Sublingual Microvascular Changes in Patients With Cerebral Small Vessel Disease

Mostafa Khalilzada, MD; Kemal Dogan, MD; Can Ince, PhD; Jan Stam, MD, PhD

Background and Purpose—It is unknown whether changes in cerebral small vessel disease (SVD) are limited to the brain or part of a generalized vascular disorder.

Methods—We examined the sublingual microcirculation of 10 healthy controls, 10 patients with large vessel disease, and 8 with SVD, with side-stream dark field imaging. We analyzed 146 video fragments masked to the origin of the videos. Imaging software measured the functional capillary density per tissue surface unit. We scored the percentage of blood vessels with abnormal flow (abnormal flow index) and the presence of extravascular erythrocyte material as presumed evidence of past microbleeds or obliterated vessels.

Results—Functional capillary density differed between the 3 groups (SVD, large vessel disease, and controls; means, 14.8, 17.0, and 16.1 mm/mm²; P<0.01). Abnormal flow was more frequent in SVD patients compared with large vessel disease patients and controls (medians, 10.5%, 6.1%, 5.5%; P=0.04). Extravascular erythrocyte material was almost exclusively present in patients with SVD (P=0.004).

Conclusions—We found evidence of pathological changes in the sublingual microcirculation in patients with cerebral small vessel disease, which suggests that cerebral SVD is part of a generalized vascular disorder. (Stroke. 2011;42:00-00.)

Key Words: cerebral small vessel disease ■ microcirculation ■ sublingual

Cerebral ischemic disorders can be divided in 2 categories: small vessel disease (SVD) and large vessel disease (LVD). SVD is attributed to alterations in the intima and media of cerebral arterioles, with hyaline deposition and fibrotic hardening,1 and is associated with leukoaraiosis, lacunar infarcts, and intracerebral hemorrhages.2,3 Cerebral LVD is caused by atherosclerosis of medium and large arteries, causing thromboembolic occlusion of cerebral arterioles. Large vessel atherosclerosis is a generalized disease that affects many organs, such as brain, heart, and kidneys. Whether cerebral SVD is part of a generalized disorder is controversial. An association with changes in retinal and renal arterioles has been shown.4,5 We hypothesized that if cerebral SVD is part of a generalized microvascular disorder, then pathological changes might be detected in the easily accessible sublingual circulation.

Subjects and Methods

We studied 28 age-matched subjects (10 controls, 8 SVD patients, and 10 LVD patients). Cerebral SVD was defined as a lacunar stroke with exclusion of a large infarct by clinical examination and CT or MRI, and the absence of cardioembolic causes or large vessel

Figure 1. Sublingual microcirculation images of a control subject (A), a patient with a large cerebral infarct (B), and a patient with a lacunar infarct (C). White arrows indicate extravascular erythrocyte material appearing as nonmoving erythrocytes.
atherosclerosis. Cerebral LVD was defined as large cerebral infarct in the absence of cardioembolic disease. Controls were age-matched healthy volunteers.

We used a hand-held side-stream dark field imaging microscope, with a 5/100 objective lens system (MicroVision Medical), connected to a video and recording unit. Focus and illumination were adjusted to optimize image quality. Video sequences were digitized to Digital Video-Audio Video Interleaved files. Artifact-free sequences of 20 seconds were selected, labeled with a random number, and scored by an observer blinded to its origin. We analyzed 146 video sequences (4–6 per subject) in random order. We assessed flow with the microvascular flow index, which is a validated scoring system. Flow was scored as absent (0), intermittent (1), sluggish (2), or continuous (3). The percentage of blood vessels in a video frame with a score is called the abnormal flow index. We quantified the functional capillary density with software designed for side-stream dark field imaging. Functional capillary density is the total length of perfused blood vessels per surface unit. We also observed nonmoving extravascular granular material around vessels with the same illumination properties as erythrocytes (Figure 1). Because the wavelength we use for side-stream dark field imaging (530 nm) is selectively absorbed by hemoglobin, we hypothesized that this material consists of free hemoglobin or degraded erythrocytes and labeled it extravascular erythrocyte material. Intragroup differences were analyzed with Kruskal-Wallis ANOVA; differences between groups were analyzed with the Mann-Whitney U test.

