Association of Cerebral Microbleeds in Acute Ischemic Stroke With High Serum Levels of Vascular Endothelial Growth Factor

Pooja Dassan, MD, MRCP; Martin M. Brown, MD, FRCP; Simone M. Gregoire, MD; Geoffrey Keir, PhD, FRCPath; David J. Werring, PhD, MRCP

Objective: To determine whether vascular endothelial growth factor (VEGF) levels are associated with the presence of cerebral microbleeds (CMBs) in patients after acute ischemic stroke.

Design: A cross-sectional study that used blood samples obtained within 24 hours of symptom onset from patients who experienced acute stroke to measure VEGF levels by enzyme immunoassay. A validated CMB rating scale was used to analyze acutely acquired magnetic resonance images, with the rater blind to clinical details and VEGF levels.

Setting: Accident and Emergency Department at University College Hospital, London, England.

Patients: Twenty patients who experienced acute ischemic stroke.

Main Outcome Measures: Presence of CMBs and serum level of VEGF.

Results: Five of the 20 patients with acute ischemic stroke (25%) had CMBs. The median VEGF level in the CMB group was significantly higher than that in the group without CMBs (P = .003).

Conclusion: An increase in vascular permeability secondary to a raised VEGF level may have a role in the genesis of CMBs in patients with acute ischemic stroke.


Cerebral microbleeds (CMBs) are focal perivascular collections of blood breakdown products and are visualized as small areas of signal loss on T2*-weighted gradient echo magnetic resonance image (MRI) sequences. Cerebral microbleeds are commonly found in patients with ischemic stroke or primary intracerebral hemorrhage; histologically, they are found adjacent to small vessels affected by hypertensive arteriopathy or cerebral amyloid angiopathy.

Cerebral microbleeds are found in approximately 30% of patients with acute ischemic stroke. An MRI follow-up study found that about 25% of patients with ischemic stroke/transient ischemic attack developed new CMBs after 5 years. Recent data have shown that CMBs develop within days of acute ischemic stroke, remote from the symptomatic acute infarct, but the mechanisms underpinning new CMB formation remain unknown. Although CMBs are known to be associated with focal small-vessel damage, another potential contributing factor is an active widespread microangiopathy with leakage of the blood-brain barrier.

Vascular endothelial growth factor (VEGF) is a potent angiogenic glycoprotein that is upregulated as a result of the ensuing hypoxia after an acute ischemic stroke; VEGF has an important role in the control of vascular permeability. To investigate the hypothesis that VEGF plays a role in the formation of CMBs in acute ischemic stroke, we compared VEGF levels of patients with CMBs with those without CMBs.

### METHODS

#### PATIENTS

Serum VEGF levels were determined in 20 patients with clinically and radiologically confirmed acute ischemic stroke. We prospect-
tively recruited all patients within 24 hours of symptom onset from the Accident and Emergency Department at University College Hospital, London. Serum VEGF levels were also measured in 15 healthy control subjects with no medical history of stroke (7 [47%] women; mean [SD] age, 59.3 [6.4] years).

Figure 1. Schematic illustrations of axial brain magnetic resonance images showing the index infarct (solid gray color) and the location of the cerebral microbleeds (CMBs) (black dots with a faint gray halo) for the 5 patients (numbered 1-5) with CMBs.

LABORATORY ANALYSIS

Venous samples were obtained from patients on admission. Blood was centrifuged within 30 minutes of collection (1500g for 10 minutes), and the serum was frozen at −80°C for later
Our results show that VEGF levels were significantly higher in patients who experienced ischemic stroke with CMBs compared with those without CMBs. In agreement with previous reports,7 we also confirm that VEGF levels in patients with acute ischemic stroke are significantly elevated compared with levels in healthy controls. Confounding factors—in particular, infarct volume and stroke severity7—are unlikely to account for the difference in serum VEGF expression between the groups with and without CMBs because the groups did not differ with respect to these characteristics.

It is generally assumed that, to form CMBs, blood degradation products must leak from focal damage, increasing the fragility of small vessels. However, CMBs are not an invariable consequence of pathologic damage to such vessels. Thus, in addition to structural small-vessel damage,1 there may be other triggers for vascular leakage in the pathogenesis of CMBs in some patients. One possibility is that abnormal vascular permeability could potentiate CMB formation. Vascular endothelial growth fac-

### Table. Clinical Characteristics of Patients Who Experienced Stroke With and Without CMBs

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Stroke With CMB (n=5)</th>
<th>Stroke Without CMB (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>69.4 (11.2)</td>
<td>65.3 (17.2)</td>
</tr>
<tr>
<td>Female sex, No. (%)</td>
<td>1 (20)</td>
<td>7 (47)</td>
</tr>
<tr>
<td>NIHSS score, mean (SD)</td>
<td>7.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Infarct volume, mean (SD), cm³</td>
<td>2.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Risk factors, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>3 (60)</td>
<td>9 (60)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>2 (40)</td>
<td>8 (53)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (60)</td>
<td>7 (47)</td>
</tr>
<tr>
<td>Cause of stroke, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>1 (20)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Large artery atherosclerosis</td>
<td>2 (40)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Lacunar</td>
<td>0</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>2 (40)</td>
<td>4 (27)</td>
</tr>
</tbody>
</table>

Abbreviations: CMB, cerebral microbleed; NIHSS, National Institute of Health Stroke Scale.

a There was no significant difference between the 2 groups for any of the characteristics.

b The maximum total NIHSS score is 42.

