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

Barbe Pieters, MD; Julie Staals, MD; Iris Knottnerus, MD; Rob Rouhl, MD; Paul Menheere, MD, PhD; AIFons 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 that underlies ischemic 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;40:00-00.)

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 before4: 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 >150/90 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; Powtrak 6000 Philips; scan parameters: 1.5 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 scale5: 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...
Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Category</th>
<th>N of patients</th>
<th>Male/Female</th>
<th>Mean age (SD), yr</th>
<th>Vitamin B12, median and range, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>N of patients</td>
<td>124</td>
<td>75/49</td>
<td>66.0 (11.9)</td>
<td>196 (52–431)</td>
</tr>
<tr>
<td>Male/Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periventricular WML</td>
<td>78</td>
<td>62.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep WML</td>
<td>85</td>
<td>68.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>75</td>
<td>60.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>49</td>
<td>39.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol &gt;5 mmol/L</td>
<td>97</td>
<td>78.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>51</td>
<td>41.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular family history</td>
<td>68</td>
<td>54.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

excluded, leaving 124 patients for the study. Vitamin B12 was assayed using a solid-phase time-resolved fluoroimmunoassay on an Auto Delfia immunoanalyzer (PerkinElmer). In the onchip 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 antinmouse 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 14683 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 frozen and 2 lyophilized controls is ∼5% for concentrations ∼200 pmol/L and ∼7% for concentrations ∼700 pmol/L. Among 45 of the eligible patients who refused participation, there were more women (30/45) and their age was older (73.0; SD, 11.8 years), but they did not differ in vascular risk factors or stroke severity (Canadian Stroke Severity Scale) from study patients.

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, 1.773; 95% CI, 1.001–3.003) for periventricular WML, and an OR (per unit) of 1.003 (95% CI, 0.998–1.007; odds ratio/100pmol/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
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. Elevated HCys levels are considered to damage vascular endothelium and have, apart from stroke, been associated with WML. However, vitamin B6 and folate also influence HCys levels, but analyses on the relation between these vitamins and WML were not accounted for in 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, Scalabrio 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. Therefore, vitamin B12 deficiency may assert an effect on the cerebral white matter apart from elevated HCys. 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. 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.

Table 3. Ordered Multivariate Logistic Regression Analysis With WML as Dependent (ordinal) Variable

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Periventricular WML OR (95% CI)</th>
<th>Deep WML OR (95% CI)</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>0.709 (0.501–1.003)</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>

Our study has shortcomings. First, we did not measure HCys or methyl malonic acid as a measure of biological significance of the measured vitamin B12 level. However, defining vitamin B12 activity by its effect on HCys levels limits the detection of consequences of vitamin B12 deficiency apart from this effect. Second, not all our consecutive lacunar stroke patients entered the study. Those who did were younger, which would rather bias toward an underestimation of the relation we found, because older age relates to WML, and also to lower vitamin B12 levels. Third, vitamin B12 measurements were performed early in some and later than 3 months after stroke in others. However, vitamin B12 levels may not vary significantly over such period, apart from eventual prescribed parenteral treatment, which none of our patients received, whereas patient characteristics between these 2 groups did not differ, including vitamin B12 levels and the grading of WML. Fourth, our visual WML rating may be criticized, but such rating correlated acceptably with quantitative measurements. Fifth, in 19% we were unable to identify with certainty the symptomatic lacunar lesion, which may relate to short duration of symptoms (but at least >24 hours) in some, but also to the rather long MRI delay in some cases, blurring the distinction between recent and eventual concomitant old lacunar lesions. However, this did not lead to unrightfully included patients, because clinically they experienced lacunar stroke. Sixth, using only T2 and FLAIR images, we may not always with certainty have distinguished a fresh, small, deep infarct from a small area of WML, especially in the deep white matter regions. However, in such cases would have been only 1 small WML area added to the total of WML to estimate the WML category, which would unlikely have resulted in a significant estimate change. Finally, the absence of a control group did not allow us to conclude on any specific relation between vitamin B12 deficiency and symptomatic small vessel stroke. However, this was not our primary aim, although others found such association at least for stroke in general. Some studies indicated that vitamin B12 levels show circadian variation and may vary over time. However, the estimation of the degree of such variation may not be reliable because of small patient numbers in the studies. We have no repeated B12 measurements.

Because vitamin B12 has been implicated in maintaining the integrity of the blood–brain barrier, deficiency may lead to blood–brain barrier damage, which has been suggested to be an early phenomenon in the development of small vessel disease leading to WML. Our data and those of others suggest that such effect may be largely independent of vitamin B12 effect on HCys lowering. Vitamin B12 may be one of the hitherto unknown, or rather neglected, factors in the “complex relationship between WML and cognition.” Because both parenteral and even daily high oral-dose vitamin B12 raises low serum levels to normal values, such therapy may eventually lower the chance of WML occurrence or slow its progression. However, considering the discussed shortcomings, the small size of our study, and the marginal degree of statistical significance in the tested associations, our findings need confirmation. The role of vitamin B12 in cerebral small vessel disease should further be clarified.
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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