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Neuron-specific Enolase in Cerebrospinal Fluid of Patients with Metastatic and Non-metastatic Neurological Disease

Nathalie A.A. Beelen, Albert Twijnstra, Marjan van de Pol and Paul P.C.A. Menheere

Neuron-specific enolase (NSE) activities were measured in cerebrospinal fluid (CSF) in 361 patients with various neurological diseases. CSF was collected as part of the diagnostic procedure both in the control group, which consisted of 189 subjects with low back pain, and in the patient group (172 patients). The mean CSF NSE level in 189 control subjects was 7.14 ± 1.94 μg/l. Slight elevations of CSF NSE (≥ 11.0 μg/l) were observed in 9 patients with non-malignant diseases and in 2 patients with malignant diseases. The findings of this study indicate that measurement of NSE in CSF cannot be used as an adjunctive diagnostic test for CNS metastases.
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INTRODUCTION

NEURON-SPECIFIC enolase (NSE) is a glycolytic enzyme found in the central nervous system (CNS) and in association with the amine precursor uptake and decarboxylation (APUD) system. It is expressed by neural and neuroendocrine cells [1–3]. Levels of NSE were found to be a marker in the sera of patients with malignant and non-malignant diseases [4–7]. In the search for better diagnostic methods, various biochemical markers have been reported to be significantly elevated in the cerebrospinal fluid (CSF) of patients with CNS metastases [8–11]. Few data are available concerning CSF NSE values in normal subjects and in patients with metastatic and non-metastatic diseases. Moreover, the results of some of these studies are conflicting [1, 12, 13].

The present study was set up to investigate the clinical usefulness of CSF NSE in the diagnosis of patients with leptomenigeal metastases (LMM).

PATIENTS AND METHODS

Controls

The patients in the control group had low back pain with or without leg pain, and were otherwise healthy. The group consisted of 189 subjects with an average age of 44.3 years, range 20.0–82.1. There were 113 males and 76 females. Myelography showed a lumbar disc herniation in 131 subjects and no abnormalities in the 58 remaining subjects.

Patients

The patient group consisted of 172 patients seen at the University Hospital during 1988 to 1990, with an average age of 55.1 years, range 15.0–84.7. There were 87 females and 85 males. We compared the CSF NSE levels in 12 patient groups (Table 1).

Correspondence to A. Twijnstra.
A. Twijnstra, N.A.A. Beelen and M. van de Pol are at the Department of Neurology; and P.P.C.A. Menheere is at the Department of Clinical Chemistry, University Hospital of Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands.
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Methods

CSF was collected by lumbar puncture (LP), with the patient in the lateral recumbent position and performed as a part of the regular clinical diagnostic procedure only. CSF samples were immediately analysed for protein, glucose, lactate dehydrogenase (LD) and cell counts, and were evaluated cytologically after cytocentrifugation. CSF NSE was frozen and kept at −70°C until analysed. All analyses were made without knowledge of the clinical diagnosis. For those patients who underwent several punctures, the first sample of CSF was used.

The protein and LD levels were determined in CSF using commercially available tests, according to the IFCC recommendations. The glucose content was determined using a glucose oxidase method on an ESAT 6660 analyser (Merck). NSE concentrations were measured by means of a commercially available radio-immunoassay (Pharmacia Diagnostics AB), using an antiserum raised in rabbit. Bound and free NSE were separated by adding a sheep antirabbit IgG coated on sepharose. The standards were calibrated against NSE purified according to a method described by Påhlman *et al.* [14]. The intra-assay coefficient of variation was better than 5.1%.

Statistical analysis

In evaluating the diagnostic value of CSF NSE the terminology described by Griener *et al.* [15] was applied. Initially, the mean plus two standard deviations (S.D.) was selected as a cut-off point.

The regression coefficient was computed to determine whether there is a correlation between CSF NSE and age. The strength of the linear correlation was determined by using the Pearson correlation coefficient.

