Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

Differentiating ischemic from hemorrhagic stroke using plasma biomarkers: The S100B/RAGE pathway☆

Joan Montaner[a,b,*], Maite Mendioroz[a], Pilar Delgado[a], Teresa García-Berrocoso[a], Dolors Giralt[a], Cristina Merino[a], Marc Ribó[b], Anna Rosell[a], Anna Penalba[a], Israel Fernández-Cadenas[a], Francisco Romero[c], Carlos Molina[b], Jose Alvarez-Sabín[b], Mar Hernández-Guillamon[a]

[a]Neurovascular Research Laboratory, Vall d'Hebron University Hospital, Research Institut (VHIR), Universitat Autònoma de Barcelona, Barcelona, Spain
[b]Neurovascular Unit and Neurology Department, Vall d'Hebron University Hospital, Research Institut (VHIR), Universitat Autònoma de Barcelona, Barcelona, Spain
[c]Neuroradiology Department, Vall d'Hebron University Hospital, Research Institut (VHIR), Universitat Autònoma de Barcelona, Barcelona, Spain

ARTICLE INFO

Article history:
Received 16 November 2011
Accepted 27 January 2012
Available online 11 February 2012

Keywords:
Stroke
Ischemic
Hemorrhagic
S100B
sRAGE

ABSTRACT

Although neuroimaging is useful in differentiating ischemic (IS) from hemorrhagic (ICH) stroke in the Emergency Department, a wide-available rapid biochemical test would add advantages in the pre-hospital triage and management of stroke patients. Our aim was to examine the predictive value of a panel of blood-borne biomarkers to differentiate IS from ICH. Admission blood samples obtained within 24 h from stroke symptoms onset were tested by ELISA for CRP, D-dimer, sRAGE, MMP9, S100B, BNP, NT-3, caspase-3, chimerin-II, secretagogin, cerebellin and NPY. The complete protocol was achieved in 915 patients (776 IS, 139 ICH). Among blood samples obtained <6 h from symptoms onset (n=337), S100B levels were increased in ICH (107.58 vs 58.70 pg/mL; p<0.001) whereas sRAGE levels were decreased (0.77 vs 1.02 ng/mL; p=0.009) as compared to IS. In this subset of patients S100B (OR 3.97 95% CI 1.82–8.68; p=0.001) and sRAGE (OR 0.22 95% CI 0.10–0.52; p<0.001) were independently associated with ICH. A regression tree was created by CART method showing good classification ability (AUC=0.762). Similar results were found for samples obtained within 3 h. In conclusion, a combination of biomarkers including those of the S100B/RAGE pathway seems promising to achieve a rapid biochemical diagnosis of IS versus ICH in the first hours from symptoms onset. This article is part of a Special Issue entitled: Translational Proteomics.

1. Introduction

Of all strokes, 87% are ischemic and 13% are hemorrhagic strokes [1]. Overall, the prognosis for intracerebral hemorrhage (ICH) remains poor: 37–38% of hemorrhagic strokes result in death within 30 days, whereas 8% to 12% of ischemic strokes die, according to the Atherosclerosis Risk In Communities (ARIC) study [2]. Differentiating between both types of stroke is a critical step when planning stroke attention within the first minutes following symptoms onset. In fact, specific

☆ This article is part of a Special Issue entitled: Translational Proteomics.

* *Corresponding author at:* Neurovascular Research Lab, Hospital Universitari Vall d'Hebron, Institut de Recerca (VHIR), Edifici Mediterrània Pg Vall d'Hebron 119-129, 08035 Barcelona, Spain. Tel.: +34 934894073; fax: +34 934894015.
E-mail address: 31862jmv@comb.cat (J. Montaner).