### Figure 2

Box and whisker plots of serum vascular endothelial growth factor (VEGF) levels in patients who experienced ischemic stroke with cerebral microbleeds (CMBs) compared with those without CMBs. The horizontal line inside each box indicates the median; the top and bottom of each box denote the 75th and 25th percentiles, respectively; and the ends of the whiskers indicate the minimum and maximum values (the outlier is indicated by an open circle). A statistically significant difference existed between these 2 subgroups (P = .003). This difference remained significant even after excluding the outlier high VEGF value from the group with CMBs (P = .01).

### IMAGING PROTOCOL

Magnetic resonance imaging, including axial T2-weighted fast spin echo and axial gradient-recalled echo T2* sequences, was carried out at a 1.5-T field strength using 2 MRI systems: (1) the Genesis Signa system (GE Healthcare) and (2) the Magnetom Avanto system (Siemens). The axial gradient-recalled echo T2* sequence settings were as follows: Genesis Signa system: repetition time, 300 milliseconds; echo time, 40 milliseconds; flip angle, 20°; field of view, 24 × 18 pixels; section thickness, 5 mm; section gap, 1.5 mm; number of excitations, 1; Magnetom Avanto system: repetition time, 800 milliseconds; echo time, 26 milliseconds; flip angle, 20°; field of view, 24 × 18 pixels; matrix, 512 × 448 pixels; section thickness, 5 mm; section gap, 1.5 mm; number of excitations, 1.

### RESULTS

All 20 patients underwent MRI within 5 days of acute ischemic stroke; 5 (25%) had at least 1 CMB. The CMBs were located distant from the index (recent) infarct in each patient (Figure 1). Eleven CMBs were noted: 3 in infratentorial regions (1 cerebellar and 2 brainstem), 2 in deep regions (thalamus), and 6 in lobar regions. When patients with and without CMBs were compared, there was no significant difference between the 2 groups with respect to age, sex, National Institute of Health Stroke Scale (NIHSS) score, infarct volume, or the prevalence of vascular risk factors (Table).

The median VEGF concentration in the patients experiencing stroke, irrespective of the presence of CMBs, was significantly higher than that in the healthy controls (2010 vs 546 pg/mL; P < .001). The median VEGF level in the group with CMBs was significantly higher than the level in the group without CMBs (P = .003, Mann-Whitney test) (Figure 2). Even when the outlier in the cohort with CMBs was excluded, the difference remained statistically significant (P = .01, Mann-Whitney test). The patient with the highest serum VEGF level (9813 pg/mL) also had the most CMBs (n = 6). We noted a modest correlation between serum VEGF level and maximum NIHSS score (r = 0.339; P = .02).

### COMMENT

The image analysis was performed with the rater (S.M.G.) blind to VEGF levels and clinical details. A validated CMB rating scale was used by a trained observer (S.M.G.).

Our results show that VEGF levels were significantly higher in patients who experienced ischemic stroke with CMBs compared with those without CMBs. In agreement with previous reports,7 we also confirm that VEGF levels in patients with acute ischemic stroke are significantly elevated compared with levels in healthy controls. Confounding factors—in particular, infarct volume and stroke severity7—are unlikely to account for the difference in serum VEGF expression between the groups with and without CMBs because the groups did not differ with respect to these characteristics.

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### Figure 1

Cause of stroke, No. (%)

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tor, a potent inducer of vascular leakage, is upregulated after acute ischemic stroke and may trigger or potentiate CMB genesis. Animal model studies have also shown that hypoxia-induced VEGF surges in the brain can exacerbate vascular leakage.

Strengths of our study include the recruitment of patients with imaging-proved ischemic stroke and the use of a validated microbleed rating scale by a rater blind to both the clinical data and the VEGF levels. In addition, although our study was small, the difference in VEGF levels between patients with and without CMBs was highly significant (P = .003). However, because this was a cross-sectional study, it is difficult to infer causality, that is, whether elevated VEGF levels were a cause or an effect of a CMB-associated cerebral microvasculopathy. Furthermore, because we have MRIs from a single time point only, we cannot determine how many of the CMBs observed occurred after rather than before the ischemic stroke. Finally, although the intergroup differences in serum VEGF level do not appear to be driven by the small differences in stroke volume or NIHSS score, our sample size is not large enough to adjust for these potential confounding factors. This study should therefore be considered preliminary: it needs to be extended to larger cohorts of patients with acute stroke in which serial MRI is used to establish the consistency of our findings and to clarify whether high VEGF levels are independently associated with new CMB formation.

Agents to pharmacologically block the harmful effects of an increased VEGF level, including vascular leakage, after cerebral ischemia are of current interest. Our findings suggest that studies of such treatments might usefully incorporate MRI to monitor the development of CMBs. Furthermore, VEGF level, as a potential marker for vascular leakage and microbleeding, deserves further investigation as a prognostic marker for bleeding risk in acute stroke (eg, before thrombolytic therapy).

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Author Contributions: Study concept and design: Dassan, Brown, Gregoire, and Werring. Acquisition of data: Dassan. Analysis and interpretation of data: Dassan, Brown, Keir, and Werring. Drafting of the manuscript: Dassan and Werring. Critical revision of the manuscript for important intellectual content: Brown, Gregoire, Keir, and Werring. Statistical analysis: Dassan and Werring. Obtained funding: Brown. Administrative, technical, and material support: Gregoire, Keir, and Werring. Study supervision: Brown and Werring.

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Additional Contributions: Andreas Charidimou, MSc, Clinical Research Fellow at UCL Institute of Neurology, provided the illustrations.

REFERENCES