**Results**

Functional capillary density differed among the groups ($P<0.01$), mainly because of a lower functional capillary density in SVD patients compared to LVD patients ($P<0.01$; Table, Figure 2). In SVD patients, 10.5% of all blood vessels had abnormal flow compared to 6.2% in the LVD patients and 5.5% in control subjects ($P<0.04$). This difference was mainly caused by absent or sluggish flow in SVD patients. Extravascular erythrocyte material was present in 22.5% of the videos from SVD patients but was virtually absent in control subjects and LVD patients ($P<0.004$).

**Discussion**

The lower number of perfused blood vessels in SVD patients may be caused by temporary occlusion or obliteration of arterioles or capillaries. The exact cause of the impaired flow could not be inferred from the video fragments. It may be caused by occluded or narrowed arterioles upstream that are not visible in the recorded images.

We found a novel abnormality, extravascular erythrocyte material, which is strongly associated with cerebral SVD and

### Table. Baseline Characteristics and Microvascular Parameters of Controls and Patients With Cerebral Large Vessel Disease and Small Vessel Disease

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=10)</th>
<th>LVD (n=10)</th>
<th>SVD (n=8)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57.7 (±4.7)</td>
<td>63.2 (±12.0)</td>
<td>55.8 (±8.7)</td>
<td>0.20</td>
</tr>
<tr>
<td>Hypertension (N)</td>
<td>0</td>
<td>4</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes (N)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>FCD (mm/mm²)</td>
<td>16.1 (±1.4)</td>
<td>17.0 (±1.4)</td>
<td>14.8 (±1.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>AFI (%)*</td>
<td>5.5 (±6.5)</td>
<td>6.2 (±7.2)</td>
<td>10.5 (±12.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>No flow (%)</td>
<td>0.9 (±1.7)</td>
<td>1.1 (±3.0)</td>
<td>3.5 (±4.8)</td>
<td></td>
</tr>
<tr>
<td>Intermittent low (%)</td>
<td>0.2 (±0.7)</td>
<td>1.6 (±2.1)</td>
<td>1.1 (±2.9)</td>
<td></td>
</tr>
<tr>
<td>Sluggish flow (%)</td>
<td>3.6 (±4.2)</td>
<td>3.5 (±5.5)</td>
<td>6.4 (±6.4)</td>
<td></td>
</tr>
<tr>
<td>EEM (%)*</td>
<td>0.0 (±0)</td>
<td>0.0 (±5)</td>
<td>22.5 (±51.8)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

AFI indicates abnormal flow index; EEM, extravascular erythrocyte material; FCD, functional capillary density; LVD, large vessel disease; NS, not significant; SD, standard deviation; SVD, small vessel disease.

Mean (SD) for continuous data; median percentage* (interquartile range) for categorical data.

$P$ are for the simultaneous comparison of groups (Kruskal-Wallis).

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Figure 2. Results of sublingual microvascular imaging in control subjects (CTRL) and in patients with cerebral large vessel disease (LVD) and small vessel disease (SVD). Box plots with median, interquartile range (filled rectangles), and full range. A, Functional capillary density. B, Abnormal flow index. C, Extravascular erythrocyte material. $P$ are for pair-wise comparisons.
probably consists of agglomerations of hemoglobin because it absorbs the green light (wavelength 530 nm) we used. Extravascular erythrocyte material may be remnants of obliterated capillaries or caused by extravasation of erythrocytes. This phenomenon may be related to the microbleeds found with MRI in cerebral SVD.3,9

A weakness of our study is the classification of cerebral SVD and LVD mainly based on clinical characteristics, history, and (other) manifestations of large vessel atherosclerosis. Ideally, SVD should be confirmed with MRI, but this is not routinely available for stroke patients in our center.

Other studies demonstrated impairment of the renal microcirculation5,10 and abnormalities in the retinal vasculature in cerebral SVD.4,11 Also, erythrocyte velocity in nail-fold capillaries was reduced during reactive hyperemia in patients with cerebral SVD.12 Our results suggest that patients with cerebral SVD have a generalized disorder of the microcirculation.

Disclosures
C.I. is the inventor of side-stream dark-field imaging and a shareholder in Microvision Medical. Patents have been submitted by the Amsterdam Medical Centre (owner) for side-stream dark-field imaging.

References
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