Vitamin B12 and folate concentrations in serum and cerebrospinal fluid of neurological patients with special reference to multiple sclerosis and dementia

T Q Nijst, R A Wevers, H C Schoonderwaldt, O R Hommes, A F J de Haan

Abstract
Vitamin B12 and folate concentrations were measured in serum and cerebrospinal fluid (CSF) in 293 neurological patients. Serum and CSF vitamin B12 concentrations showed a positive correlation. In individual patients CSF B12 concentrations varied considerably for a given serum concentration. The median serum vitamin B12 concentration of the Alzheimer's type dementia group was significantly lower compared with that of a control group. Lower median CSF vitamin B12 concentrations were found in groups of patients with multiple sclerosis and Alzheimer's type dementia. Five patients with heterogeneous clinical pictures had unexplained low serum and CSF B12 concentrations without macrocytosis. Two patients had very high serum B12 and low-normal CSF concentrations which could be explained by a blood-brain barrier transport defect. Serum and CSF folate concentrations did not show significant differences between the various groups.

Vitamin B12 deficiency may cause neurological complications.1 These complications can appear independently of possible haematological changes.2 During the 1950s and 60s, several studies3-6 were carried out on vitamin B12 concentrations in multiple sclerosis because of the suspected role of this vitamin in myelin formation. Using the now obsolete microbiological assay techniques, the results were conflicting and interest waned.

Recently Reynolds8 discussed a possible relation between low serum B12 concentrations and multiple sclerosis. The connection between low serum vitamin B12 concentrations and dementia has been investigated more recently. Cole9 and Karnaze10 independently found significantly lower serum B12 concentrations in Alzheimer's type dementia. CSF vitamin B12 levels were not determined in their studies. It is important to recognise this relation because there are indications that some of the patients may improve after receiving high doses of parenteral vitamin B12.11

To examine the relation between vitamin B12 and neurological disorders, cerebrospinal fluid (CSF) vitamin B12 could provide important additional information. The vitamin B12 status of the brain may be better represented by the CSF vitamin B12 concentration. This study analysed retrospectively the vitamin B12 and folate concentrations in serum and CSF in 293 neurological patients.

Patients
Since January 1985 the investigation of neurological disorders of unknown origin in our department has included the determination of vitamin B12 and folate concentrations in serum and CSF. In October 1987, 337 patients (age > 10 years) have had retrospectively such investigation and they form the basic frame of this study. We excluded from the study patients receiving vitamin B12 or folate medication and patients with elevated amounts of erythrocytes, free haemoglobin (> 0.10 μmol/l) or bilirubin (> 0.15 μmol/l) in the CSF. Others not included were patients who had partial gastrectomy or a history of pernicious anaemia. Thus 293 patients remained (129 males and 164 females, age range: 11–83 years). Almost all patients were non-institutionalised and receiving symptomatic drug treatment. The patients were divided into the following groups:

1) Multiple sclerosis: 58 definite MS patients.
2) Myelopathy: 15 patients (posterior column and/or pyramidal tract lesions of unknown cause).
3) Cervical spondylosis (3), arteriosclerosis of the spinal cord (2), radiation myelopathy (1), Friedrich's disease (1), Paget's disease (1).
4) Alzheimer's-type dementia: 10 patients.
5) Non-Alzheimer's-type dementia: 15 patients (organic brain syndrome (4), multi-infarct dementia (5), normal pressure hydrocephalus (5), Creutzfeldt Jakob disease (1)).
6) Reference group: 157 patients with a neurological disease from which no association with low vitamin B12 or low folate concentrations is known.

A heterogeneous group of 38 patients remained and was not included in the calculations. These patients had neurological disorders that can be caused by low vitamin B12 or folate concentrations (subacute combined system degeneration, metabolic encephalopathy, polyneuropathy, epilepsy and depression).

Methods
Fasting CSF samples for diagnostic purposes were obtained by lumbar puncture between...
8am and 9am. Vitamin B12 was measured with the Quantaphase B12/folate radio assay (Biorad 1911002, California, USA), using immobilised affinity-purified porcine intrinsic factor and folate binding proteins. The method was carried out according to the instructions of the manufacturer with the following exceptions: CSF samples (4 ml) were evaporated until dryness in a Vortex evaporator/Buchler Instruments Inc, New York, United States and subsequently taken up in 0.5 ml of the Bio-rad zero standard. In the assay only 50 μl instead of 100 μl of the microbead reagent were used, both for blood and CSF determinations. After a one hour incubation period and subsequent centrifugation, we added a washstep with 1 ml saline. Samples were always determined in duplicate. Two additional standards (18 and 37 pmol/l) were used for the low vitamin B12 concentration range. For the determination of CSF folate the sample was diluted with the Bio-rad zero standard (50% v/v).

