increased in Patients With Symptomatic Atherosclerotic Carotid Plaques and Is Related to Plaque Instability

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Background and Purpose—The risk for cardiovascular events is related to the composition and stability of an atherosclerotic plaque driven by inflammation and deposition of lipids. Scavenger receptors are a family of cell surface receptors involved in lipid uptake and inflammation. Recently, we found that soluble CD36 is increased in plasma from patients with diabetes strongly correlated with insulin resistance.

Methods—We tested whether soluble CD36 is a marker of plaque stability in patients with high-grade internal carotid stenoses (n=62). The patients were classified according to plaque symptomatology and plaque echogenicity on ultrasound examination.

Results—When patients were divided into 3 groups according to the latest clinical symptoms from plaques (ie, symptoms within the last 2 months [n=16], symptoms within the last 2 to 6 months [n=15], or asymptomatic [n=31]), the former group had significantly raised plasma levels of soluble CD36 as compared with the other 2 groups. In contrast, we found no differences in plasma levels of C-reactive protein, β-thromboglobulin, lipid parameters, or HbA1C between these groups. The patients with echolucent carotid plaques (n=20) tended to have higher soluble CD36 levels in plasma compared with those with echogenic/heterogenic plaque (n=39; P=0.087). By immunohistochemistry, CD36 was localized to macrophages-rich area of intima within the atherosclerotic lesion.

Conclusion—We propose that sCD36 may be a marker of plaque instability and symptomatic carotid atherosclerosis, possibly at least partly as a result of CD36 release to the circulation from the foam cells within the atherosclerotic lesion. (Stroke. 2008;39:3092-3095.)

Key Words: atherosclerosis ■ carotid artery disease ■ inflammation ■ lipids ■ stroke

Atherosclerosis is a progressive and chronic inflammatory disease in which lipids, immune cells, vascular smooth muscle cells, and extracellular matrix accumulate in the subendothelial space to form the growing atherosclerotic lesion. The risk for of cardiovascular events is related to the composition and stability of the plaque rather than to the degree of arterial stenoses, and recent studies suggest that inflammation is a critical determinant of plaque stability.1,2

CD36 is a transmembrane glycoprotein expressed in a variety of tissues and has been shown to be involved in angiogenesis, inflammation, lipid metabolism, and atherosclerosis and has also recently been linked to platelet activation. Membrane CD36 in monocytes and macrophages is upregulated by oxidized low-density lipoprotein and is an early step in the differentiation of macrophages into foam cells.3 The importance of monocyte/macrophage-related CD36 in the initiation and growth of atherosclerotic lesions has been supported by the reduction of the size of atherosclerotic lesions by inactivation of CD36 in ApoE-deficient mice.4

We have recently identified a soluble form of CD36, soluble CD36 (sCD36), in cell-free plasma. Like in intact monocytes, we found increased plasma levels of sCD36 in patients with diabetes, tightly correlated with insulin resistance,5 potentially released into the circulation as part of low-grade inflammation in insulin resistance. Based on the role of CD36 in inflammation, lipid metabolism, and platelet activation, we hypothesized that sCD36 could be a marker of plaque instability.
Table. Baseline Variables in Patients According to Clinical Symptoms and Months After Symptoms (n=62)*

