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

Aase Handberg, MD, DMSc; Mona Skjelland, MD; Annika E. Michelsen, MSc; Ellen Lund Sagen, BSc; Kirsten Krohg-Sørensen, MD, PhD; David Russell, MD, PhD; Arve Dahl, MD, PhD; Thor Ueland, PhD; Erik Øie, MD, PhD; Pål Aukrust, MD, PhD; Bente Halvorsen, MSc, PhD

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.
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. Values are median (range) or numbers (percentages).

### 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 precerbral color duplex and CT angiography according to consensus criteria. The plaques were also classified as echolucent or echo-genic/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 mouse 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 anti-rat von Willebrand factor IgG (Cedarlane, Ontario, Canada). Omission of the primary antibody served as a negative control.

### Methods

**Omission of the primary antibody served as a negative control.**

### 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,
CD36 is expressed not only on monocytes and macrophages, but also on endothelial cells and platelets. Recently, Podrez and coworkers proposed that platelet-related CD36 could be a sensor of oxidative stress and a modulator of platelet activation. Although β-thromboglobulin, an established marker of platelet activation, showed no differences among the 3 subgroups of patients, we cannot exclude that formation of lipid-engorged macrophage foam cells. Indeed, previous studies have reported accelerated CD36 expression in parallel with the progression of atherosclerosis, especially located on foamed, large-sized macrophages. 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. Moreover, foam cell apoptosis has been linked to plaque destabilization and thrombus formation with subsequent development of acute ischemic events, 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 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

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
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.

Acknowledgments
The assistance of Stine Bjørnsen and Lene Dabelstein Petersen is greatly appreciated.

Sources of Funding
This work was supported by Helse Sør, Medinnova Foundation, Freia Found, Eckbo’s Foundation, the Novo Nordisk Foundation, the Danish Diabetes Association, the Danish Medical Research Council, and Rikshospitalet Medical Center.

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

Aase Handberg, Mona Skjelland, Annika E. Michelsen, Ellen Lund Sagen, Kirsten Krohg-Sørensen, David Russell, Arve Dahl, Thor Ueland, Erik Øie, Pål Aukrust and Bente Halvorsen

Stroke. 2008;39:3092-3095; originally published online August 21, 2008;
doi: 10.1161/STROKEAHA.108.517128

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/39/11/3092

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/