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Short-term prognostic value of serum neuron specific enolase and S100B in acute stroke patients

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ABSTRACT

Objective: To explore the value of blood markers for brain injury as outcome predictors in acute stroke.

Design and methods: The study included 61 patients with acute stroke (44 ischemic and 17 hemorrhagic) and a high risk control group (79 individuals with no known history of neurological disease).
Serum neuron specific enolase (NSE) and S100B were determined by immunoassay (CanAg Diagnostics, Sweden). Outcome at 60 days was evaluated with clinical scales.

Results: Higher concentrations of NSE and S100B were measured in patients compared to high risk controls, but they were not related to stroke severity on admission. NSE was associated with functional neurological outcome at 60 days and to the degree of recovery, whereas S100B exhibited a strong correlation with depression symptoms at 60 days.

Conclusions: The measurements of serum concentrations of NSE and S100B after acute stroke may be clinically relevant for predicting functional neurological outcome and post-stroke depression, respectively.

Introduction

Stroke is the main neurological cause of mortality and the third most common cause of death worldwide. The most important risk factor for stroke is hypertension [1]. For many years research has been directed toward the identification of biomarkers for establishing the differential diagnosis, etiology and prognosis of stroke. These, span from the application of clinical scores to the use of complex imaging techniques. Blood based biomarkers have received an important attention, especially during the last decade. Serum molecular markers for neural damage have been used for estimating the extent of cerebral injury, as well as for long term clinical outcome in cerebrovascular diseases and other neurological conditions [2–6].

Despite the numerous attempts that have been made to develop a blood test to diagnose stroke using one or several proteins, to date no blood-based biomarker has proven to be of diagnostic relevance [5,6]. In our experience, serum concentrations of NSE and S100B did not improve the diagnosis of acute stroke [7]. On the other hand, the clinical usefulness of blood biomarkers for predicting prognosis is still controversial and is yet to be definitely established [6,8]. Of the blood-based biomarkers, neuron specific enolase (NSE) and protein S100B have been studied most often in clinical settings [2,3,8,9].

The association of these biomarkers with outcome studies has centered primarily on short and long term neurological deficit, and on stroke recurrence; however, their association with post-stroke depression symptoms has never been explored.

Recently serum S100B and NSE were found to be associated with hypertension and diabetes mellitus [10,11], two important risk factors for stroke; however, most authors compare post-stroke biomarker concentrations with those of age matched healthy controls [2,3].

Considering the above mentioned features, the object of this work was 1) to evaluate post-stroke serum concentrations of NSE and S100B with respect to a control group comprised of high risk individuals (presence of hypertension and/or diabetes mellitus without known neurological disease, as these are comorbidities frequently present in stroke), and 2) to determine whether serum concentrations of NSE and S100B on admission could predict short term neurological deficit and depression symptoms in stroke patients.

Patients and methods

Patients

Sixty one patients with stroke (32–88 years of age), admitted in the Stroke Unit were consecutively recruited between 8 and 48 h of symptom onset.

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CT scans were obtained on admission and 48 h after onset to define the size and location of the ischemic or hemorrhagic lesion. Forty four patients were diagnosed as ischemic stroke (IS) and 17 as hemorrhagic stroke (HS). All the patients were treated according to the Cuban Guidelines for Cerebrovascular Diseases [12].

All patients completed an interview aimed to ascertain their personal pathological history and medication used. Those with peripartum stroke or clinical evidence of other neurological diseases, known malignancies, severe chronic diseases, recent infection or trauma were not included.

The control group consisted of 79 age-matched individuals with a similar frequency of major comorbidities (hypertension and/or diabetes mellitus), who were referred to our institution by the general practitioner mainly because of headache, suspected epilepsy, lumbosacral pain and dizziness. No neurological disease was confirmed, and there was no history of previous neurological, chronic inflammatory or malignant diseases. Those referring recent infection or trauma were not included.

The study was conducted according to the revised Declaration of Helsinki (1998) and the protocol was approved by the ethics committee of the Institute of Neurology and Neurosurgery La Habana, Cuba. The patients (or relatives) and the control subjects gave signed informed consent prior to entering the study.

Clinical variables

Data on admission

Neurological deficit was measured on admission by trained stroke physicians employing the National Institutes of Health Stroke Scale ($NIHSS_0$) [13]. Comorbidities were collected from clinical records, considering the following criteria: hypertension (>140 mm Hg systolic, >90 mm Hg diastolic or currently prescribed anti-hypertensive medication); diabetes mellitus (spontaneous blood sugar level >200 mg/dL or currently prescribed diet or anti-diabetic medication); hyperlipidemia (>220 mg/dL total cholesterol or >150 mg/dL triglyceride).

IS was classified according to the Oxford Shire scale [14], and stroke etiology was classified according to the TOAST criteria [15]. Patients with HS were all classified as intracranial hemorrhage (ICH).

