

Serum neuron-specific enolase and S100 calcium binding protein B biomarker levels do not improve diagnosis of acute stroke

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ABSTRACT

Background: The high sensitivities and specificities reported for blood biomarkers as a supportive test in the diagnosis of acute stroke do not correspond with their performance for decision-making in emergency situations.

Methods: Seventy-two patients with suspected stroke were recruited: 44 with ischaemic stroke, 17 with haemorrhagic stroke and 11 stroke mimics, as well as a high-risk control group of 79 individuals. Serum neuron-specific enolase (NSE) and S100 calcium binding protein B (S100B) biomarker levels were determined on admission, using immunoassay kits. The sensitivities and specificities of NSE and S100B for distinguishing acute stroke from stroke mimics and high-risk controls were calculated.

Results: For cut-off values (NSE ≤ 14 micrograms per litre and S100B ≤ 130 nanograms per litre) the sensitivity was 53% and 55% respectively. Specificity was 64 for both versus the stroke mimic group. Specificity was higher (79% and 86% respectively) when calculated on the basis of the control group.

Conclusions: This study supports the evidence indicating that serum levels of NSE and S100B do not improve the diagnosis of acute stroke.

KEYWORDS Neuron-specific enolase, S100 calcium binding protein B, stroke, specificity, sensitivity, biomarker

DECLARATION OF INTERESTS No conflicts of interest declared.

INTRODUCTION

The assessment of stroke in emergency settings has typically been done by clinicians in combination with neuroimaging. However an expert clinician is not always available and while computed tomography (CT) scans accurately distinguish brain ischaemia and haemorrhage, they may take some time to perform and the results are frequently normal even in cases of confirmed ischaemic stroke.^{1,2} The availability of a blood biomarker to support a clinical diagnosis of stroke would be valuable, especially in specific situations where imaging resources are limited, such as in pre-hospital facilities where the physician has to rapidly identify ischaemic stroke in order to accelerate access to treatment.

In Cuba, a network of 22 stroke units treat many patients with acute stroke. They are located in secondary or tertiary hospitals and have trained staff and basic equipment for the diagnosis and management of stroke patients. However, these patients are also treated by emergency services, in the ambulance and in primary care centres by general practitioners.

The diagnosis of stroke can be challenging because of the variety of aetiological processes involved in stroke pathophysiology, the numerous symptoms that can be displayed and the variety of stroke mimics such as brain

tumours, seizures, hemiplegic migraine or hypoglycaemia which often appear in an emergency department. The levels of several biomarkers in the blood following brain injury have been evaluated for use in distinguishing acute stroke from stroke mimics, but the successful translation to a biomarker useful in clinical practice has proven difficult, as they are not specific and many disease processes can damage brain tissue. The two molecules which have been most extensively assessed for the diagnosis and prognosis of stroke are S100 calcium binding protein B (S100B) and neuron-specific enolase (NSE) but the high sensitivities and specificities reported in the scientific literature often do not correspond with the practical performance of these and other blood markers.³ Many studies have concentrated on distinguishing a stroke patient from a healthy control subject when evaluating blood markers of brain injury. Perhaps a more relevant issue is whether stroke can be distinguished from stroke mimics.^{3,4}

Another difficulty in the use of these biomarkers for the diagnosis of acute ischaemic stroke is the reported elevation of S100B and NSE levels in the blood of hypertensive patients when compared to healthy controls.⁵ Since hypertension is the most frequent risk factor for stroke, this issue should be considered and could provide additional diagnostic validation.

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The object of our study was to evaluate the specificity and sensitivity of serum S100B and NSE for distinguishing stroke patients from: 1) patients with suspected stroke on arrival to the emergency room but a final diagnosis of transient ischaemic attack (TIA) or stroke mimics, and 2) a control group with a similar percentage of hypertensive individuals as the stroke group, but without a history of neurological disease.

PATIENTS AND METHODS

Patients

Seventy-two patients with suspected stroke (from 8–48 hours of symptom onset) were consecutively recruited in the stroke unit. 'Suspected stroke' was defined by the medical practitioner on duty in the emergency room based on the sudden appearance of a focal neurological deficit. The patient was then sent to the stroke unit where a trained neurologist determined admission based on clinical examination and a computed tomography (CT) scan of the brain. The diagnosis was later confirmed with the subsequent identification of a lesion consistent with the symptoms. All the patients were treated according to the Cuban Guidelines for Cerebrovascular Diseases.⁶

A total of 44 patients were confirmed as having an ischaemic stroke, 17 with a haemorrhagic stroke and 11 patients were diagnosed as having a TIA or as stroke mimics. The patients presenting as stroke mimics had one of two diagnoses: brain metastasis or hypertensive emergency. Ischaemic and haemorrhagic stroke patients were combined as a stroke group to evaluate the effectiveness of NSE and S100B levels in differentiating stroke from stroke mimics and TIA.

