Neuron-Specific Enolase Concentrations in Blood as a Prognostic Parameter in Cerebrovascular Diseases

Henrike Schaarschmidt, MD; Hilmar W. Prange, MD; Hansotto Reiber, PhD

Background and Purpose  To investigate the clinical relevance of plasma concentrations of neuron-specific enolase (NSE) in patients with severe cerebrovascular diseases, serial analyses were performed during the first 10 days after the acute event.

Methods  Plasma samples taken from 61 patients (30 with brain infarction, 13 with intracerebral hemorrhage, 11 with cardiogenic hypoxia-ischemia, and 7 with myocardial infarction as control group) were analyzed for NSE concentration using an enzyme immunoassay. The time course of plasma NSE was correlated with clinical findings, clinical outcome, cranial computed tomography, intracranial pressure, and other laboratory data.

Results  In cases of hypoxia-ischemia there was close correlation between plasma NSE values during the first 72 hours and the clinical outcome. In brain infarction and intracerebral hemorrhage, high plasma NSE mostly indicated an unfavorable outcome, but low values did not permit a reliable prognostic estimation. In cases of cerebral infarction and intracerebral hemorrhage with secondary neuronal destruction (for example, due to malignant edema), increasing NSE concentrations in plasma preceded the change of clinical or other diagnostic parameters.

Conclusions  The course of plasma NSE levels is seen as a relevant parameter for assessing the prognosis of cerebral hypoxia-ischemia. Additionally, it may prove to be a useful tool for monitoring space-occupying brain infarctions and intracerebral hemorrhages and therefore may contribute to improved therapeutic management of severe cerebrovascular diseases.

Key Words  • blood • hypoxia • intracerebral hemorrhage • prognosis

Subjects and Methods

Patients  Table 1 summarizes information regarding the number of cases, disease, sex, mean age, and age range of all patients investigated in this study. The sex, age, volume of infarction or hemorrhage on cranial computed tomography (CT), Glasgow Outcome Scale (GOS) score, maximal and mean NSE concentration in plasma during the observation, and localization of the lesion of exemplary subjects are listed in Table 2. All patients were treated in the critical care units of the Departments of Neurology and Cardiology (University Hospital Göttingen).

Clinical Investigations and Therapy  Neurological state was investigated on admission according to a special protocol including, among others, the Glasgow Coma Scale score, then on a daily basis during treatment in the critical care unit, and at discharge from the hospital. The GOS score was also calculated. GOS scores are defined as follows: 1, death; 2, vegetative state; 3, severe disability; 4, moderate disability (but independent); and 5, good outcome.

Deterioration in the clinical condition included loss of brain stem reflexes, appearance of pupillary abnormalities, abnormal extensor mechanisms, and decrease in the level of consciousness. The findings were carefully registered using a special flow sheet.

Initial cranial CT and repeated control scans were performed during the course of the treatment. The volume of parenchymal lesions was computed in brain infarctions and intracerebral hemorrhages according to Steiner et al with the Evaluskop EVA 1 (Siemens Comp). Intracranial pressure was monitored in four cases.

Routine analyses of the blood included partial thromboplastin time, thrombin time, platelet count, hemoglobin, hematocrit, sodium, potassium, glucose, osmolality, creatinine, lipase, and amylase. For specific therapy, the following substances were used when clinically indicated: 10% glycerol infusions, 20% mannitol,
Neuron-Specific Enolase Assay

Neuron-specific enolase concentrations in plasma were measured with an enzyme immunoassay (EIA) (NSE EIA kit, Hoffmann La Roche). The monoclonal anti-human NSE antibodies were specifically for the gamma subunit. The assay was performed as described by Hoffmann La Roche, with five standards and one control (level II) together with the plasma samples. Samples with values above 200 ng/mL were diluted 1:2 with the zero standard. To a 25-μL sample with 250 μL anti-gamma-enolase solution (polyclonal) the antibody-coated bead (monoclonal anti-gamma-enolase) was added. After 60 minutes' incubation at 37°C the bead was washed carefully (EIA washer, Hoffmann La Roche), and 250 μL of anti-immunoglobulin G peroxidase conjugate was added. After 60 minutes' incubation at 37°C the bead was washed, and 250 μL substrate (o-phenylenediamine) was added. After an additional 30 minutes the reaction was stopped with 1 mL H₂SO₄. The absorbance of the samples was read in the EIA photometer (Hoffmann La Roche). The NSE concentration of blood samples was calculated directly with the EIA photometer program by interpolation of the standard dilution values.