Statistics
Using an appropriate linear model, contrast tests were applied for testing equality of slopes of the regression equations. For comparing the levels of the parameters between the first five groups, a Kruskal-Wallis test was performed with confidence level alpha = 0.05, followed (in case of a significant result) by two tailed Wilcoxon tests with confidence level alpha = 0.025, correcting for multiple comparisons.

Results
Age and sex dependency
In the reference group, neither vitamin B12 and folates serum and CSF concentrations nor CSF:serum ratios changed significantly with increasing age, B12 and folate concentrations and CSF:serum ratios were not sex-related.

Correlation between serum and CSF concentrations
As shown in table 1, there is a positive correlation between serum and CSF vitamin B12 concentrations in the various groups. In individual patients, however, CSF B12 concentrations vary considerably for a given serum B12 concentration. The regression equations of the various groups do not differ significantly compared with the regression equation of the reference group. The intercepts do not deviate significantly from zero.

Serum and CSF folate concentrations showed a positive correlation (all patients with serum folate < 35 nmol/l: \( r = 0.43, p < 0.01 \); regression equation: \( Y = 0.032X + 0.40 \) with \( Y = \text{CSF} \text{ folate} \) and \( X = \text{serum folate} \) concentration).

Differences between the diagnostic groups
Table 2 shows the median and the 5%, 50%, and 95% range of the vitamin B12 and folate concentrations in CSF and serum of the various groups and the CSF:serum ratio of both vitamins. The median serum B12 concentration in the Alzheimer’s type dementia group was lower than in the reference group \( (p = 0.016) \). In one multiple sclerosis group \( (P = 0.014) \) and the Alzheimer’s type dementia group \( (P = 0.024) \) had lower median CSF B12 concentrations compared with that of the reference group.

Serum and CSF folate concentrations did not show significant differences between the various groups.

Individual patients
Table 3 shows some individual patients with their neurological diagnoses, who deserve further attention. The 13 patients with a low CSF:serum ratio for vitamin B12 (below 0.41 = 5%, value of the reference group) had various neurological disorders (table 3A). In 11 cases the low ratio could be explained by a low serum B12 concentration and in two cases by an extremely high serum B12 concentration. In all, six patients had a serum vitamin B12 concentration above 700 pmol/l (table 3B) although none of them was known to have a disorder which could be responsible for such a high vitamin B12 concentration. Twenty seven patients had a serum and/or CSF vitamin B12 concentration below the 5% value of the reference group. None of them had macrocytic anaemia (MCV range: 81–96 fl. and Hb range: 7–9–8 mmol/l). Only five patients had both a serum concentration below 142 pmol/l and a CSF concentration below 2–1 pmol/l. Their neurological disorders are shown in table 3C.

Discussion
Lazar12 concluded that there is no correlation between serum and CSF vitamin B12 levels. This study, however, showed a clear relationship between both concentrations \( (r = 0.61 \)

<table>
<thead>
<tr>
<th>Number</th>
<th>Correlation coefficient (Pearson)</th>
<th>Regression equation</th>
<th>Significance of the correlation (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>153</td>
<td>0.58</td>
<td>Y = 0.032X + 0.40</td>
</tr>
<tr>
<td>MS</td>
<td>57</td>
<td>0.66</td>
<td>Y = 0.024X + 0.05</td>
</tr>
<tr>
<td>ALZ DEM</td>
<td>10</td>
<td>0.41</td>
<td>Y = 0.019X + 1.68</td>
</tr>
<tr>
<td>NON A DEM</td>
<td>15</td>
<td>0.78</td>
<td>Y = 0.026X + 0.90</td>
</tr>
<tr>
<td>MYEL</td>
<td>14</td>
<td>0.59</td>
<td>Y = 0.019X + 4.38</td>
</tr>
<tr>
<td>ALL</td>
<td>249</td>
<td>0.61</td>
<td>Y = 0.029X + 0.90</td>
</tr>
</tbody>
</table>