<table>
<thead>
<tr>
<th></th>
<th>≤2 Months (n=16)</th>
<th>&gt;2 and ≤6 Months (n=15)</th>
<th>≥6 Months or No Symptoms (n=31)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>65 (56–83)</td>
<td>67 (50–79)</td>
<td>65 (44–81)</td>
<td>0.72</td>
</tr>
<tr>
<td>Male†</td>
<td>12 (75)</td>
<td>10 (66.7)</td>
<td>21 (67.7)</td>
<td>0.85</td>
</tr>
<tr>
<td>Degree of stenoses, %</td>
<td>80 (80–95)</td>
<td>80 (70–95)</td>
<td>80 (70–95)</td>
<td>0.66</td>
</tr>
<tr>
<td>Echolucent carotid plaque†</td>
<td>7 (43.8)</td>
<td>4 (26.7)</td>
<td>9 (29)</td>
<td>0.52</td>
</tr>
<tr>
<td>Ischemia on cerebral MRI† (n=53)</td>
<td>11 (68.8)</td>
<td>10 (66.7)</td>
<td>21 (67.7)</td>
<td>0.96</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7 (20.4–35.5)</td>
<td>25.2 (21.2–28.2)</td>
<td>26.6 (19–35.5)</td>
<td>0.73</td>
</tr>
<tr>
<td>Carotid endarterectomy treated†</td>
<td>15 (93.8)</td>
<td>11 (73.3)</td>
<td>27 (87.1)</td>
<td>0.26</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>151 (110–196)</td>
<td>160 (110–193)</td>
<td>150 (110–194)</td>
<td>0.58</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73 (32–99)</td>
<td>85 (58–101)</td>
<td>78 (49–99)</td>
<td>0.06</td>
</tr>
<tr>
<td>Statin treatment†</td>
<td>13 (81.3)</td>
<td>13 (86.7)</td>
<td>28 (90.3)</td>
<td>0.68</td>
</tr>
<tr>
<td>Current smoking†</td>
<td>7 (43.8)</td>
<td>9 (60)</td>
<td>16 (51.6)</td>
<td>0.70</td>
</tr>
<tr>
<td>Neopterin, nmol/L</td>
<td>8.7 (4.9–18.0)</td>
<td>11.7 (7.6–30.9)</td>
<td>8.6 (5.7–60.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>3.5 (1.0–39.0)</td>
<td>4.9 (1–21)</td>
<td>5 (0.6–28.0)</td>
<td>0.88</td>
</tr>
<tr>
<td>Total leucocyte count, 10⁹/L</td>
<td>8.3 (4.0–12.1)</td>
<td>8.3 (4.8–10.6)</td>
<td>7.5 (3.8–11.8)</td>
<td>0.39</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>4.0 (2.9–6.6)</td>
<td>3.9 (2.8–6.9)</td>
<td>4.0 (2.6–5.5)</td>
<td>0.99</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.3 (3.6–5.9)</td>
<td>4.5 (3.7–6.3)</td>
<td>4.2 (2.8–7.5)</td>
<td>0.11</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol, mmol/L</td>
<td>1.3 (0.7–1.9)</td>
<td>1.2 (0.8–2.2)</td>
<td>1.3 (0.8–2.5)</td>
<td>0.92</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.4 (0.6–3.8)</td>
<td>1.9 (0.7–3.7)</td>
<td>1.4 (0.7–3.5)</td>
<td>0.59</td>
</tr>
<tr>
<td>Low-density lipoprotein (n=49)</td>
<td>2.6 (2.0–4.0)</td>
<td>2.7 (1.8–4.6)</td>
<td>2.6 (1.5–5.1)</td>
<td>0.29</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.7 (5.2–6.9)</td>
<td>5.8 (4.4–7.8)</td>
<td>5.7 (9.0–8.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>β-thromboglobulin, IU/mL</td>
<td>53.3 (26.7–142.1)</td>
<td>56.8 (37.7–266.5)</td>
<td>53.0 (15.4–139.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>Platelet count, 10⁹/L</td>
<td>225 (132–391)</td>
<td>252 (187–441)</td>
<td>261 (168–441)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*Clinical symptoms include stroke, transient ischemic attack, or amaurosis fugax ipsilateral to the stenotic internal carotid artery. Values are median (range) or †numbers (percentages).

Methods

Patients

Sixty-two consecutive patients with high-grade internal carotid stenoses (≥70%) were treated with carotid endarterectomy (53 patients) or carotid angioplasty with stenting (9 patients; Table). The patients were classified into 3 groups: (1) symptoms within 2 months before blood collection; (2) symptoms 2 to 6 months before blood collection; and (3) asymptomatic patients (Table). Several studies have used 6 months for the inclusion of patients with symptomatic carotid stenosis (eg, the SPACE trial). To examine the ability of sCD36 to reflect plaque instability, we wanted to focus on those with the most recent symptoms. Accordingly, the symptomatic patients were divided into 2 equal groups in relation to the time since their latest clinical symptoms. Symptomatic patients had clinical symptoms such as stroke, transient ischemic attack, or amaurosis fugax ipsilateral to the stenotic internal carotid artery within the past 6 months. The carotid stenoses were diagnosed and classified using preoperative color duplex and CT angiography according to consensus criteria. The plaques were also classified as echolucent or echogenic/heterogeneous depending on plaque echogenicity on ultrasound examination. The protocols were approved by the regional ethics committee and signed informed consent was obtained from all individuals. Platelet-poor plasma was collected and stored as previously described.

Immunohistochemistry

Immunohistochemistry was performed as previously described using monoclonal antihuman CD36 (FA6–152, Novus Biologicals, Littleton, Colo), antihuman CD3 IgG (DakoCytomation, Glostrup, Denmark), antihuman smooth muscle actin (DakoCytomation), affinity purified polyclonal mouse antihuman macrophages (calprotectin) IgG (MCA874G; Serotec Ltd, Oxford, UK), and sheep antirat von Willebrand factor IgG (Cedarlane, Ontario, Canada). Omission of the primary antibody served as a negative control.

Miscellaneous

Plasma levels of sCD36 were measured by enzyme-linked immunoassay. Plasma levels of neopterin and β-thromboglobulin were measured by enzyme-linked immunoassays obtained from IBL Hamburg (Hamburg, Germany) and Diagnostica Stago (Asnières, France), respectively.

Statistical Analyses

For comparisons of 2 groups of individuals, the Mann-Whitney U test was used. When comparing 3 groups of individuals, the nonparametric Kruskal-Wallis test was used. If a significant difference was found, Mann-Whitney U test was used to calculate the difference between each pair of groups. Coefficients of correlation were calculated by the Spearman rank test. Fisher exact test was used when comparing proportions. The relationship between variables was calculated using the linear and binary regression analysis for continuous and categorical dependent variables, respectively. Probability values (2-sided) were considered significant at value of <0.05.