Results

Reference Range and Precision of the Neuron-Specific Enolase Assay

The normal range in frozen plasma from 20 control patients from our Department of Neurology who were not suffering from vascular or inflammatory processes of the central nervous system was 2 to 20 ng/mL (mean ± 2 SD, 10.8 ± 4.5 ng/mL). NSE concentrations above 30 ng/mL were considered definitely pathological. Day-to-day imprecision was calculated using the control sample (39.6 ng/mL) in n = 10 series, with a coefficient of variation equal to 8.5%. Plasma samples must be centrifuged before freezing with a sufficiently high speed to avoid contamination with platelets. Unfrozen plasma had slightly lower values than frozen plasma or frozen and unfrozen serum (mean, 2 ng/mL).

Time Course of Neuron-Specific Enolase Concentrations After Brain Infarction

Fig 1 shows a representative time course of NSE concentrations from a 69-year-old patient with a large infarction in the anterior and middle cerebral artery territory who developed brain edema with subsequent brain death on the ninth postinfarction day. This case was representative of the severest type of brain infarction, which was characterized as a GOS score of 1.

The first blood analysis, 20 hours after the stroke, showed an increased value of 81 ng/mL, which returned to the normal range 4 hours later. Up to this point cranial CT merely revealed a diffuse slight hypodensity within the afflicted hemisphere but did not provide any information regarding the extent of neuronal destruction. NSE values from the following 2 days were normal. During the fourth day after admission a massive increase in NSE began. From this time on, NSE values were persistently high, with a maximal value at day 6 and a subsequent slight decrease during the following 3 days.

The most important aspect of this case was that a deterioration of the clinical-neurological condition did not become evident until 24 hours after the start of the increase in NSE concentration; ie, on the fifth day the pupillary reaction was diminished. Six days after the onset of stroke, the pupils were fixed to light, and electrocardiographic changes were noted as a result of diabetes insipidus, hyperglycemia, and extensor posturing was noted. This deterioration was induced by a malignant brain edema, which was seen on cranial CT on day 6. The patient died of brain death.

Correlation Between Neuron-Specific Enolase Concentration and Glasgow Outcome Scale

Fig 2 illustrates 30 cases of ischemic stroke and 13 cases of intracerebral hemorrhage stratified according to the clinically defined GOS scores. The most important results for the characterization of prognostic relevance are summarized for brain infarction in Table 3 and for intracerebral hemorrhage in Table 4.

Brain Infarction

**GOS = 1**

This group comprised 11 patients who died as a consequence of the primary insult or from the resulting cerebral edema. Ten of the 11 patients demonstrated pathological NSE concentrations (Fig 2a). Only 4 of the 11 patients had pathological NSE values within the first 24 hours after the stroke. The highest values (peaks) were found between 24 and 48 hours in 5 patients and between 48 and 72 hours in 4 patients. The increased NSE values persisted in 6 patients over 72 hours (Table 3). For 7 patients who...
Table 2. Maximal and Mean Neuron-Specific Enolase Plasma Concentration, Diagnosis, Glasgow Outcome Scale Score, and Other Clinical Data

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<th>Volume of Destruction, mL</th>
<th>GOS Score</th>
<th>Max/Mean NSE, ng/mL</th>
<th>Localization of Lesion</th>
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GOS indicates Glasgow Outcome Scale; Max, maximum; NSE, neuron-specific enolase plasma concentration; CK, creatine kinase; CK-MB, creatine kinase-MB; BI, brain infarction; ICH, intracerebral hemorrhage; CH-I, cerebral hypoxia-ischemia; MI, myocardial infarction; BA, basilar artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; and PRIND, prolonged reversible ischemic neurological deficit.

demonstrated one or more NSE peaks, NSE elevation was the first parameter to indicate a forthcoming deterioration of the cerebral condition. Only 1 patient had no NSE increase during the course of his disease. However, in this case three of the planned plasma samples could not be collected.

**GOS=3**

Twelve patients who survived with severe deficits from their ischemic stroke were classified with a GOS score of 3 (Fig 2b). Seven of these 12 patients demonstrated pathological NSE values of more than 30 ng/mL. The concentrations from the other 5 patients remained in the normal range. NSE increases occurred within 24 hours after the initial event in 3 patients. In 3 patients NSE peaks were recorded 24 to 48 hours and in 2 additional patients 48 to 72 hours after the stroke. In 6 of these 7 patients with increased NSE values the rise of NSE concentration in blood preceded the deterioration in clinical condition (Table 3). In 1 patient the NSE increase
Of the 7 patients who survived the intracerebral hemorrhage with a GOS score of 3, 5 had increased NSE values. NSE peaks were common 24 to 48 hours after the onset of the hemorrhage. The time course of NSE measurements for these patients is shown in Fig 2f. Despite massive neurological deficits, 2 patients had no increases in the NSE concentration in plasma.

