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Correlation between serum level of neuron-specific enolase and long-term functional outcome after acute cerebral infarction: prospective study

血清神經元特異性烯醇化酶的水平與急性腦梗死遠期功能成效的相關性：前瞻性研究

Objective. To determine the value of measuring serum levels of neuron-specific enolase in predicting extent of disease and short- and long-term functional outcome after acute cerebral infarction.

Design. Prospective study.

Setting. Neurology departments at two university teaching hospitals, Shanghai.

Patients. Thirty-eight patients who presented for acute cerebral infarction between October 1998 and October 2000 were divided into two groups: those whose infarction extended to the cerebral cortex in the carotid artery region (cortical group) and those with an infarction in the subcortical carotid artery region (subcortical group).

Main outcome measures. Using a solid-phase enzyme immunoassay, we measured serum levels of neuron-specific enolase on admission and on days 2, 3, and 15. Infarct volume was measured by computed tomography on day 5. The Activities of Daily Living scale was used to assess the clinical outcome at 1-, 3-, and 6-month follow-up after onset.

Results. Mean (standard deviation) serum neuron-specific enolase levels were significantly higher among patients with acute cerebral infarction than among controls (18.48 [16.61] ng/mL versus 9.00 [2.70] ng/mL; $P < 0.001$). The neuron-specific enolase level was also higher in the cortical group than in the subcortical group (33.54 [29.71] ng/mL versus 15.97 [5.91] ng/mL; $P < 0.01$). Levels peaked after 2.11 (0.86) days and correlated positively with the infarct volume ($r = 0.81$; $P < 0.01$) and negatively with clinical outcome at 1 month ($r = -0.37$; $P < 0.05$), 3 months ($r = -0.45$; $P < 0.01$), and 6 months ($r = -0.65$; $P < 0.001$), as assessed on the Activities of Daily Living scale.

Conclusion. Serum neuron-specific enolase levels after cerebral infarction may be a useful marker to predict infarct volume and short- or long-term functional outcome.

Key words:

Cerebral infarction;
Phosphopyruvate hydratase;
Prognosis

關鍵詞：

腦梗死；
Phosphopyruvate hydratase；
預後

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目的：確定使用血清神經元特異性烯醇化酶水平評估病況，以及急性腦梗死遠近期的功能成效。

設計：前瞻性研究。

安排：兩所大學教學醫院的神經內科病房，上海。

患者：在1998年10月至2000年10月期間，38位因腦梗死而入院的患者，把他們分成兩組進行研究分析：皮層組（即梗死伸延至頸動脈大腦皮層區）及皮層下組（即於皮質下頸動脈區發現梗死）。

主要結果測量：以固相酶聯免疫吸附法檢測患者發病後第1、2、3及15天的血清神經元特異性烯醇化酶水平，並於發病後第5天用電腦斷層照相術量度腦動脈梗死的體積。此外，利用日常生活活動能力量表分析患者於發病後第1、3及6個月的臨床結果。

結果：患者的血清神經元特異性烯醇化酶水平的中位值（標準差）明顯地較正常對照組高（18.48 [16.61] ng/mL 比 9.00 [2.70] ng/mL; $P < 0.001$ ）。而皮層組患者的血清神經元特異性烯醇化酶水平亦明顯高於皮層下組患者（33.54 [29.71] ng/mL 比 15.97 [5.91] ng/mL; $P < 0.01$ ）。根據日常生活活動能力評分，血清神經元特異性烯醇化酶在第2.11（0.86）天達峰值水平，其峰值水平與梗死體積呈正相關。

($r=0.81$; $P<0.01$)，但與1個月($r=-0.37$; $P<0.05$)、3個月($r=-0.45$; $P<0.01$)，及6個月($r=-0.65$; $P<0.001$)的臨床結果則呈負相關。

結論：血清神經元特異性烯醇化酶水平有助評估腦梗死體積，以及遠近期的功能成效。

Introduction

In recent years, several techniques have been developed to measure levels of biochemical markers that might be used to evaluate neuronal injury. Neuron-specific enolase (NSE) is one such biochemical marker and has been the subject of many clinical and experimental studies.^{1,2} Previous studies³⁻⁷ have focused on the release and the kinetics of NSE after acute cerebral infarction, mostly in the cerebrospinal fluid (CSF). However, daily sampling of CSF is difficult and is associated with a high risk of complications. Thus, measuring serum levels of NSE could allow frequent testing with a relatively low risk of complications. In this study, we evaluated whether serial measurements of serum levels of NSE are useful in predicting the extent of brain damage and prognosis in patients with acute cerebral infarction.

