Periventricular White Matter Lucencies Relate to Low Vitamin B12 Levels in Patients With Small Vessel Stroke

Barbe Pieters, MD; Julie Staals, MD; Iris Knothnerus, MD; Rob Rouhl, MD; Paul Menheere, MD, PhD; Alfons Kessels, MD, MSc; Jan Lodder, MD, PhD

Background and Purpose—Blood–brain barrier dysfunction may be an early phenomenon in the development of the small vessel disease, which underlies white matter lesions. Because vitamin B12 plays a role in maintaining the integrity of the blood–brain barrier, we studied serum vitamin B12 level in relation to such lesions.

Methods—In 124 patients with first lacunar stroke, we measured serum vitamin B12 level and rated the degree of white matter lesions on MRI.

Results—Mean vitamin B12 level was 202 pmol/L (SD, 68.9). Thirty-nine patients (31.5%) had a vitamin B12 level less than the lower reference value of 150 pmol/L. Lower vitamin B12 level was (statistically significant) associated with more severe periventricular white matter lesions (odds ratio/100 pmol/L decrease, 1.773; 95% CI, 1.001–3.003), but not with deep white matter lesions (odds ratio/100 pmol/L decrease, 1.441; 95% CI, 0.881–2.358; ordered multivariate regression analysis).

Conclusions—More severe periventricular white matter lesions in lacunar stroke patients relate to lower vitamin B12 levels. A possible causal relationship should now be studied prospectively. (Stroke. 2009;40:1623-1626.)

Key Words: lacunar stroke ■ vitamin B12 ■ white matter lesions

Blood–brain barrier dysfunction has been suggested as an early phenomenon in the development of the small vessel disease that underlies ischemic white matter lesions (WML) as imaged by MRI.1 Experimental and human evidence support the idea that vitamin B12 plays a role in maintaining the integrity of the blood–brain barrier.2,3 Therefore, we studied the relationship between serum vitamin B12 level and the severity of WML in a population with a high frequency of WML: patients with symptomatic cerebral small vessel disease as clinically manifested by a first lacunar stroke.

Patients and Methods

We included patients with a first lacunar stroke between May 2003 and November 2006. Of 184 patients, 45 refused to participate. All patients had standard blood and urine analyses, a 12-lead ECG, a chest X-ray, ultrasound studies, and a cerebral MRI. Echocardiography, 24-hour (Holter) monitoring, and cerebral angiography were performed in selected patients. Lacunar stroke, vascular risk factors, and ancillary investigations were defined as described before; in addition to age and sex, the following vascular risk factors were recorded: hypertension (known hypertension, treated or not, or at least 2 blood pressure recordings ≥160/90 mm Hg before stroke or >1 week after stroke), diabetes mellitus (known diabetes, treated or not; fasting serum glucose >7 mmol/L; or a postprandial level >11 mmol/L on at least 2 separate occasions before or at least 3 days after stroke), current smoking, first-degree family history of vascular disease, and hypercholesterolemia when blood cholesterol >5 mmol/L. Lacunar stroke was an acute lacunar stroke syndrome, lasting >24 hours, with or without a compatible lesion with a diameter <15 mm on MRI (T2 and FLAIR; Gyroscan ACS-NT; Powertrak 6000 Philips; scan parameters: 1.5 or 3.0 Tesla; field of view, 23×23 cm; matrix, 512×512; standard axial T2 [repetition time shortest, echo time 100 ms], and axial FLAIR [repetition time 8000 ms, echo time 120 ms]). Images were made with slice thickness of 5 mm and gaps of 0.5 mm. As before this study, 2 vascular neurologists independently assessing MR images of 101 patients with first-ever stroke had Cohen kappa of 0.89 for symptomatic infarct, 0.96 for ≥1 asymptomatic lacunar infarcts, 0.77 for periventricular WML, and 0.84 for deep WML; for this study, the same neurologists assessed MR images by consensus. If no symptomatic lacunar lesion was visible, then we used the established criteria of unilateral motor or sensory signs that involved the whole of at least 2 of the 3 body parts (face, arm, leg), without disturbance of consciousness, visual fields, language, or other cortical functions, compatible with lacunar syndrome. We graded periventricular and deep WML based on the Fazekas scale; periventricular: (1) none; (2) smooth halo or pencil-thin lining; (3) restricted lesion toward the deep white matter; and (4) lesions extending into the deep white matter; and deep: (1) none; (2) punctated; (3) restricted, partially confluent; and (4) large confluent. To increase the chance that the lacunar stroke resulted from small vessel disease and not from cardiac or large vessel thromboembolism, patients (N=15) with evidence of a cardiac embolic source (atrial fibrillation, myocardial infarct <6 weeks, prosthetic cardiac valve, endocarditis, cardiomyopathy, mitral stenosis, left ventricular aneurysm, or thrombus) or signs of severe (pre-) cerebral large vessel disease (at least 1 internal carotid artery stenosis of >50% on ultrasound investigation) were excluded.