Hypertension is the main risk factor for all types of stroke so we established a control group ('high-risk') with similar age and co-morbidities as the stroke patients (hypertension and/or diabetes mellitus). This high-risk control group consisted of 79 individuals who were referred by their general practitioner mainly because of headache, suspected epilepsy, lumbosacral pain and dizziness. Fifty-four patients with essential hypertension and 25 normotensive patients were included in this group in order to obtain a prevalence of approximately 70% with hypertension (as occurs in stroke). No neurological disease was confirmed and there was no history of previous neurological disorders.

Although the levels of S100B and NSE biomarkers are highly specific for brain tissue, they are also expressed in other cell types under certain physiological and pathological conditions. For this reason, patients and members of the control group with clinical evidence of other neurological diseases, known malignancies, chronic inflammatory diseases, recent infection or trauma were not included.

We interviewed all patients and members of the control group in order to establish their personal pathological history and medication used. The patients or their relatives and the control subjects gave signed informed consent prior to entering the study. It was conducted according to the revised Declaration of Helsinki (1998) and approved by the Ethics Committee of the Institute of Neurology and Neurosurgery in the Cuban Ministry of Public Health in Havana.

Data collection

Computed tomography scans were performed on admission and within 48–72 hours after the onset of symptoms in order to define the size and location of the ischaemic or haemorrhagic lesion.

Patient co-morbidities were collected from clinical records using the following criteria: hypertension (>140 millimetres of mercury [mmHg] systolic, >90 mmHg diastolic or currently prescribed anti-hypertensive medication); diabetes mellitus (spontaneous blood sugar level >200 milligrams per deciliter [mg/dL] or currently prescribed diet or anti-diabetic medication); hyperlipidaemia (>220 mg/dL total cholesterol or >150 mg/dL triglyceride).

Blood sampling and analytical procedures

Blood was drawn from patients (12–48 hours after symptom onset) and from control subjects and was collected in ethylenediaminetetraacetic acid and dry tubes. Each participant received a consecutive number which was assigned to the blood samples. This blood was tested for general haematological results and an erythrocyte sedimentation rate. Serum was obtained by centrifugation and stored at –20°C until analysis was done. Routine haematological and hemochemical analyses were performed (haemoglobin, haematocrit, leukocyte count, glucose, creatinine, urates, lipid profile, total proteins, aspartate aminotransferase, alanine aminotransferase and γ -glutamyltransferase). The results of the blood workup in control subjects and stroke patients did not reveal other possible associated pathologies. Serum S100B and NSE levels were determined using immunoassay kits CanAg S100 EIA (708–10) and CanAg NSE EIA (420–10) from CanAg Diagnostics AB (Sweden) as described elsewhere.⁵

Statistical analysis

Demographic, clinical and laboratory frequency variables were calculated. Continuous variables were tested for normal distribution using the Kolmogorov-Smirnov test. Medians and interquartile ranges were calculated for biomarker concentrations and differences between groups were assayed using the Mann-Whitney U, Wilcoxon matched pair or Kruskal-Wallis tests. The

relationship between sensitivity and specificity for stroke versus TIA+mimics and vs controls was calculated by receiver-operating characteristics (ROC) analysis. The 'optimum' cut-off values from the ROC curve (considered as the point at which the sum of sensitivity and specificity is maximal) were calculated for each protein. These values were also used to distinguish stroke from high-risk control subjects. Statistical calculations were performed with Statistica 6.0 for Windows. Significance was considered in all instances when $p < 0.05$.