Methods

Sample

Eligible study participants were patients with acute cerebral infarction who were admitted to the neurology departments at the Shanghai First People's Hospital and Hua Shan Hospital between October 1998 and October 2000. We initially selected 68 patients who were admitted within 24 hours of the onset of infarction and whose lesion was confined to the carotid artery according to neurological examination on admission and computed tomography (CT) or magnetic resonance imaging 4 to 6 days after admission. However, we excluded 22 patients who had documented or clinical evidence of brain infarction, haemorrhage, head trauma, or central nervous system (CNS) infection within the 3 months before admission, or a history of nervous system tumour, subarachnoid haemorrhage, or other neurological disorders. Furthermore, eight patients were excluded because of haemolytic specimens in which platelet or erythrocyte lysis would have influenced the laboratory results.

In all, 38 patients with complete data—21 men and 17 women, whose ages ranged between 41 and 83 years (mean age, 66.2 years; standard deviation [SD], 12.5 years)—were included in this study. The time of onset for 16 patients was within 12 hours and that for other patients was within 12 to 24 hours. All participants underwent a standardised neurological examination on admission and were divided into two groups according to the extent of infarction. One group consisted of patients whose infarction extended to the cerebral cortex in the carotid artery region (the cortical group); another group consisted of patients with an infarction in the subcortical carotid artery region (the subcortical group). Randomly selected control subjects included 27 healthy blood donors (17 men and 10 women) whose mean age was 40.3 years (SD, 7.1 years;

range, 25-53 years) and whose blood samples were used to determine reference values for concentrations of NSE.

Outcomes

Five days (SD, 2.5 days) after infarction, patients underwent CT to measure the infarct volume. A neuroradiologist, who was blinded to the treatment and clinical information, studied the CT images and ensured the correct diagnosis. A neurologist, who was also blinded to all clinical information (including treatment), measured the infarct volume, which was calculated by using the following formula: $(A \times B \times C)/2$, where A, B, and C were the largest diameters in the three axes, as determined by summing the widths of the slices in which the lesion was visible.⁸ The functional outcome of the patients was evaluated using the Activities of Daily Living (ADL) scale at 1 month, 3 months, and 6 months after onset. A neurologist blind to all patient information performed the ADL evaluation and neurological tests (not reported in this article).

Measurement of neuron-specific enolase levels

Immediately after admission, the first blood sample was obtained from all patients. Subsequent blood samples were collected on days 2, 3, and 15 after the acute cerebral infarction. Within 4 hours of collection, all blood samples were allowed to clot, and after centrifugation (5000 rpm, 10 minutes) the serum portions were stored at -80°C until analysis for NSE concentrations.

Serum levels of NSE were measured by using solid-phase enzyme immunoassay. The assay used a highly specific monoclonal antibody to NSE. During the incubation, the serum NSE reacted with the antibody, which was immobilised on polystyrene beads, and then with a rabbit polyclonal antibody to form a 'sandwich'. The beads were washed to remove any unbound rabbit antibody and incubated with a highly purified goat antibody against rabbit immunoglobulin, which was conjugated to horseradish peroxidase. The beads were rewashed to remove any unbound antibody enzyme conjugate and incubated with an enzyme substrate solution. The change in substrate colour was measured with a Cobas Photometer (F Hoffmann-La Roche Ltd, Basel, Switzerland), and was directly proportional to the amount of NSE present.