*Y = CSF B12, X = SERUM B12
REF = Reference group, MS = Multiple sclerosis group, ALZ DEM = Alzheimer’s type dementia group, NON A DEM = non Alzheimer’s type dementia group, MYEL = Myelopathy group.
and P < 0.01). A correlation between serum and CSF folate levels has been described previously.\textsuperscript{13}

There have been previous studies on serum vitamin B12 concentrations in dementia. Karnaze\textsuperscript{10} reported a 29% prevalence of subnormal serum vitamin B12 concentrations (<180 pmol/l) in 17 patients with primary degenerative dementia. Cole\textsuperscript{8} found a 30% rate (serum B12 < 150 pmol/l) in 20 patients with Alzheimer's type dementia. In both studies this prevalence was higher than in the group with other forms of dementia. The mean serum vitamin B12 concentration of the group with primary degenerative dementia was lower than that in the group with the other forms of dementia.

Our study can partly confirm the results of Cole and Karnaze. In the group of patients with Alzheimer's type dementia we found a 30% prevalence of serum B12 concentrations below 155 pmol/l (which is the 10% value of the reference group) against 13% in the group with "Non Alzheimer's type dementia".\textsuperscript{14} This difference, however, was not significant. Median serum and CSF vitamin B12 concentrations in the Alzheimer's type dementia group were significantly lower compared with those of the reference group and tended to be lower than those of the non Alzheimer's type dementia group. These findings stress the importance of the vitamin B12 status of the brain in Alzheimer's type dementia and may justify vitamin B12 supplementation in these patients.

Renvoize\textsuperscript{14} reported a 44-8% prevalence of folate deficiency in 150 patients with a diagnosis of dementia. Reynolds\textsuperscript{15} found a significant increase of organic brain syndrome and pyramidal tract damage in a group of patients with folate deficiency. In our study the serum and CSF folate concentrations were not significantly different between the groups.

Bauer\textsuperscript{1} and O'Connor\textsuperscript{2} found reduced serum vitamin B12 concentrations in patients with multiple sclerosis using microbiological assay techniques (MBA). Basil\textsuperscript{3} and Schrumpl\textsuperscript{4} described slightly lower CSF B12 concentrations (MBA) in patients with multiple sclerosis. Our study, using a RIA technique to determine vitamin B12 concentrations, showed a lower median CSF B12 concentration in the group of patients with multiple sclerosis (P = 0.014). The overlap in CSF vitamin B12 levels between the multiple sclerosis group and the reference group is such that CSF vitamin B12 cannot be used as a diagnostic parameter in multiple sclerosis.

The median CSF:serum ratio and the regression equations of the various groups did not differ significantly. This reflects an undisturbed blood-brain barrier function. Two

<table>
<thead>
<tr>
<th>Table 2 Vitamin B12 (pmol/l) and folate (nmol/l) concentrations in serum and CSF (median and 5th-95th range)</th>
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<tbody>
<tr>
<td>Vitamin B12</td>
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<td>-----------------------------------------------</td>
</tr>
<tr>
<td>REFERENCE</td>
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<tr>
<td>MS</td>
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<tr>
<td>0-9-22</td>
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<tr>
<td>MYELOPATHY</td>
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<td>ALZH DEM</td>
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<tr>
<td>NON A DEM</td>
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<td>FOLATE</td>
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<td>1.5-19.4</td>
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*P = < 0.025 versus reference.

MS = Multiple Sclerosis, ALZH DEM = Alzheimer's type dementia, NON A DEM = non Alzheimer's type dementia.

<table>
<thead>
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<th>Table 3 Neurological diagnosis</th>
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<tr>
<td>Sex</td>
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<tr>
<td>A Patients with low CSF:Serum vitamin B12 ratios. (ratio &lt; 0.01, CSF and serum vitamin B12 in pmol/l)</td>
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<tr>
<td>1</td>
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<td>B Patients with serum vitamin B12 level above 700 pmol/l</td>
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<tr>
<td>C Patients with low serum and CSF vitamin B12 levels (Serum &lt; 142 pmol/l and CSF &lt; 2.1 pmol/l)</td>
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<tr>
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<tr>
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<td>4</td>
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<td>5</td>
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</tbody>
</table>
patients, however, had an extreme discrepancy between serum and CSF vitamin B12 values (table 3B: case 4 and 5) which could be explained by a blood-brain barrier transport defect.

We thank M J T Jansen and A van Rens for their technical assistance.