GOS=4

Of the 2 patients with a GOS score of 4, 1 had an increase 4 hours after the initial event without any further NSE increases (Fig 2g).

Summary of Patients With Intracerebral Hemorrhage

Altogether, 9 of the 13 patients with intracerebral hemorrhage demonstrated NSE peaks 24 to 72 hours after the initial hemorrhagic event (Table 4), which in 8 cases preceded clinical evidence of neurological deterioration (deepening of coma or signs of a tentorial herniation syndrome). In 1 patient the NSE peak was recorded simultaneously with the worsening clinical condition.

Cerebral Hypoxia-Ischemia

The 11 patients in this group were all admitted after cardiopulmonary resuscitation due to cardiac arrest; they were comatose and required respiratory support on admission. Their clinical state was characterized by a Glasgow Coma Scale score of 3. Again, the patients were grouped according to their clinical outcome to establish NSE values as a prognostic parameter for clinical outcome.

GOS=1

Elevated NSE values on admission with further increases during the first 3 days were found in all 5 patients. The maximal NSE plasma concentrations were between 124 and 445 ng/mL (Fig 5). The time courses of NSE values from these patients are shown in Fig 3a. Two of these patients died within the first 1.5 days from cerebral hypoxia. Three patients who initially were in stable condition died of the sequelae of hypoxic cerebral damage at a later point. No patient recovered consciousness during the course of observation. However, 1 patient did attain a Glasgow Coma Scale score of 6 for a short period, whereas all others attained scores of 3.

GOS=3

Both patients of this group demonstrated increased NSE values on admission (time course in Fig 3b). In the beginning 1 patient had maximum NSE values of 95 ng/mL, which returned to normal on the third day. The other patient presented constantly elevated values of 41 to 50 ng/mL. Both patients attained consciousness with massive neurological deficits. Maximum NSE values for both patients were below 100 ng/mL (Fig 5).

GOS=5

All 4 patients of this group (Fig 3c), who were also in a coma on admission and required respiratory support (Glasgow Coma Scale score of 3), had normal NSE values (less than 30 ng/mL) in the first 24 hours and no subsequent increase. These patients recovered within hours or days. After a maximum of 5 days after cardiopulmonary arrest all neurological symptoms had disappeared.
Myocardial Infarction Patients as a Control Group

None of the 7 patients in this group required cardiopulmonary resuscitation, nor was there any evidence of central nervous system disturbances. With the exception of 1 patient who had borderline NSE values up to a maximum of 32 ng/mL, all other patients presented NSE concentrations below 30 ng/mL throughout the course of observation (Fig 4).

Maximal Neuron-Specific Enolase Concentration in Blood as a Prognostic Parameter

None of the survivors from all three groups—brain infarction, intracerebral hemorrhage, and hypoxia-ischemia—showed NSE concentrations above 115 ng/mL. In contrast, NSE data of patients who died after brain infarction or intracerebral hemorrhage were found to range between normal NSE plasma values and values up to 180 ng/mL, which indicates a great overlap between the maximal NSE values measured among patients who survived and those who died. Among the patients suffering from cerebral hypoxia-ischemia, NSE values of the survivors were markedly different from NSE values of those who died (Fig 5). NSE values above 120 ng/mL were seen only in patients who died. An increased chance for survival was associated with values below 100 ng/mL. The ranges of NSE values in both of these groups (124 to 445 ng/mL or 10 to 95 ng/mL) did not overlap. Together with the observation that cerebral hypoxia-ischemia with normal NSE values in the blood is associated with a 100% recovery of neurological functions, the early NSE value in the blood is a clear-cut prognostic parameter for clinical outcome of the patient after cerebral hypoxia-ischemia.

Correlation Between Neuron-Specific Enolase Concentration and Cranial Computed Tomography

There was no statistically significant correlation of the maximal NSE concentration measured during the course...
Correlation Between Neuron-Specific Enolase Concentration and Intracranial Pressure

Intracranial pressure monitoring was performed in 4 patients with infarction in the area of the middle cerebral artery, which was combined in 1 patient with an ipsilateral infarction in the area of the posterior cerebral artery. In all 4 patients, despite an initial intracranial pressure value below 30 mm Hg, increased NSE values were observed 48 to 72 hours (3 patients) or 24 to 48 hours (1 patient) after the initial event. The intracranial pressure increase to values above 30 mm Hg had a delay of up to 36 hours after the initial NSE increase (3 patients) and up to 6 days after the initial NSE increase (1 patient with moderately increased intracranial pressure).

Laboratory Parameters and Neuron-Specific Enolase Concentration

Using repeated hematologic examinations (partial thromboplastin time, thrombin time, platelet count, hemoglobin, hematocrit, sodium, potassium, serum osmolality, creatinine, glucose, lipase, and amylase), we were able to exclude platelet destruction with release of α2γ-enolase and hemoconcentration as an explanation for increased NSE levels.