Statistical analyses

All results were expressed as the mean (SD) unless stated otherwise. The NSE peak levels of each individual in the first 3 days were used for statistical analysis. Group comparisons were analysed with the independent sample *t* test and analysis of variance. Regression analyses were also performed, and correlations were determined by calculating Spearman's rank correlation coefficients; SAS version 6.12 software (SAS Institute Inc, Cary, US) was used for

all statistical analyses. A value of $P < 0.05$ was considered significant.

Results

Analysis of NSE levels in the serum showed no relationship with age or sex either in patients or in controls. The levels of NSE were, however, significantly higher in patients than in controls (18.48 [16.61] ng/mL versus 9.00 [2.70] ng/mL; $P < 0.001$). For the 38 patients with acute infarction in the carotid artery region, we measured NSE levels 4 times; the level peaked at a mean of 2.11 (0.86) days after infarction. For most of patients, NSE levels returned to normal on day 15. The NSE level was significantly higher in the cortical group than in the subcortical group (33.54 [29.71] ng/mL versus 15.97 [5.91] ng/mL; $P < 0.01$).

The patient with the highest concentration of NSE recorded in the study (158.50 ng/mL) had an infarct in the middle cerebral artery that had a volume of 289 mL (Fig 1). The peak NSE level correlated positively with the infarct volume ($r = 0.81$; $P < 0.01$) [Fig 2].

Our results also indicated that higher peak levels of NSE predicted increasingly worse functional outcome, as reflected by the ADL score. This was true both at 1 month after the infarction, ($r = -0.37$; $P = 0.022$) and at 3 months ($r = -0.45$; $P = 0.005$). Moreover, the peak level of serum NSE and the longer-term clinical outcome at the 6 months' follow-up were inversely correlated ($r = -0.65$; $P < 0.001$).

Discussion

Neuron-specific enolase is the $\gamma\gamma$ -isoenzyme of the glycolytic enzyme enolase; it has a molecular weight of approximately 80 000 Da and is present predominantly in neurons and neuroendocrine cells.¹ It has been found in several acute CNS insults, such as cerebral infarction, head trauma, hypoxia, and seizure, during which changes in the brain-blood barrier and astroglial disintegration cause NSE to leak into the CSF and serum.⁴ Increased NSE levels in the serum and CSF indicate neuronal damage. We evaluated the serum NSE level rather than the CSF level because the daily serum sampling was practical and posed no risk for older patients.

In the previous reports, levels of NSE in the serum peaked within the first 96 hours of cerebral infarction, and in some cases as late as day 6 after infarction.^{3,9-14} The half-life of NSE in the serum has been reported to be about 48 hours¹⁵; hence, serum levels of NSE would be expected to rise as long as damage due to the infarction continued and NSE was washing out of the brain tissue. Cunningham et al⁹ found that the level of NSE peaked later for larger infarcts, and concluded that the peak level best reflects the final infarct volume. The time to the peak serum level of NSE in our study was 2.11 (0.86) days

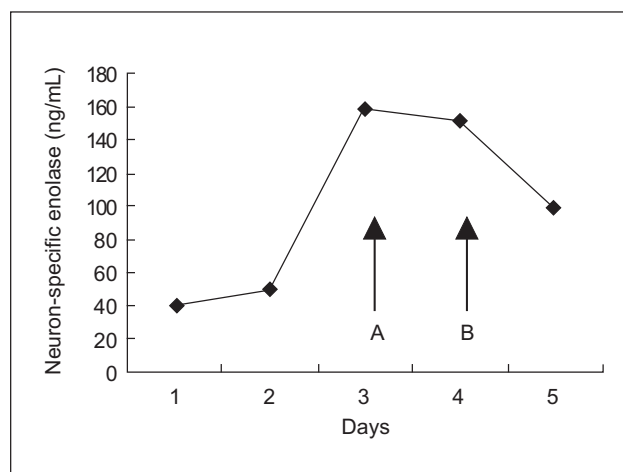


Fig 1. Serum neuron-specific enolase levels of one patient with acute infarction in the middle cerebral artery 'A' indicates the peak level and 'B' indicates that the patient became unconscious on day 4