TABLE 1 Clinical and demographic characteristics of the study groups

Variable	Stroke (IS+HS) (n=61)	High-risk control (n=79)	TIA+mimics (n=11)
Age (years)**	68.0 (32–88)	61.3 (30–98)	65.7 (42–83)
Female gender n (%)†	23 (38.3%)	46 (58.2%)	4 (44.4%)
Hypertension n (%)	46 (75.4%)	54 (68.5%)	9 (81.8%)
Diabetes mellitus‡ n (%)	14 (27.5%)	22 (35.5%)	1 (11.1%)
Obesity‡ n (%)	17 (34.0%)	31 (49.2%)	3 (37.5%)
Previous cardiac disease‡ n (%)**	31 (56.4%)	10 (13.8%)	3 (37.5%)

IS= Ischaemic stroke; **HS**= Haemorrhagic stroke;
TIA= Transient ischaemic attacks
 Age= mean (range)
 **Analysis of variance between groups (ANOVA) (Age): $F=5.04$; $p=0.0076$. Post hoc (Tukey): Stroke versus control $p=0.0045$ (different letters denote significant difference)
 †Pearson χ^2 test (female gender) $\chi^2=7.09$; $p=0.03$
 ‡Pearson χ^2 test (previous cardiac disease) $\chi^2=26.40$; $p=0.0000$
 †Percentages were calculated in the cases where co-morbidity information was available.

RESULTS

The clinical and demographic characteristics of the patients with confirmed stroke, high-risk controls and TIA+mimics are presented in Table 1.

Although differences were observed with respect to age and gender between the study groups, no differences in serum NSE and S100B levels were previously reported for these characteristics.⁵ The higher percentage of previous cardiac disease in the stroke patients was an expected finding.

The median and interquartile range of serum NSE and S100B levels in the three study groups are presented in Table 2. When analysed independently (four study groups), the Kruskal-Wallis (post hoc) test only revealed a significant difference between stroke and control groups,

TABLE 2 Serum NSE and S100B concentrations in the study groups

Marker levels	Stroke (IS+HS) (n=61)	High-risk control (n=79)	TIA+mimics (n=11)
NSE* (µg/L)	15.0 (9.7–22.4)	9.5 (5.8–13.0)	6.0 (5.4–21.5)
S100B* (ng/L)	142.5 (103.0–214.3)	76.0 (53.6–106.6)	117.0 (97.0–153.0)

IS= Ischaemic stroke; **HS**= Haemorrhagic stroke;
TIA= Transient ischaemic attacks
NSE and S100B= median (interquartile range)
 µg/L= micrograms per litre
 ng/L= nanograms per litre
 **NSE= Kruskal-Wallis: $H(2, n=152)=22.09$; $p=0.0000$
 Post hoc: stroke vs control: $p=0.0000$
 **S100B= Kruskal-Wallis: $H(2, n=150)=40.21$; $p=0.0000$
 Post hoc= Stroke vs control: $p=0.0000$;
 TIA+mimics vs control: $p=0.0265$

but not with TIA and mimics. Thus, due to the small sample size TIA and mimics were combined into one group. Concentrations of NSE were significantly higher in patients with stroke (IS+HS) than in control subjects, while there were no differences in TIA+mimics. Concentrations of S100B in serum were significantly higher in patients with stroke than in controls, and also in TIA+mimics with respect to controls (Table 2). No difference between stroke and TIA+mimics was found. Levels of NSE and S100B were significantly correlated in stroke patients (Figure 1), but not in controls ($p=0.235$), nor in stroke mimics ($p=0.715$).

For confirmed stroke vs high-risk control subjects, the optimum cut-offs for the two analytes as obtained by ROC curve analysis were 14 µg/L for NSE and 130 ng/L for S100B. Patients in the three study groups were classified as having a positive or negative result with respect to these cut-off values (Table 3).

TABLE 3 Number of patients with positive or negative results according to NSE and S100B cut-off values in the study groups

Study groups	NSE (µg/ml)		S100B (ng/ml)	
	+ (>14)	- (≤14)	+ (>130)	- (≤130)
Stroke (61)	35	26	34	27
TIA+mimics (11)	4	7	5	6
Control (79)	19	60	16	63

TIA= Transient ischaemic attacks
 µg/L= micrograms per litre
 ng/L= nanograms per litre

