Age-Related Changes of Neuron-Specific Enolase, S-100 Protein, and Myelin Basic Protein Concentrations in Cerebrospinal Fluid

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Studies on cerebrospinal fluid (CSF) concentrations of neuron-specific enolase (NSE), S-100 protein, and myelin basic protein (MBP) in patients with neurological lesions indicate a quantitative relation between the degree of cell damage in the central nervous system (CNS) and the concentration of these CNS-specific proteins in CSF. Thus NSE, S-100, and MBP could be of use as markers for destructive processes in the CNS. We collected 937 specimens of CSF from children and adults (from newborns to age 91 years) who were undergoing a diagnostic lumbar puncture for several clinical indications. Of these, 79 samples from subjects ranging in age from 0.7 to 66 years could be used retrospectively to construct a reference interval according to our criteria. In these 79 samples no sex dependency existed. The relative increase of NSE, S-100, and MBP with age was similar (1% per year), suggesting a common underlying mechanism. These results emphasize the necessity of using age-matched reference values when the CNS-specific proteins are to be evaluated in neurological diseases. We also present three case histories to discuss the possible clinical relevance of the measurement of NSE, S-100, and MBP in children and adults.

Additional Keyphrases: reference interval · nervous-system disorders

The clinician dealing with neurological disorders has to answer three questions. Is there a disease involving the nervous system? If so, where is the disease located? What kind of disease is it, and what is its pathological nature? The first question is often the most difficult one to answer. To answer it, but also the third question, the assessment of damage to the central nervous system (CNS) by determination of neuron-specific enolase (NSE), S-100 protein (S-100), and myelin basic protein (MBP) in cerebrospinal fluid (CSF) may be helpful.

NSE, S-100, and MBP are regarded as nervous-system-specific proteins. NSE, the \( \gamma \)-isoenzyme of enolase, is a soluble cytoplasmic protein localized mainly in neurons (1). S-100, named after its solubility in 100% saturated ammonium sulfate at neutral pH, constitutes a major component of the cytosol predominantly of glial cells (2, 3). MBP is detectable in developing oligodendroglia and is bound to cellular membranes of central and to a lesser extent peripheral myelin (4, 5). Thus, increased concentrations of NSE, S-100, and MBP in CSF indicate CNS damage and may help to identify the cell type or part (neuron, glia, or myelin) affected by the pathological process. NSE, S-100, and MBP can now be measured in CSF with sufficient sensitivity by immunoassays (1-4). However, data on sex- and age-related reference values are lacking.

We measured the concentrations of NSE, S-100, and MBP in CSF from 937 children and adults, ranging in age from newborn to 91 years, who were undergoing a diagnostic lumbar puncture for several clinical indications. To establish possible sex- and age-related effects in the concentrations of NSE, S-100, and MBP, we retrospectively selected, according to certain criteria, 79 of the patients as a reference group.

We also present three case histories to discuss the possible clinical relevance of the measurement of NSE, S-100, and MBP in CSF.

Materials and Methods

Reference Group

From 1985 to 1988, 937 CSF samples were obtained from patients undergoing a diagnostic lumbar puncture for conventional clinical indications such as suspected CNS infection or neurological disorder.

Of each CSF sample, 0.5 mL was used for the present investigation. Retrospectively, we were able to use 79 of the samples (from 37 females and 42 males, ages 0.7–66 years) for the formation of reference ranges. These samples were selected according to the following criteria: no use of medication; no evidence of an organic neurological disorder, an inherited metabolic disease, or a malignant disease; and normal concentration of total protein in CSF. The diagnoses selected in this way, comprise predominantly somatoform disorders (6).

To get normal control subjects in a hospital population remains a problem: although we started with nearly 1000 cases, only 79 could be used for the formation of reference ranges. Relaxing our entry criteria led to qualitative differences in the reference ranges; e.g., when we included patients with migraine, the overall reference ranges significantly increased. So we used strict entry criteria.

Determinations in CSF

NSE in serum and CSF were determined by RIA according to instructions of assay manufacturer (Pharmacia Diagnostics AB, Uppsala, Sweden). This double-antibody assay contains human NSE as antigen and
rabbit anti-human antiserum. Sepharose–anti-rabbit IgG raised in sheep is used as precipitating reagent. The detection limit for NSE is 2 μg/L.

S-100 concentrations in CSF were determined by a particle-counting immunoassay (PACIA), essentially as described by Sindic et al. (3). The S-100 immunoassay kits were received from C.J.M. Sindic of the Department of Experimental Medicine, University of Brussels. The detection limit is 0.5 μg/L.