RESULTS

Reference values for CSF NSE levels were calculated from the results obtained in the control subjects (Table 1). No differences in the mean and distribution of CSF NSE levels were observed in the control subjects with a normal total protein content (≤ 0.50 g/l) or in those with abnormal protein content (> 0.50 g/l) nor in CSF with normal erythrocytes count (≤ 2 per mm^3) and raised count (> 2 per mm^3) (Table 2). Therefore, we used all data of the controls for the calculation of the reference range of CSF NSE levels.

Table 1. Distribution of NSE in controls and patients with malignant and non-malignant neurological diseases

Group	*n*	Age (years)	Male/female	Mean (S.D.) (μg/l)	Range (μg/l)	Increased values* *n* (%)
Control group	189	44.3	113/76	7.14 (1.94)	2.9–14.0	6 (3.2)
Patient groups						
1. Brain metastasis	7	56.8	2/5	8.44 (2.07)	4.8–10.4	0 (0.0)
2. Epidural metastasis	16	65.0	10/6	6.86 (2.01)	2.5–10.0	0 (0.0)
3. Leptomeningeal metastasis	21	53.2	5/16	6.63 (3.90)	1.6–21.3	1 (4.8)
4. Primary tumours of the CNS	9	50.5	4/5	8.78 (5.60)	5.5–23.4	1 (11.1)
5. Other tumours without metastasis to CNS	33	56.3	17/16	6.73 (1.69)	3.7–10.7	0 (0.0)
6. Infections	21	40.2	11/10	7.23 (3.40)	3.8–15.0	3 (14.3)
7. Demyelinating disorders	11	52.6	4/7	7.89 (2.66)	4.3–13.2	2 (18.2)
8. Cerebrovascular accident	20	60.4	13/7	7.65 (2.48)	4.0–14.1	2 (10.0)
9. Neuropathy	18	53.6	12/6	7.58 (2.18)	3.8–12.1	1 (5.6)
10. Epilepsy	6	58.7	2/4	7.47 (2.44)	5.0–11.3	1 (16.7)
11. Extrapyramidal disorders	4	52.9	1/3	4.85 (2.16)	3.0– 7.9	0 (0.0)
12. Dementia	6	74.0	4/2	8.10 (1.59)	5.4–10.1	0 (0.0)

*Neuron-specific enolase level of ≥ 11.0 μg/l was considered to be increased. CNS, Central nervous system.

The mean CSF NSE level (±S.D.) in 189 control subjects was 7.14 ± 1.94 μg/l (Table 1). The upper limit of the normal range (±2 S.D.) was taken as 11.0 μg/l.

We found no difference in mean and distribution in females and males (Table 2). We found no correlation between age and CSF NSE level [regression coefficient 0.03 (95% confidence interval 0.01–0.05), Pearson correlation coefficient 0.18].

11 out of 172 patients had a raised CSF NSE level (≥ 11.0 μg/l) (Table 1). The range for CSF NSE values in the patient groups was 1.6–21.3 μg/l. Only 1 of the 15 subjects with leptomenigeal spread from solid tumours had an increased CSF NSE level. None of the 6 patients with LMM from haematological tumours had CSF NSE activities of 11.0 μg/l or over (Table 3). None of the patients with spinal epidural metastases and none of the patients with cerebral metastases showed pathological values of CSF NSE. CSF NSE levels were elevated in 1 out of 9 (11%) patients with primary CNS tumours, concerning a patient with a recurrent glioblastoma multiforma. None of the 33 patients with a primary tumour without metastasis to the CNS had raised CSF NSE levels. CSF NSE was found to be elevated in various non-malignant diseases (Table 1). However, the elevation was never statistically significant.

