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 Reynolds⁷ 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. (7), cervical spondylosis (3), arteriosclerosis of the spinal cord (2), radiation myelopathy (1), Friedreich's disease (1), Paget's disease (1)).
- 3) Alzheimer's-type dementia: 10 patients.
- 4) Non Alzheimer's-type dementia: 15 patients (organic brain syndrome (4), multi-infarct dementia (5), normal pressure hydrocephalus (5), Creutzfeldt Jakob disease (1)).
- 5) 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

**Institute of Neurology,
University Hospital
Nijmegen, Nijmegen**

T Q Nijst
R A Wevers
H C Schoonderwaldt
O R Hommes

**Department of
Statistical
Consultation,
University of
Nijmegen, Nijmegen,
The Netherlands**
A F J de Haan

Correspondence to:
Dr H C Schoonderwaldt,
Institute of Neurology,
University Hospital
Nijmegen, Box 9101, 6500
HB Nijmegen, The
Netherlands

Received 29 March and in
final revised form 8 January
1990.

Accepted 23 January 1990

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 μ l instead of 100 μ l 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: $r = 0.53$, $p < 0.01$; regression equation: $Y = 0.93 X + 14.97$ with $Y = \text{CSF folate}$ and $X = \text{serum folate concentration}$).

Differences between the diagnostic groups

Table 2 shows the median and the 5th–95th 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 ($p = 0.016$). Both the 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 0.01 = 5th value of the reference group) had various neurological disorders (table 3A). In 11 cases the low ratio could be explained by a low CSF 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 5th value of the reference group. None of them had macrocytic anaemia (MCV range: 81–96 fl. and Hb range: 7.8–9.8 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 1 The correlation between serum and CSF vitamin B12 concentrations. (Patients with serum vitamin B12 below 700 pmol/l. N = 249)

	Number	Correlation coefficient (Pearson)	Regression equation*	Significance of the corr/coefficient (P)
REF	153	0.58	$Y = 0.032 X + 0.40$	<0.01
MS	57	0.66	$Y = 0.024 X + 0.95$	<0.01
ALZ DEM	10	0.41	$Y = 0.019 X + 1.68$	0.24
NON A DEM	15	0.78	$Y = 0.026 X + 0.90$	<0.01
MYEL	14	0.59	$Y = 0.019 X + 4.38$	<0.05
ALL	249	0.61	$Y = 0.029 X + 0.90$	<0.01

*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.

Table 2 Vitamin B12 (pmol/l) and folate (nmol/l) concentrations in serum and CSF (median and 5°-95° range)

	Vitamin B12		
	CSF	SERUM	CSF/SERUM (× 100)
REFERENCE	8.7	284	3.0
MS	2.1-22.9	142-541	1.0-6.6
	6.9*	255	2.8
MYELOPATHY	0.9-22.5	124-618	0.5-6.4
	11.4	349	3.8
ALZH DEM	4.3-41.9	177-835	1.1-5.0
	6.3*	229*	2.6
NON A DEM	0.0-9.2	125-320	0.0-4.9
	7.6	286	2.7
	1.5-19.4	109-614	1.1-7.5
	FOLATE		
	CSF	SERUM	CSF/SERUM
REFERENCE	24.8	10.4	2.4
MS	14.4-42.1	5.5-19.1	1.3-4.3
	27.0	10.7	2.3
MYELOPATHY	14.4-38.0	6.1-22.4	1.4-3.6
	22.2	8.4	2.4
ALZH DEM	13.2-39.4	5.7-24.0	1.2-3.8
	23.5	10.1	2.4
NON A DEM	14.0-36.2	4.7-19.4	1.7-3.4
	21.2	9.5	2.4
	10.4-33.4	3.6-19.7	1.0-3.6

*P = <0.025 versus reference.

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

and $P < 0.01$). A correlation between serum and CSF folate levels has been described previously.¹³

There have been previous studies on serum vitamin B12 concentrations in dementia. Karnaze¹⁰ reported a 29% prevalence of sub-normal serum vitamin B12 concentrations (<180 pmol/l) in 17 patients with primary degenerative dementia. Cole⁹ 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". 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.

Renvoize¹⁴ reported a 44.8% prevalence of folate deficiency in 150 patients with a diagnosis of dementia. Reynolds¹⁵ 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.