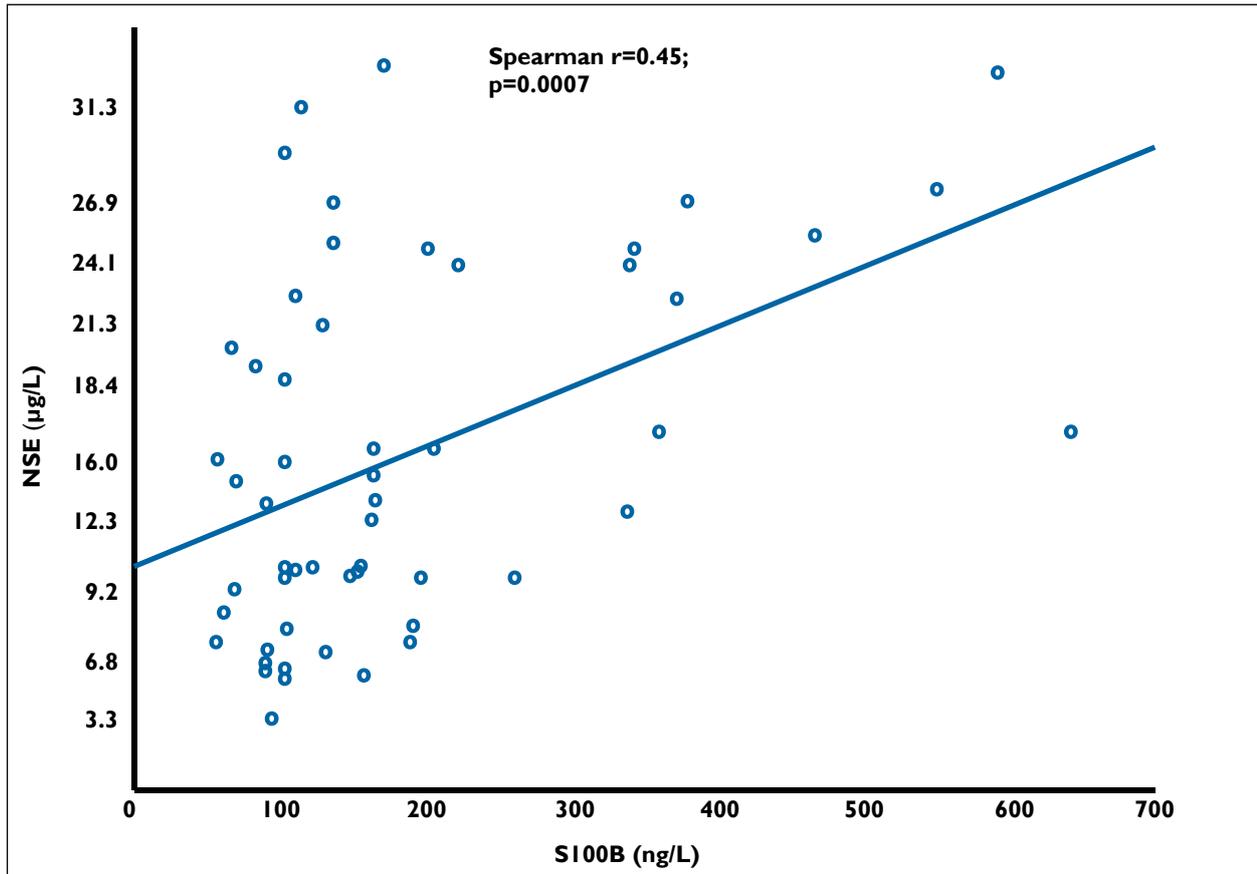


FIGURE 1 Correlation between neuron-specific enolase and S100 calcium binding protein B levels in stroke patients.

The sensitivity and specificity for NSE and S100B for distinguishing stroke from TIA+mimics and stroke from high-risk control subjects were determined by ROC curve analysis. The area under the ROC curves showed that NSE and S100B differentiated confirmed stroke patients from high-risk controls with a sensitivity of 53% and 55% and a specificity of 79% and 86% respectively, but did not differentiate them from TIA+mimics (Table 4).

As expected, specificity was higher for NSE and S100B when comparison was versus the control group than versus the TIA+mimics group (64% for both proteins).

DISCUSSION

Though many proteins are associated with stroke, successful translation into a biomarker useful in clinical practice for differential diagnosis has proven difficult.⁷ Some of the challenges for the diagnosis of stroke are the heterogeneity of stroke itself and the number and variety of stroke mimics.⁸ Raised levels of NSE and S100B were found in this study when compared to high-risk control subjects (with a prevalence of arterial hypertension and diabetes mellitus), where an elevation of these proteins had been previously reported.⁵ Nevertheless, serum NSE and S100B in cases of

TABLE 4 Sensitivity and specificity of NSE and S100B for diagnosis of stroke

Study groups	Protein (cut-off)	Sensitivity % (95% CI)	Specificity % (95% CI)	AUC (95% CI)
Confirmed stroke vs TIA+mimics	NSE (14 µg/L)	53% (44–70)	64% (35–92)	0.68 (0.49–0.89)
	S100B (130 ng/L)	55% (43–68)	64% (25–84)	0.60 (0.44–0.76)
Confirmed stroke vs control	NSE (14 µg/L)	53% (45–70)	79% (66–85)	0.73* (0.65–0.81)
	S100B (130 ng/L)	55% (43–68)	86% (71–89)	0.81* (0.74–0.88)