Table 2. Distribution of NSE in controls regarding protein content, erythrocytes count and sex

	Mean (μg/l)	S.D. (μg/l)	Mean ±2 S.D. (μg/l)
Protein			
Normal content (≤ 0.50 g/l)	6.96	1.85	3.26–10.7
Raised content (> 0.50 g/l)	7.47	2.06	3.35–11.6
Erythrocyte count			
Normal count (≤ 2 per mm³)	7.17	1.94	3.29–11.1
Raised count (> 2 per mm³)	7.08	1.95	3.18–11.0
Sex			
Male	7.43	1.94	3.55–11.3
Female	6.71	1.87	2.97–10.5

DISCUSSION

NSE is an enzyme which can be measured in various biological fluids by means of radioimmunoassay (RIA) techniques. The method of analysis described by Rider and Taylor is not comparable with the RIA nor the enzymoimmunoassay technique [16]. Although the control groups are comparable regarding age and sex distribution as well as neurological disorders, remarkable differences are observed in the mean and the distribution of CSF NSE. Royds *et al.* [1] found a mean (2 S.D.) of 0–8.0 μg/l. The mean (2 S.D.) found by Jacobi *et al.* was 2.8–19.8 μg/l and Cutler calculated a mean of 15.0 μg/l [12, 17]. In contrast to our study, the studies mentioned in Table 4 consisted of a rather small control group.

Some authors suggest that NSE levels are influenced by lysis of blood cells [1, 3]. We did not find any correlation between erythrocyte counts and CSF NSE, but we selected only those samples with < 100 erythrocytes/mm³. In just 1 case of proven leptomeningeal metastasis, CSF NSE level was found to be elevated. In patients with other metastases to CNS no sample had an increased level of CSF NSE. Raised CSF NSE levels found at diagnosis in 2 (10%) of our patients with a cerebrovascular accident and in 3 (14.3%) of our patients with meningitis,

Table 3. Classification of patients with leptomeningeal metastases from solid and haematological tumours

	n	No. with increased NSE*
Solid tumours	15	1
Breast cancer	10	
Lung cancer	4	
Primary CNS tumours	1	
Haematological tumours	6	0

* NSE level of ≥ 11.0 μg/l was considered to be increased.

was in agreement with other studies [1, 12 13, 18]. CSF NSE was found to be elevated in various neurological diseases. However, the difference in distribution of CSF NSE between the individual diseases was in no case statistically significant.

Our results suggest that the use of CSF NSE does not improve the diagnostic yield for CNS involvement in either systemic or primary CNS malignancies.

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Tumour Uptake of 57-Cobalt-bleomycin in Patients with Breast Cancer

L. Pace, G. D'Aiuto, C. Acampora, P. Oliviero, G. Botti, F. Tatangelo, M. Cerra and M. Salvatore

17 patients with breast carcinoma were studied with 57-cobalt-bleomycin scintigraphy. Scans showed increased tumour uptake in all patients. Results expressed as percentage of the injected dose (ID) normalised by the size of the tumour region (% ID/pixel) showed higher tumour uptake in patients with T3–T4 breast carcinomas ($n = 5$) than in patients with T1–T2 breast cancer ($n = 12$) ($8.4 \pm 0.55 \times 10^{-3}$ vs. $5.25 \pm 1.71 \times 10^{-3}$ % ID/pixel, respectively, $P < 0.05$). An inverse correlation between tumour uptake of 57-cobalt-bleomycin and progesterone receptor concentration was also found in all tumours tested ($r = -0.60$, $P < 0.05$, $n = 10$) and was confirmed in the group of patients with T2 breast carcinomas ($r = -0.89$, $P < 0.05$, $n = 6$). We conclude that a quantitative analysis of 57-cobalt-bleomycin uptake can give additional information suitable for the presurgical characterisation of a tumour.
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INTRODUCTION

RADIOLABELLED bleomycin is an agent used for tumour detection [1–5]. When given intravenously, radiolabelled bleomycin localises in tumours with a high tumour to normal tissue ratio [3, 6].

On the other hand, unlabelled bleomycin is clinically used in the treatment of several human tumours. DNA strand scission by bleomycin is believed to be responsible for its therapeutic effect. Although the mechanism of DNA damage by bleomycin is not completely understood, there is evidence of a specific recognition and cleavage of DNA by bleomycin [7, 8].