MBP was determined by a double-antibody RIA kit according to the instructions of the manufacturer (cat. no. DSL 1500; Diagnostic Systems Labs., Webster, TX). Human MBP (whole molecule) is used as the antigen and rabbit anti-human MBP as antiserum. Goat anti-rabbit gamma globulin is the MBP precipitation reagent. The detection limit is 0.2 μg/L.

Statistics

After logarithmic transformation, we performed a regression analysis to yield age-related reference intervals for NSE, S-100, and MBP. We estimated the median value and the reference limits as the 5th and 95th percentiles. P-values for sex and age dependency were calculated.

The scatter diagrams of NSE and MBP against age contained no clear outliers. The scatter diagram of S-100 against age contained one clear outlier (7 μg/L at age 7 years), which we removed before calculating the reference interval for S-100.

**Results**

**Age and Sex Dependency**

NSE, S-100, and MBP in CSF increase with age. Table 1 lists the age-related reference intervals (μg/L) and Figure 1 illustrates the NSE, S-100, and MBP concentrations as functions of age (years). The P-values for age-dependency were 0.03 for NSE, <0.001 for S-100, and 0.001 for MBP. The 95% confidence intervals for the relative increase of NSE, S-100, and MBP with age overlapped and were as follows (percentage increase per year): NSE, 0.1–1.4%; S-100, 0.4–1.5%; MBP, 0.5–1.9%.

There were no significant differences between males (n = 42) and females (n = 37) in age or in NSE, S-100, and MBP concentrations (0.20 < P < 0.80). Therefore, we combined the data for further analysis.

| Table 1. Reference Values (μg/L) for NSE, S-100, and MBP in CSF |
|---|---|---|---|---|---|---|---|
| Age, years | NSE | S-100 | MBP |
| | PS<sup>*</sup> | P50 | P95 | P5 | P90 | P95 | PS | P50 | P95 |
| 1 | 2.2 | 5.0 | 10.2 | 0.9 | 1.5 | 2.6 | 0.12 | 0.30 | 0.72 |
| 20 | 2.7 | 5.8 | 12.0 | 1.1 | 1.8 | 3.3 | 0.17 | 0.40 | 0.95 |
| 40 | 3.1 | 6.5 | 13.8 | 1.3 | 2.2 | 4.0 | 0.22 | 0.52 | 1.21 |
| 60 | 3.8 | 7.6 | 16.0 | 1.6 | 2.7 | 5.0 | 0.30 | 0.70 | 1.57 |

<sup>*</sup> P, percentile.

**Clinical Examples**

Serial analysis of NSE, S-100, and MBP in CSF has been recommended in the literature as a very sensitive but unspecific screening variable for pathological organic CNS processes (2, 4, 7–9). In our opinion, these measurements have even greater clinical relevance and potentials. To illustrate this, we present three case histories (Table 2).

**Transverse myelopathy.** Patients with an acute transverse myelopathy show distinct nervous-system-specific protein profiles in CSF, depending on the different...
underlying causes and corresponding to different prognosis.

Patient 1, with acute transverse myelopathy after infection with *Borrelia burgdorferi* treated with penicillin G, and patient 2, affected after adenovirus infection, show MBP values solely above the reference interval. The values of NSE and S-100 in the acute stage of the disease are within the respective reference intervals. This combination indicates acute and isolated demyelination. If this process terminates spontaneously (patient 2) or after treatment (patient 1), remyelination and clinical recovery occur. No neurological deficit remains because there was no irreversible damage to the neuron compartment.

In these cases, the determination of NSE, S-100, and MBP may serve to assess which compartment (neuron, glia, or myelin) is affected. By elucidating which functional structure is involved in an acute neurological disorder, the CNS-specific proteins can serve as a diagnostic but also as a prognostic tool.

*Panencephalitis.* In the CSF of a child (case 3) with an acute disease with fever, focal epilepsy, and hemiparesis, we found clearly increased values for NSE, S-100, and MBP. Such an acute and severe panencephalitis has to be treated as a herpes encephalitis. The remarkably high values of NSE correspond to a very bad prognosis when therapy is delayed. After therapy was started, NSE and S-100 values decreased in our patient (Table 2). Thirty days after the start of the therapy, MBP was still clearly above the reference interval, indicating ongoing demyelination.

