

Neuron-specific enolase and S 100B protein as predictors of outcome in ischaemic stroke

Neuronalna enolaza i białko S 100B jako czynniki prognostyczne w udarze niedokrwiennym mózgu

Małgorzata Kaca-Oryńska¹, Ryszard Tomasiuk², Andrzej Friedman³

¹Neurological Ward, Bródno Voivodship Hospital, Warsaw, Poland

²Biochemistry Laboratory, Bródno Voivodship Hospital, Warsaw, Poland

³Department of Neurology, Medical University of Warsaw, Warsaw, Poland

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Abstract

Background and purpose: As neuron-specific enolase (NSE) and S 100B protein are brain origin proteins, the aim of this study was to assess whether a single NSE and S 100B measure may predict clinical outcome of patients with cerebral ischaemic infarct.

Material and methods: Seventy-one patients with ischaemic stroke and 41 controls were studied. All patients had computed tomography of the brain performed after admission and on the third day and volume of the infarct was assessed by the volumetric method from the second examination. NSE and S 100B protein were analysed by immunochemiluminescence on the fourth day after admission. Clinical state of the patients was determined with the NIH stroke, Barthel and Rankin scales on admission, discharge from the hospital, and after one and 3 months from the onset of stroke.

Results: NSE levels in blood were significantly higher in stroke patients than in the control group – 36.9 ± 24.0 vs. $14.3 \pm 9.7 \mu\text{g/L}$. Also, the levels of the S 100B protein were significantly higher in the patient group (0.85 ± 1.74 vs. $0.10 \pm 0.03 \mu\text{g/L}$) but only the levels of S 100B protein correlated with the calculated size of the infarct (Spearman coefficient = 0.77). No such correlation was identified for NSE level (Spearman coefficient = 0.25).

Streszczenie

Wstęp i cel pracy: Celem pracy było zbadanie, czy jednorazowy pomiar stężeń dwóch białek pochodzenia mózgowego – enolazy neuronalnej (NSE) i białka S 100B – może mieć znaczenie dla przewidywania stanu klinicznego chorych na udar niedokrwienny mózgu.

Materiał i metody: Zbadano 71 chorych na udar niedokrwienny mózgu i 41 osób z grupy kontrolnej. U wszystkich pacjentów wykonano tomografię komputerową (TK) mózgu w chwili przyjęcia i 3. dnia po udarze. Wielkość udaru oceniano na podstawie badania TK metodą wolumetryczną. Stężenie NSE i białka S 100B w surowicy badano przy użyciu metody immunochemiluminescencyjnej 4. dnia po przyjęciu do szpitala. Stan kliniczny oceniano przy użyciu skali NIHSS, skali Barthel i Rankin w dniu przyjęcia, wypisu, po miesiącu i po 3 miesiącach od udaru.

Wyniki: Stężenie NSE było istotnie większe u chorych z udarem niż w grupie kontrolnej ($36,9 \pm 24,0$ w porównaniu z $14,3 \pm 9,7 \mu\text{g/l}$). Stężenie białka S 100B również było większe u chorych ($0,85 \pm 1,74$ w porównaniu z $0,10 \pm 0,03 \mu\text{g/l}$); tylko stężenie białka S 100B korelowało z wielkością zawału (współczynnik Spearmana = 0,77). Nie stwierdzono takiej korelacji dla NSE (współczynnik Spearmana = 0,25).

Correspondence address: Prof. Andrzej Friedman, Klinika Neurologii Wojewódzkiego Szpitala Bródnowskiego, ul. Kondratowicza 8, 03-242 Warszawa, tel. +48 22 326 58 15, fax +48 22 326 58 15, e-mail: friedman@amwaw.edu.pl

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Conclusions: Although significant differences in NSE and S 100B levels between stroke patients and the control group were found, only S 100B protein level correlated with stroke volume, neurological status at admission and functional outcome. NSE did not correlate with stroke volume, neurological status or clinical outcome.

Key words: neuron-specific enolase, S 100B protein, stroke, predictors.

Introduction

Recently, many techniques have been investigated for their usefulness in monitoring patients' neurological status and predicting the outcome from ischaemic stroke.