Results

When patients were divided into 3 groups according to their latest clinical symptoms (ie, symptoms within the last 2 months [n=16], symptoms within the last 2 to 6 months [n=15], or asymptomatic plaques [n=31]), the former group had significantly raised plasma levels of sCD36 as compared with the other 2 groups (Figure 1). Moreover, data on plaque morphology were available in 59 of the patients, and notably,
patients with echolucent plaques (n=20) tended to have higher sCD36 levels compared with those with echogenic/heterogeneous plaques (n=39; 1.96 versus 1.25; P=0.087).

In contrast to sCD36, none of the parameters described in the Table, including the degree of stenosis, lipid parameters, HbA1c, the use of statins, smoking habits, the fraction of patients with echolucent plaques, ischemia on cerebral MRI before surgery, and plasma levels of β-thromboglobulin and C-reactive protein, being reliable markers of platelet activation and systemic inflammation, respectively, were different among the 3 groups. In contrast, the differences in neopterin levels, another marker of monocyte/macrophage activation, reached statistical significance (Table). However, although the increase in sCD36 was restricted to those with the most recent symptoms (within 2 months; Figure 1), the difference in neopterin levels reflected an increase in those with symptoms between 2 and 6 months before blood collection (Table), suggesting that sCD36 may more accurately reflect plaque instability than neopterin.

We have recently identified CD36 as one of 87 genes that was markedly upregulated in symptomatic carotid plaques,7 suggesting that plaque in itself could contribute to the raised plasma levels of sCD36 in these patients. Staining of serial sections of these atherosclerotic lesions showed anti-CD36 immunostaining in plaques from symptomatic patients that were localized to the lipid-rich core of the plaque (Figure 2A) with strong immunostaining against calprotectin-positive macrophages (Figure 2B). CD36 immunostaining was also seen in lipid-rich calprotectin-positive regions in lesions from patients with asymptomatic disease, but in these patients, the atherosclerotic plaques were less advanced, and the region with CD36-positive immunostaining was smaller than in lesions from patients with symptomatic disease.

**Figure 1.** Plasma levels of sCD36 in patients with atherosclerotic carotid stenosis. The patients were divided into groups according to months after their last clinical symptoms. Clinical symptoms within the last 2 months (open bar, n=16), 2 to 6 months (squared bar, n=15), or >6 months (ie, asymptomatic; black bar, n=31). *P*≤0.0219 versus 2 months; #P≤0.0263 versus 2 months.

**Discussion**

CD36 is believed to play a critical role in the initiation and progression of atherosclerosis through its ability to bind and internalize modified low-density lipoprotein, facilitating the formation of lipid-engorged macrophage foam cells.8,9 Indeed, previous studies have reported accelerated CD36 expression in parallel with the progression of atherosclerosis,4 especially located on foamed, large-sized macrophages.10 Our finding in the present study of strong immunostaining of CD36 in symptomatic as compared with asymptomatic plaques, primarily located to lipid-loaded macrophages in the fatty core of the atherosclerotic plaque, further support a relationship between CD36 and advanced atherosclerosis. Furthermore, plasma levels of sCD36 were markedly elevated in those with symptoms related to their carotid stenosis within the last 2 months as compared with other patients. The mechanisms for release of CD36 in its soluble form are at present unclear, but it has been suggested that plasma levels of sCD36 could serve as a biomarker of conditions with altered CD36 expression such as elevated levels of modified lipoproteins and low-grade inflammation.9 Moreover, foam cell apoptosis has been linked to plaque destabilization and thrombus formation with subsequent development of acute ischemic events,11 and it may be speculated that apoptosis of lipid-loaded macrophages may lead to enhanced release of CD36. It is therefore tempting to hypothesize that the increased plasma levels of sCD36 in patients with recent symptomatic carotid plaques, with a time-dependent relationship with the acute symptoms, at least partly reflects intensified release of sCD36 during plaque destabilization.

CD36 is expressed not only on monocytes and macrophages, but also on endothelial cells12 and platelets.13 Recently, Podrez and coworkers proposed that platelet-related CD36 could be a sensor of oxidative stress and a modulator of platelet activation.13 Although β-thromboglobulin, an established marker of platelet activation, showed no differences among the 3 subgroups of patients, we cannot exclude that
activated platelets, induced by brain ischemia, could contribute to the raised levels of sCD36 in those with the most recent symptoms. It is possible that the ability of sCD36 to reflect plaque instability and symptomatic disease may reflect its capacity to mirror several pathogenic processes involved in plaque destabilization such as activation of platelets and monocytes/macrophages as well as pathogenic events within the lesion. Through interaction with thrombospondin-1, CD36 may be related to enhanced release of matrix metalloproteinases-9, another marker of plaque instability, further supporting a link between CD36 and plaque destabilization.

The present study has some limitation such as its retrospective nature as well as the low number of patients in every clinically subgroup. Nevertheless, the results may suggest that sCD36 should be further investigated as a marker of plaque instability. This should include studies in larger patient populations as well as studies that examine the ability of sCD36 to predict forthcoming cardiovascular events.

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Disclosures
A.H., M.S., P.A., and B.H. are listed as coinventors on a pending patent application for the use of sCD36 as a prognostic marker in cardiovascular disease.

References
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