Specific Therapy and Neuron-Specific Enolase Concentration

Some of the drugs required for therapy could lead to platelet instability and, at least theoretically, could cause an increase in basal NSE plasma values. There was no correlation between the amount of medication and the measured NSE concentration for any of the drugs used.

**FIG 3.** Line graphs show time courses of plasma neuron-specific enolase (NSE) concentrations in patients with cardiac arrest hypoxia in correlation to Glasgow Outcome Scale (GOS) score (panels a through c). The dotted line refers to the upper limit of the reference range with 30 ng/mL.
Discussion

The present study deals with the role of NSE as a parameter for estimation of parenchymal damage in severe cerebrovascular diseases, thereby monitoring the course of disease. It is important to have a sufficiently sensitive marker for brain damage that can be determined in blood instead of CSF, because blood samples can be taken more frequently and more independently of raised intracranial pressure than CSF samples. In addition, cerebral damage with increasing distance from CSF space shows decreasing CSF protein levels.14 Earlier investigations reported single determinations of plasma NSE would not be expected in cases of brain stem infarctions with impressive clinical dysfunctions because these are often elicited by small tissue lesions with a minor loss of neurons.

Nonetheless, the monitoring of NSE values over the first days may provide the clinician with valuable information with regard to rising intracranial pressure; this may be a useful tool for adequate timing and management of treatment to decrease intracranial pressure. NSE increases have frequently been seen at three distinct times (Fig 2). The first, very early rise in blood NSE occurs for only a few hours during the first day; it may reflect the initial cerebral damage. The more frequently noted second short-term NSE increase emerging from 24 through 72 hours afterwards mostly precedes a deterioration of the neurological state. The long-lasting third NSE elevation after approximately 48 hours up to several days (Tables 3 and 4) is of remarkable clinical relevance because it seems to be a very early indicator of secondary cell damage due to edema and rising intracranial pressure as documented by clinical course, cranial CT, and intracranial pressure measurements. In more than 80% of the investigated brain infarction and intracerebral hemorrhage patients these "second" and "third" NSE increases herald the clinical deterioration.

Starting the forced antiedematous therapy at this time may largely prevent brain parenchyma from secondary damage. According to several investigations,15-19 the secondary "delayed" cell death evolves from a sum of different factors. One of these factors is the growing cytotoxic and vasogenic edema leading to increased intracranial pressure, thus inducing a vicious circle. The latter may be averted by the correct timing of antiedematous treatment.

Patients with uncomplicated myocardial infarction were chosen as a control group because acute cell death also occurs in these patients and because to a certain extent they receive a treatment similar to that of stroke patients, eg, high-dose heparin (Fig 4). The absence of increased NSE plasma values with myocardial infarction excludes nonspecific nonneuronal cell death, local platelet activation within the necrotizing tissue, or high-dose heparin administration as the origin of the increased NSE levels in the plasma of brain infarction and intracranial hemorrhage patients. Monitoring the platelet count and creatinine levels and simultaneously discarding hemolytic samples were adequate to eliminate false high NSE values.

In contrast to brain infarction and intracerebral hemorrhage, hypoxia-ischemia after cardiac arrest shows a more unique pathology in that this event always impairs
the same areas of the brain, probably those with the most intense metabolism of oxygen and need for energy (Fig 3). Consequently, we observed a unique connection between the peak of pathological plasma NSE values and the clinical outcome in patients with hypoxia-ischemia, which is the most important result of this retrospective study. Values less than 30 ng/mL during the first 24 hours indicate a 100% recovery of neurological functions, whereas elevated or increasing values at that time parallel unfavorable outcome. Moreover, the time course of NSE concentrations during the first 5 days is relevant for the prognosis and reflects the extent of cerebral damage. All the patients in our study presenting with maximal NSE levels above 120 ng/mL during the first 3 days died (Fig 5). This figure was confirmed in an extended study which showed that all 18 patients with NSE values above 120 ng/mL within the first 5 days (maximal) died or survived in a persistent vegetative state (Schaarschmidt H, Prange HW. 1993. Unpublished data). NSE in blood is the earliest parameter for prognostic classification, earlier than any other method such as regularly repeated neurological examinations, neuroimaging (cranial CT or magnetic resonance imaging), or electrophysiological assays. The estimation of creatinine kinase-BB concentrations in serum and CSF was far less reliable for prognostic discrimination because of the large overlap between the patient groups with different outcomes. In clinical practice we analyze the patient's blood as early as possible after the hypoxic event (within the first 24 hours) and then once per day each morning for 5 days (or longer in cases of increased NSE values).

References
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