In this case, the CNS-specific proteins may again serve as a diagnostic and a prognostic tool. Another possible use is for evaluation and measurement of the effect of therapeutic interventions.

**Discussion**

NSE, S-100, and MBP in these CSF samples increased with age from 0.7 until 66 years. It is remarkable that the relative increases of NSE, S-100, and MBP with age are similar. The 95% confidence limits of the relative increases of NSE, S-100, and MBP overlap. The median increase of the CNS-specific proteins is 1% per year.

We found no prior reports on age dependency of CNS-specific proteins in the literature.

A supposed age-dependent increase in blood–brain barrier permeability, resulting in an increase in CSF total protein with age, cannot be the explanation for the age-dependency of the CSF CNS-specific proteins. Because they originate from the nervous system, there is no transudation of these proteins from the serum to the CSF across the blood–brain barrier. At least three explanations for increases in NSE, S-100, and MBP with age are possible: (a) the age-dependency reflects increasing cell and myelin loss with age, (b) the NSE, S-100, and MBP concentrations in the cells and myelin increase with age, whereas the turnover of the cells and myelin remains constant, or (c) the same relative increase of NSE, S-100, and MBP (1% per year) with age could be the result of a common underlying mechanism, e.g., an increased half-life attributable to a reduced CSF bulk flow at older ages. The CSF bulk flow reportedly decreases with age (10), resulting in a synchronous increase of the concentrations of proteins (10–12).

Some investigators have reported about NSE, S-100, and MBP in CSF of children and adults in relation to various neurological diseases: head injury (2, 9); CNS tumors (4, 7, 8, 13–15); cerebral ischemia, including stroke (2, 7, 8, 14, 16); inflammatory diseases of the CNS, such as encephalitis or myelitis (3, 7, 13, 14); multiple sclerosis (3, 7, 8); Guillain–Barre syndrome (3, 7); epilepsies (8, 14); migraines (8); cervical myelopathy (7, 8); amyotrophic lateral sclerosis (7); Huntington disease (17); Creutzfeldt–Jacob disease (18); dementia (8); and subarachnoidal and intracerebral bleeding or hematoma (2, 7). We shall discuss the clinical potentials and restrictions of the CNS-specific proteins in relation to neurological diseases more in general.

Many modern imaging techniques yield important information concerning the status of the brain or spinal cord but cannot distinguish between irreversibly damaged brain or spinal cord (infarcts) on the one hand and reversible changes in viable tissue (edema) on the other. Although the neurological deficit depends on the size as well as on the site of the lesion—a small strategically placed lesion can lead to substantial clinical deficit—quantification of CNS-specific proteins may contribute to a more-detailed estimation of the actual structural and irreversible brain or spinal cord damage in various clinical situations (19). Therefore assessment of CNS-specific proteins can be of diagnostic and prognostic value (2, 7–9).

Markers of CNS cell damage can also be of clinical value in evaluating the effect of therapeutic measures to reduce cerebral cell damage caused by vascular reconstruction, hemodilution, hyperventilation, and treatment with barbiturates, calcium blockers, or mannitol (2).

Although the nervous-system-specific proteins are very sensitive indexes (13), normal values for NSE,
S-100, and MBP do not exclude disease. The time of CSF sampling in relation to the ictus of the cytolytic process is of crucial importance: in ischemic stroke patients, concentrations of NSE and S-100 were increased only between 18 h and 4 days after the ictus (2). In demyelinating diseases, MBP is cleared within 5–7 days after the ictus (5). Therefore, serial measurements can elucidate the dynamics of the pathological process in relation to therapy.

From our own clinical examples and review of the literature, we think the significance of the assessment of NSE, S-100, and MBP in clinical (child)neurology could be

- to help to determine if the disease is involving the nervous system;
- to differentiate between damage to the glia or neuron compartment (clinical symptoms and signs, neuro-radiology, and neurophysiology also contribute to this differentiation but in a less direct and specific way);
- to differentiate between irreversible damage or reversible changes in nervous tissue (in addition to a diagnostic value, there is a potentially prognostic one); and
- to help in evaluating the effect of treatment.

In this study we obtained reference values for NSE, S-100, and MBP in children and adults of different ages and both sexes. The availability of these age-matched reference values allows interpretation of CNS-specific proteins in CSF of patients with different neurological disorders, perhaps leading to a better understanding of their basic mechanisms.

In a second paper we intend to use age (but not sex) matching to investigate whether the CNS-specific proteins behave independently and show distinct profiles in various neurological disorders.

References