Neuroradiological imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) may identify the location and volume of the infarct and in this way predict the outcome of the stroke, but their cost and availability, especially MRI, promote a search for other techniques which could also predict the long-term prognosis. Therefore, identification of biochemical markers of stroke severity and possible outcome is certainly needed. There are many studies in the literature dealing with biochemical methods in stroke patients. Several proteins normally located within the brain tissue are released during stroke to the cerebrospinal fluid and blood and may be detected by biochemical methods. It seems, therefore, that they could serve as markers of the extent of the damage. As the results of such studies are not uniform we decided to examine two brain-specific proteins, neuron-specific enolase (NSE) and S 100B protein, in patients admitted to our department due to first-ever ischaemic stroke, and to correlate the results with the volume of brain infarct and clinical outcome of the patients.

These two proteins have already been extensively studied in the literature. S 100B protein is functionally related to a family of calcium binding proteins. It consists of two sub-units, alpha and beta, in dimeric forms: alpha-alpha, alpha-beta, beta-beta. The protein, in particular the form 100 beta beta (S 100B) is present in high concentrations in glial and Schwann cells. In normal conditions it is not detected in cerebrospinal fluid or in blood [1].

NSE is the neuronal form of the glycolytic enzyme enolase. It is found mainly in the cytoplasm of neurons and cells of neuroendocrine origin. It has also been found in erythrocytes and platelets, but in smaller con-

Wnioski: Stwierdzono istotne zwiększenie stężeń białek NSE i S 100B u chorych na udar niedokrwienny mózgu w porównaniu z grupą kontrolną, jednak tylko stężenie białka S 100B korelowało z objętością zawału oraz stanem klinicznym zarówno przy przyjęciu, jak i w odległym czasie. Nie stwierdzono takich korelacji dla NSE.

Słowa kluczowe: neuronalna enolaza, białko S 100B, udar mózgu, determinanty.

centrations. It is always present in cerebrospinal fluid and blood in small amounts [2].

Elevated concentrations of S 100B protein and NSE have been noted in ischaemic and haemorrhagic stroke [3-6], head injuries [7,8], and brain tumours, and measurement of NSE and S 100B protein is used in angioplasty and cardiosurgery to assess the state of nervous tissue during surgical procedures requiring temporary circulatory arrest [9,10].

In a study examining 275 patients with ischaemic stroke who had received thrombolytic therapy, elevation of serum S 100B levels was found to be an indicator of a higher risk of haemorrhagic transformation of the infarct [11]. In another study, measuring S 100B across the first three days after subarachnoid haemorrhage was useful in prediction of outcome and risk of vasospasm [12].

Our study aimed to assess whether the concentration of NSE and S 100B proteins in blood correlates with the size of the infarct and whether it could predict the clinical outcome of stroke patients.

Material and methods

Seventy-one patients (29 females and 42 males) with first-ever ischaemic stroke hospitalized in the Department of Neurology of Brodno Voivodship Hospital in 2007-2009 and 41 controls (27 females and 14 males) entered the study. The two groups did not differ significantly regarding age (71 ± 8 years for patients and 64 ± 7 years for controls; range: 45-77 for patients and 48-77 for controls). Although the difference regarding the sex between the two groups was significant, this should not interfere with the assessment of NSE and S 100B levels as sex does not influence the level of these proteins [13,14].

Exclusion criteria included haemorrhagic stroke, brain stem infarct, head injury, brain tumour and history of previous stroke. We also excluded patients with concomitant diseases which could influence NSE and

S 100B protein levels in blood, such as neuroendocrine tumours and melanoma.

All participants gave informed and written consent to enter in the study.

The following risk factors in the stroke group were identified: 75% of the patients suffered from hypertension, 48% had hyperlipidaemia, 47% were diagnosed with ischaemic heart disease, and 25.3% had diabetes. In the control group these risk factors were also present: 43.9% of controls suffered from hypertension, 12.2% had hyperlipidaemia, 19.5% had ischaemic heart disease and 14.6% were diabetics.

Venous blood samples were taken only once, on the fourth day after admission, when the highest concentration of both proteins is observed, and S 100B and NSE were analysed using immunochemiluminescence (Liaison NSE and Liaison S100 tests). Samples were centrifuged and stored at -20°C until assayed.