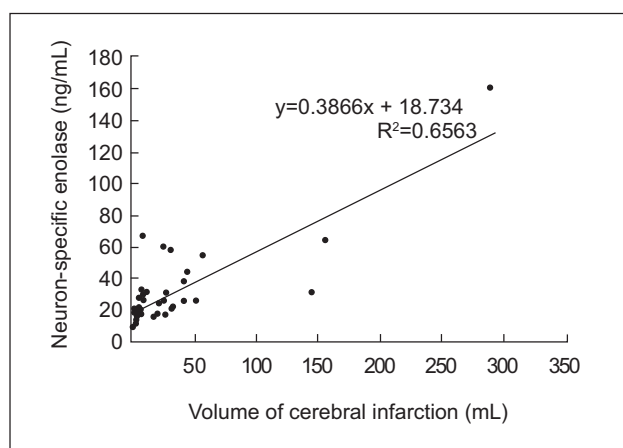


Fig 2. Scatter-plot of peak neuron-specific enolase level versus infarct volume

Shown is the equation according to a regression analysis; $r = 0.81$ ($P < 0.01$)

after infarction, which compares well to the 48-hour half-life reported in the literature; we also noted a trend for the peak level and increasing infarct volume.

Although the mean age of the control group was younger than that of the patients, our analysis confirmed the findings of Missler et al,¹³ who showed that the serum level of NSE was not related with age or sex. Compared with control subjects, patients with acute cerebral infarction in the carotid artery territory had clearly elevated serum levels of NSE. Our results indicate that the measurement of serum NSE level during the acute phase of stroke is valuable for evaluating the neurological dysfunction of cerebral infarction.

A report suggested that patients with total anterior circulation stroke had higher initial NSE serum levels

than patients with partial anterior circulation stroke or lacunar infarction.¹⁶ The results of our study showed that the peak NSE level was significantly higher in patients in the cortical group than in the subcortical group, which again indicates that the peak NSE level may be correlated with the infarction size. The results of several animal studies also indicate a semi-quantitative relationship between elevated CSF levels of NSE and the infarct volume.⁴⁻⁷ Furthermore, the peak NSE level between 48 and 96 hours after onset was strongly correlated with the infarct volume.⁹ Our study also showed that the serum level of NSE is a better indicator of the extent of cerebral infarction ($r=0.81$, $P<0.01$).

As shown in a previous study,¹⁰ a higher NSE level predicts a worse clinical outcome after cerebral infarction. Butterworth et al¹¹ studied the ratio of NSE to carnosinase levels in 124 patients with ischaemic or haemorrhagic stroke, and found a statistically significant correlation between the ratio and the outcome after ischaemic stroke, as assessed with the modified Barthel Index ($r=0.34$, $P<0.001$) and the modified Rankin scale ($r=0.30$, $P<0.01$). However, some reports have failed to demonstrate the correlation between NSE level and neurological outcome after cerebral infarction.^{12,17} Therefore, it is still debatable whether NSE is a reliable biochemical marker of neurological outcome in patients with acute cerebral infarction.

One recent study showed a positive correlation between functional outcome (the modified Rankin scale score at discharge) and serum NSE concentration after stroke in 28 patients, though no details were given of the mean hospital stay.¹⁸ In our study, we found a moderate inverse correlation between peak NSE levels in the serum and clinical outcome at 1, 3, and 6 months assessed on the ADL scale. In another study, we also found that clinical assessment using the European Stroke Scale, Chinese Stroke Scale, and ADL scale can predict the stroke severity to some degree.¹⁹ Although NSE cannot be regarded as a diagnostic tool for cerebral infarction, our results indicated that NSE might serve as a prognostic marker of the ischaemic stroke.

In conclusion, our data show that the peak level of NSE in the serum during the acute phase of stroke reflects the extent of the cerebral infarction and can be used as a useful marker of short- or long-term clinical outcome. Although the overlap in the range of NSE levels between patients and controls and the relatively small samples are a limitation of this study, serial measurements of the NSE concentration in the blood might still be useful in monitoring the state changes of the illness following acute cerebral infarction.

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