TIA= Transient ischaemic attacks; **AUC**= area under the curve; * $p<0.0001$; **CI**= Confidence interval; **µg/L**= micrograms per litre; **ng/L**= nanograms per litre

confirmed stroke were not different when compared to TIA+mimics. This would be expected, due to the increased passage of these proteins into the systemic circulation as a consequence of release and/or secretion subsequent to glial and neuronal damage and the ensuing disruption of the blood-brain barrier. There are several reports indicating that both proteins are markers of glial and neuronal injury in ischaemic stroke and a smaller number of studies demonstrating the role of peripheral markers in haemorrhagic stroke.^{9–14} However these markers are not specific for stroke; raised levels of NSE and S100B have also been found in other neurological conditions such as Alzheimer's disease, epilepsy, brain tumours, dementia, schizophrenia and Creutzfeldt-Jakob disease, among others.^{15–18} The correlation observed between NSE and S100B levels in stroke patients supports its association with acute damage of glial and neuronal cells. It was not present in TIA+stroke mimics and control subjects.

Whiteley et al. published a detailed review of the use of blood biomarkers in the diagnosis of ischaemic stroke, where there was great variability in the sensitivity of both NSE and S100B (NSE: 55–80; S100B: 35–60) and specificity of NSE (NSE: 35–100).⁸ Our results are within the range reported in the scientific literature for the sensitivity of NSE and S100B, with lower specificity values. The low sensitivity observed is probably related to the inclusion of study subjects who had lacunar strokes, which display values of NSE and S100B similar to those of the high-risk control group. The heterogeneity of the types of stroke in most of the studies reported in the review⁸ is probably the underlying cause for the similarity with our results. The timing of blood withdrawal in relation to symptom onset can also introduce variability in the sensitivity reported in different studies. The lower specificity reported is probably due to the fact that it was defined by other authors as a 95% or 98% reference interval in subjects without disease,¹² while our values were calculated on the basis of stroke mimics and for control subjects with a high prevalence of hypertension.

The ROC analysis of serum NSE and S100B levels showed optimal values of specificity of 79% (95% confidence interval [CI]: 66–85) and 86% (95% CI: 71–89) respectively, when compared with a high-risk control group; the values decreased to 64% for both proteins when compared with TIA+stroke mimics. The biomarkers were not effective at differentiating acute stroke from other confounding conditions in our study. This could be due to two main reasons: 1) acute stroke

patients may not display markedly increased blood markers during the first 24 hours, especially when stroke severity is not high (as in a lacunar ischaemic stroke), and 2) in some neurological conditions which can be mistaken for stroke, brain damage accompanied by an increased release of brain-specific proteins to the circulation can occur by similar mechanisms as in stroke (i.e. neuronal or glial necrosis occurs in patients with brain tumours as well as in patients with ischaemic stroke).

One limitation of this study is the relatively small number of patients who initially presented with suspected stroke but were not ultimately diagnosed with it. It should be taken into account that the low prevalence of mimics could affect the total accuracy and the results cannot be accepted with certainty as representative of unbiased findings. Nevertheless, our results agree with other authors who have stated that elevated levels of NSE and S100B in the blood are not specific for stroke, as increases occur in other neurological conditions which are symptomatically similar.^{12,19} As Whiteley stated, the high sensitivities and specificities that have been reported for most blood markers are in part due to the study design which inflates their sensitivity and specificity for a diagnosis of stroke.²⁰

To date, no single or panel of blood biomarkers has proven to be of diagnostic relevance for the diagnosis of acute stroke. It was clearly established recently that none of them improved the diagnostic performance of a validated clinical stroke scale.³ These blood biomarkers may however prove useful for assessing short and/or long-term prognosis, but this is still controversial and yet to be definitely established.^{12,21} We are now evaluating the association of NSE and S100B levels with short-term neurological outcome in this cohort.

Although this study has limitations due to the diagnostic distribution and low internal validity, it has high external validity as the findings concur with those in larger series, thus supporting the evidence indicating that serum levels of NSE and S100B do not provide the clinician with appropriate decision-making information for the diagnosis of acute stroke.

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