All patients had cranial computed tomography (CT) imaging performed at admission and on the third day after stroke. Volume of infarct was assessed from the second CT examination. Measurements of the ischaemic area were performed in layer thickness of 2.5 mm. The size of the ischaemic area was assessed manually on each layer. The volume of the infarct was then calculated from the number of layers. The results were compared with computerized programs and were found to be similar. As the use of our method was easier, all lesion volumes were assessed in this way. In some cases, when there was no visible lesion on CT, the presence of stroke was confirmed by diffusion-weighted magnetic resonance imaging (DWI). DWI was not used to assess the volume of the infarct, and all those cases with stroke not seen in their CT scans were given the volume of stroke as 0 for further analysis.

All patients underwent a standardized neurological examination: on admission, discharge from the hospi-

tal, and after one and three months from onset of stroke. The neurological status was quantified using the National Institutes of Health Stroke Scale (NIHSS), Barthel Index Score and Rankin Scale.

Statistical significance of the results was assessed by Mann-Whitney U test. Spearman's rank correlation was used to analyse associations between the neurological scales and biochemical markers.

Results

NSE level above the normal value was found in 93.5% of stroke patients, while the level of S 100B protein was elevated in 63.1% of them only. Patients with small infarct size on CT had a normal S 100B level. In our laboratory, the normal value for NSE in blood is below $12.5 \mu\text{g/L}$, and for S 100B protein it is below $0.15 \mu\text{g/L}$.

There was no significant association between either of these biochemical markers and sex or age.

NSE levels in blood were significantly higher in stroke patients than in the control group (36.9 ± 24.0 vs. $14.3 \pm 9.7 \mu\text{g/L}$). Also, the levels of the S 100B protein were significantly higher in the patient group (0.85 ± 1.74 vs. $0.10 \pm 0.03 \mu\text{g/L}$), but only the levels of S 100B protein correlated with the calculated size of the infarct (Spearman coefficient = 0.77). No such correlation was identified for NSE level (Spearman coefficient = 0.25).

The levels of S 100B in blood correlated significantly with NIHSS, Barthel and Rankin scores at all examinations – at admission, at discharge, and after one and three months post stroke.

The correlations between the level of S 100B protein and size of ischaemic lesion and NIHSS are shown in Table 1.

Table 1. Correlations of S 100B protein serum concentrations with infarct volume and NIH Stroke Scale (NIHSS) scores at various time points (Spearman's rank correlation coefficient, *R*)

Variables	N	Spearman's R	t (N-2)	p-value
Size of ischaemic lesion & S 100B protein level	69	0.771	9.925	< 0.001
S 100B protein level & NIHSS on admission	71	0.408	3.707	< 0.001
S 100B protein level & NIHSS on discharge	71	0.479	4.536	< 0.001
S 100B protein level & NIHSS after 1 month	62	0.516	4.668	< 0.001
S 100B protein level & NIHSS after 3 months	61	0.491	4.333	< 0.001

Discussion

The correlation between NSE and S 100B protein and stroke is well known. Also our results confirm this observation. There is, though, some new information that emerges from the present study. Our study confirmed the significant correlation between the level of S 100B protein in blood and the volume of the infarct and also the clinical outcome reported by others [15]. The new finding of our study was that the single measurement of S 100B protein could predict the long-term prognosis. Such a correlation was not found for NSE. On the other hand, NSE was increased in almost all cases of stroke but also in 30% of the control group, while S 100B protein level increased only in patients with larger size of the stroke. It may be concluded that NSE is much less specific and therefore has much lower value as a predictor of outcome in stroke patients than S 100B protein. We have to admit that in some other studies a correlation between the volume of the infarct and the level of NSE was found [16-19]. It is important to note that in those studies the volume of the infarct was assessed as small, medium or large, while in our study it was precisely measured. Although some studies found a correlation between the clinical severity of stroke and NSE level [19-22], our study did not confirm this finding.

As a conclusion one may say that measuring the level of S 100B protein may be of clinical importance as it could predict the clinical outcome of stroke patients. The method is simple and of low cost. A single measure of S 100B concentration in stroke patients may provide information about stroke volume, stroke severity and functional outcome.

It seems profitable to add biochemical methods to other tests used in monitoring stroke patients. We examined only two biochemical markers. Studies including other brain-specific proteins in larger groups of patients should be done.

Conclusions

Although significant differences in NSE and S 100B levels between stroke patients and the control group were found, only S 100B protein level correlated with stroke volume, neurological status at admission and functional outcome. NSE did not correlate with stroke volume, neurological status or clinical outcome.

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Disclosure

Authors report no conflict of interest.

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