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excluded, leaving 124 patients for the study. Vitamin B12 was assayed using a solid-phase time-resolved fluoroimmunoassay on an Auto Delfia immunoanalyser (PerkinElmer). In the on-board chemical pretreatment step, the B12 is released from carrier proteins and converted into a stable, measurable form. The assay is of the competitive type, based on the competition of europium-labeled B12 and sample B12 for a limited amount of binding sites on intrinsic factor. The intrinsic factor is coupled to an anti-mouse IgG using an anti-IF antibody. The strong fluorescent signal from the Eu-chelates (formed after dissociation of the europium from the Eu-labeled B12 tracer by an enhancement solution) is inversely proportional to the concentration of the vitamin B12 in the sample. Using a cutoff of 2.5% at both ends, reference values for vitamin B12 are 150 to 630 pmol/L. To further substantiate the lower limit reference value, we applied the Bhattacharya technique (which allows estimates of reference values, not affected by disease or treatment, in patient samples >3000) to 14 683 vitamin B12 measurements performed in our clinic, and found the lower limit also at 150 pmol/L. The intra-assay and interassay precision as determined with 1 homemade reference data. Logistic regression analysis with vitamin B12 concentration as dependent variable and WML severity (through stroke outcome coefficient, 0.01; P = 0.94).

### Results
There were 75 men and 49 women, aged 66.0 (SD,11.9) years. Table 1 shows the patient characteristics. In 26 patients (21%) a symptomatic lacunar infarct could not with certainty be identified. Delay between stroke and MRI was 40 (median; range, 0–410 days).

Vitamin B12 blood samples were taken during hospital stay in 28 patients, and at least after 3 months in 96, whereas baseline characteristics, distribution of B12 level (Mann-Whitney U), and WML categories were similar in the 2 groups. However, the mean age in the first group was 69 (SD, 9) and 55 (SD, 13) years in the second group. This difference probably reflects that more elderly patients were admitted early to hospital and were consequently scheduled for vitamin B12 sampling earlier than younger patients who visited the outpatient clinic late. None of the patients were administered vitamin B12 substitution therapy.

The hypothesis that our data set was not normally distributed was rejected (P = 0.2). The mean vitamin B12 level was 202 pmol/L (SD, 68.9; median, 196; range, 52–431 pmol/L). Thirty-nine patients (31.5%) had a vitamin B12 level less than the lower reference value of 150 pmol/L (median, 130.5; range, 52–149 pmol/L), which indicates >15-times higher proportion of low values in our group compared with the reference data. Logistic regression analysis with vitamin B12 level dichotomized with 150 pmol/L as cutoff, comparing periventricular WML category 1 and 2 with 3 and 4, yielded a probability value of 0.040 (OR, 2.66; 95% CI, 1.04–6.80) for the association of vitamin B12 decrease with more severe WML.

Table 2 shows the number of patients within each WML category, and corresponding median vitamin B12 level with range. Comparison of vitamin B12 levels between periventricular WML categories (univariate ordered logistic regression analysis) yielded an OR (per unit) of 1.004 (95% CI, 0.999–1.009; odds ratio/100 pmol/L decrease, 1.003 (95% CI, 1.001–1.005) for periventricular WML, and an OR (per unit) of 1.003 (95% CI, 0.998–1.007; odds ratio/100 pmol/L decrease, 1.441; 95% CI, 0.881–2.358) for deep WML.

Table 3 shows the results of the multivariate analyses. Apart from age and a family history of vascular disease, vitamin B12 level showed a statistically significant association with periventricular WML (P = 0.034), but not with deep WML (P = 0.146).

Folate levels (mean, 16.4 nmol/L) in 100 patients with folate measured did not relate to vitamin B12 level (correlation coefficient, 0.01; P = 0.94).