Outcome variables

The following outcome variables were measured at 60 days after acute onset by the neurologists OFC and MAP through personal interviews:

a) Neurological deficit according to the NIHSS score ($NIHSS_{60}$).
b) Degree of change in NIHSS (δ_{NIHSS}) was evaluated as an index of recovery: $NIHSS_0$–$NIHSS_{60}$. For this analysis, patients with a low initial NIHSS ($NIHSS_0 \leq 5$) were excluded, because a slight severity on admission admits little change, and this would not indicate poor recovery.
c) Functional outcome employing Barthel index [16].
d) Geriatric depression screening (GDS) scale in IS patients (measured employing the short form) [17].

Blood sampling and analytical procedures

Ten milliliters of venous blood was obtained from all enrolled patients and controls and collected in EDTA and dry tubes. Each participant received a consecutive number which was assigned to the blood samples. Sample collection in patients was taken between 12 and 48 h of symptom onset. A continuous elevation of NSE and S100B over 48 h has been reported as specific for brain damage [4,18]. EDTA blood was employed for general hematological studies and erythrocyte sedimentation rate. Serum was obtained by centrifugation (800–1000 rpm for 10 min) and stored at −20 °C until S100B and NSE concentrations were measured.

Routine hematological and hemochemical analyses were performed (hemoglobin, hematocrit, leukocyte count, glucose, creatinine, urates, lipid profile, total proteins, aspartate aminotransferase, alanine aminotransferase,γ-glutamyltransferase). Blood analysis results in controls and stroke patients did not reveal other possible associated pathologies.

Serum S100B and NSE were determined by employing immunoassay kits CanAg S100 EIA (708-10) and CanAg NSE EIA (420-10) from CanAg Diagnostics AB (Sweden), according to the manufacturer's instruction manual. S-100B was determined by a two-step non-competitive immunoassay based on two mouse monoclonal antibodies (MAb), which measured the b-subunit concentration in both bb and ab isoforms of the protein. NSE was measured by a solid phase non-competitive immunoassay based on two mouse MAbs directed against two separate antigen determinants of the NSE molecule. After the chromogen enzyme reaction with hydrogen peroxide and 3-3′5,5′-tetra-methylbenzidine (for both NSE and S-100B), the color intensity was determined in a microplate spectrophotometer SUMA PR-521 at 620 nm and the concentrations were expressed as ng/L and μg/L respectively. Since NSE is also present in erythrocytes, hemolyzed samples were discarded.

All NSE and S-100B assays were conducted by SGG in duplicate, random order, and blinded to the nature of the sample. Intra- and inter-assay coefficients of variation were 1.7–3.5% and 3.7–5.5% for NSE and 1.3–2.5% and 1.5–2.5% for S-100B. Detection limits were <1 ug/L for NSE and ≤10 ng/L for S-100B.

Statistical analysis

Power calculations based on previous reports suggested that a sample size of 38 was able to detect a 20% difference in serum NSE and S-100B concentrations between groups, with $\alpha = 0.05$ and power 95%.

Frequencies of the demographic, clinical, and laboratory variables were calculated. Continuous variables were tested for normal distribution using Kolmogorov–Smirnov test. As NIHSS scores and serum concentrations of S100B and NSE were not normally distributed, medians and 10–90 percentiles were calculated and differences between groups were assayed employing Mann–Whitney U test, Wilcoxon matched pair or Kruskall–Wallis tests. Correlations between continuous variables were evaluated by calculating Spearman's (r) coefficient. Associations between categories of variables were measured by the χ^2 test.

The relationship between sensitivity and specificity for stroke vs controls was calculated by receiver-operating characteristics (ROC) analysis. The "optimum" cutoff values from the ROC curve were calculated for each protein.

Multivariate regression analysis was undertaken with outcome variables ($NIHSS_{60}$, δ_{NIHSS}, and GDS) as dependent variables. We included as independent variables those parameters that showed a significant association with the dependent variables in univariate analysis at 5% significance level (NSE and type of stroke for NIHSS measures; and NSE, S100B, $NIHSS_{60}$, and δ_{NIHSS} for GDS).

Statistical calculations were performed with Statistica 6.0 for Windows (StatsoftInc, 2000). Statistical significance was achieved if $p<0.05$ (two-sided p values).

Results

Clinical characterization of stroke patients

Patients with IS (n = 44) were distributed according to the Oxford Shire classification as follows: 9 patients with total infarction of the anterior circulation (20.4%), 16 with partial infarction (36.3%), 17 with lacunar infarcts (38.6%), and the posterior territory was affected in only 2 (4.6%). According to the TOAST classification [19], large vessel infarction was present in 63.0% (32.6% atherothrombotic stroke and 30.4% cardioembolic). Previous stroke was referred in 34% of