Bauer⁴ and O'Connor⁵ found reduced serum vitamin B12 concentrations in patients with multiple sclerosis using microbiological assay techniques (MBA). Basil³ and Schrupf⁶ 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 3 Neurological diagnosis

	Sex	Age (years)	Neurological diagnosis	CSF	Serum	Ratio (× 100)
A Patients with low CSF:Serum vitamin B12 ratios. (ratio <0.01, CSF and serum vitamin B12 in pmol/l)						
1	M	32	Multiple sclerosis	<0.5	297	<0.17
2	F	42	Parkinson's disease	<0.5	190	<0.26
3	F	64	Alzheimer's type dem.	<0.5	125	<0.40
4	M	74	Undiagnosed pain	5.9	>1475	<0.40
5	F	34	Multiple sclerosis	0.9	211	0.43
6	F	42	Multiple sclerosis	0.7	153	0.46
7	M	54	Shy Drager syndrome	7.6	1440	0.53
8	F	67	Vertebrobasilar ischaemia	1.0	171	0.58
9	M	85	Brain contusion	2.5	351	0.71
10	F	18	Cerebellar ataxia	2.1	264	0.80
11	M	44	Locomotor ataxia	1.7	178	0.96
12	F	31	Multiple sclerosis	1.8	183	0.98
13	F	24	Multiple sclerosis	2.4	242	0.99
B Patients with serum vitamin B12 level above 700 pmol/l						
1	F	38	Pyramidal tract lesion of unknown cause	41.9	835	5.02
2	F	53	Multiple sclerosis	29.7	925	3.21
3	F	78	Gait disturbances	31.1	1129	2.75
4	M	54	Shy Drager syndrome	7.6	1440	0.53
5	M	74	Undiagnosed pain	5.9	>1475	<0.40
6	M	49	Parkinson's disease	59.8	>1475	<4.05
C Patients with low serum and CSF vitamin B12 levels (Serum <142 pmol/l and CSF <2.1 pmol/l)						
1	F	64	Alzheimer's type dem.	<0.5	125	<0.40
2	M	59	Creutzfeldt-Jakob disease	1.5	132	1.14
3	F	77	Vertebrobasilar ischaemia	1.8	101	1.78
4	F	36	Bulbar dysarthria	1.8	102	1.76
5	M	66	Spinal cord compression	2.0	75	2.67

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.

- 1 Chanarin I. *The megaloblastic anaemias*. Oxford: Blackwell, 1979:324–27.
- 2 Strachan RW, Henderson JG. Psychiatric syndromes due to avitaminosis B12 with normal blood and marrow. *Quart J Med* 1965;135:303–18.
- 3 Basil W, Brown JK, Matthews DM. Observations on vitamin B12 in serum and cerebrospinal fluid in multiple sclerosis. *J Clin Path* 1965;18:317–21.
- 4 Bauer H, Heinrich HC. Vitamin B12 metabolism in neurological disease. In: Folch-Pi J, ed. *Chemical pathology of the nervous system*. Oxford: Pergamon Press, 1961:120–25.
- 5 O'Connor JS, Davis RL, Langworthy OR. B12 metabolism and multiple sclerosis. *Proc Soc Exp Biol Med* 1960;103:180–83.
- 6 Schrupf E, Bjelke E. Vitamin B12 in the serum and the cerebrospinal fluid. *Acta Neurol Scand* 1970;46:243–48.
- 7 Reynolds EH, Linnell JC. Vitamin B12 deficiency, demyelination, and multiple sclerosis. *Lancet* 1987;920.
- 8 Reynolds EH, Linnell JC. Multiple sclerosis and vitamin B12 metabolism—a new clue. *J Neurol Neurosurg Psychiatry* 1988;51:739.
- 9 Cole MG, Prchal JF. Low serum vitamin B12 in Alzheimer-type dementia. *Age and Ageing* 1984;13:101–5.
- 10 Karnaze DS, Carmel R. Low cobalamin levels in primary degenerative dementia. *Arch Intern Med* 1987;147:429–31.
- 11 Gross JS, Weintraub NT, Neufeld RR, Libow LS. Pernicious anemia in the demented patient without anemia or macrocytosis. *J Am Geriatr Soc* 1986;34:612–14.
- 12 Lazar GS, Carmel R. Cobalamin binding and uptake in vitro in the human central nervous system. *J Lab Clin Med* 1981;97:123–33.
- 13 Reynolds EH, Gallagher BB, Mattson RH. Relationship between serum and cerebrospinal fluid folate. *Nature* 1972;240:155–57.
- 14 Renvoize EB, Gastkell RK, Klar HM. Results of investigations in 150 demented patients consecutively admitted to a psychiatric hospital. *Br J Psychiatry* 1985;147:204–5.
- 15 Reynolds EH, Rothfeld P, Pincus JH. Neurological disease associated with folate deficiency. *Br Med J* 1973;2:398–400.