### Discussion
First, we found that that low vitamin B12 levels in our patients with a first lacunar stroke were associated with periventricular WML, but not with deep WML severity. Associations were statistically significant in univariate and multivariate ordered logistic regression analyses, and also when vitamin B12 level was dichotomized with the lower reference value of 150 pmol/L as cutoff. Our second finding
Table 3. Ordered Multivariate Logistic Regression Analysis With WML as Dependent (ordinal) Variable

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Periventricular WML</th>
<th>Deep WML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/yr</td>
<td>1.085 (1.050–1.123)</td>
<td>1.069 (1.037–1.103)</td>
</tr>
<tr>
<td>Female vs male</td>
<td>1.201 (0.580–2.484)</td>
<td>1.433 (0.713–2.881)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.397 (0.644–3.031)</td>
<td>1.071 (0.153–2.239)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.641 (0.230–1.782)</td>
<td>0.687 (0.257–1.831)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>0.993 (0.724–1.362)</td>
<td>0.811 (0.561–1.171)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.728 (0.501–1.057)</td>
<td>1.441 (0.881–2.358)</td>
</tr>
<tr>
<td>Vascular family history</td>
<td>1.647 (1.005–2.699)</td>
<td>1.568 (1.083–2.270)</td>
</tr>
<tr>
<td>Vitamin B12 level/unit decrease</td>
<td>1.006 (1.001–1.011)</td>
<td>1.004 (0.999–1.009)</td>
</tr>
<tr>
<td>Vitamin B12 level/100-unit decrease</td>
<td>1.773 (1.001–3.003)</td>
<td>1.441 (0.881–2.358)</td>
</tr>
</tbody>
</table>

was that the mean vitamin B12 level was rather low when compared with the reference data.

So far, vitamin B12 has been mainly related to cerebrovascular disease by its effect on lowering serum homocysteine (HCys) levels.6,7 Elevated HCys levels are considered to damage vascular endothelium and have, apart from stroke, been associated with WML.8,9 However, vitamin B6 and folate also influence HCys levels, but analyses on the relation between these vitamins and WML were not accounted for vitamin B12 levels in studies so far. Furthermore, lowering serum HCys levels is not the only mode of action of vitamin B12. In an experimental rat model of vitamin B12 deficiency, Scalabrio10 found an increase in myelinolytic tumor necrosis factor-α and a decrease of the neurotrophic agents epidermal growth factor and IL-6, which lead to intramyelinic and interstitial edema in the spinal marrow. These findings comply with the histopathologic findings of myelin rarefaction in periventricular WML.10,11 Therefore, vitamin B12 deficiency may assert an effect on the cerebral white matter apart from elevated HCys.12 Although we did not measure HCys, considering the high folate level in 100 cases, low folate-induced elevated HCys levels can be considered highly unlikely in our series.

The fact that we found only an association with the periventricular white matter is in line with the idea that periventricular and deep WML may have different causes, different clinical consequences, and different progression rates.1,10,13–19 However, the rather small size of our study does not rule out any association between vitamin B12 and deep WML.

The weak, although statistically nonsignificant, relation between hypertension and WML severity that we found may relate to the relative small study sample size, or to blood pressure-lowering therapy, because any effect of such therapy does not undo the diagnosis of hypertension. Although statistically not significant, the direction of the association between WML and diabetes mellitus, hypercholesterolemia, and smoking may not be explained by something else than chance in our small series, because any biological explanation seems less plausible.
before any trial measuring potential therapeutic effect of whatever intervention on the development or progression of WML and its clinical consequences should be further attempted.

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**Disclosures**
None.

**References**
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In the article by Pieters et al, “Periventricular White Matter Lucencies Relate to Low Vitamin B12 Levels in Patients With Small Vessel Stroke,” which was published ahead-of-print on March 12, 2009, and appeared in the May issue of the journal (Stroke. 2009;40:1623–1626) a correction was needed.

Methods: MRI scan parameters: 1.5 or 3.0 Tesla.

The authors became aware of the use of different MRI field strengths in their patient population after publication of the results. The authors don’t think that this affected the severity rating of white matter lesions in this study, because the Fazekas’ scale was used, which does not count individual lesions nor volume. Any increase in white matter lesion detection (due to higher field strength) would unlikely have resulted in a higher category of white matter lesion extension in the Fazekas’ scale. The authors conclude that the results and conclusions as published remain valid.