1874-3919/$ – see front matter © 2012 Published by Elsevier B.V.
doi:10.1016/j.jprot.2012.01.033

management and treatment protocols are recommended for each stroke subtype. For instance, reducing the blood pressure in acute ICH may prevent or delay hematoma growth [3] and also decrease the risk of rebleeding [4], improving the clinical outcome [5]. On the contrary, lowering blood pressure may be deleterious in ischemic stroke [6,7]. Moreover, rapid identification of ischemic stroke allows us to promptly initiate tissue plasminogen activator (tPA) treatment while timely ICH detection may improve the early monitoring and management of these patients. In addition, some therapeutic measurements would be initiated during the transfer of the patient to the hospital.

Although brain computed tomography (CT) is usually performed as part of the initial evaluation of a patient with suspected stroke in most hospitals, because of its excellent sensitivity to differentiate ischemic from hemorrhagic stroke [8], the availability of a rapid biochemical diagnostic test would add clear advantages in the management of stroke patients, particularly if used outside the stroke care centers. Our aim was to examine the predictive value of a panel of blood-borne biochemical markers to differentiate ischemic from hemorrhagic stroke.

2. Methods

2.1. Study population

Patients with an acute stroke admitted to the Emergency Department of Vall d'Hebron University Hospital within the first 24 h after symptoms onset were recruited during two years. Symptoms onset was defined as the last time the patient was known to be asymptomatic. A total of 1005 consecutive patients on suspicion of stroke were evaluated. Among these, 90 had other conditions mimicking stroke and therefore they were excluded. Finally, 915 patients with an acute confirmed ischemic or hemorrhagic stroke were included in the study.

2.2. Neuroimaging and clinical protocol

On admission, all patients underwent a brain CT scan that was reviewed by a neuroradiologist with extensive experience in acute stroke, blinded to clinical details and biomarker results. This test was used as the reference standard to classify patients into ischemic or hemorrhagic strokes. The detailed clinical protocol has been reported elsewhere [9]. This study was approved by the Ethics Committee [PR(AG)89/2003] of the Vall d'Hebron University Hospital and all patients or relatives gave informed consent.

2.3. Immunoassays

Blood samples were drawn at arrival on the Emergency Department to test a panel of biomarkers including C-reactive protein (CRP), D-dimer, soluble receptor for advanced glycation end products (sRAGE), matrix metalloproteinase-9 (MMP-9), S100 calcium-binding protein B (S100B), brain natriuretic peptide (BNP), neurotrophin-3 (NT-3), caspase-3, chimerin II, secretagogin, cerebellin and neuropeptide Y (NPY). Blood testing for biomarkers was performed before any treatment was administered to avoid drug-biomarkers interference. Blood was drawn in EDTA tubes, centrifuged at 3000 rpm for 15 min and frozen at −80 °C. All biomarkers were assayed by ELISA performed in 384-well microtiter plates with a Tecan Genesis RSP 200/8 Workstation (Tecan, Durham, NC, USA). Each sample was tested twice with biotinylated antibodies (Biosite Inc, San Diego, CA, USA).

(For extended immunoassay methodologies see Supplemental data 1).

2.4. Statistical analyses

Descriptive and frequency statistical analyses were obtained and comparisons were made using SPSS software, version 15.0. Biomarkers were not normally distributed (Kolgomorov–Smirnov and P–P plot) and values were expressed as median (interquartile range). Statistical significance for intergroup differences was assessed by Pearson's chi-square for categorical variables and for continuous variables the Student's *t* test for data with normal distribution or Mann–Whitney *U* test for not normal continuous variables was used to assess differences between ischemic and hemorrhagic stroke patients. In all tests a p-value <0.05 was considered significant.

Bonferroni correction was used as a method for correcting multiple testing in the univariate analysis. The adjusted p-value that was considered significant was 0.0042 as a result of dividing $p=0.05$ by the 12 biomarkers tested. To obtain cut-off values with the best sensitivity and specificity for each biomarker, a receiver operator characteristic (ROC) curve was configured.

Those biomarkers and clinical variables associated with stroke subtype in the univariate analysis ($p<0.10$) were entered into a forward stepwise multivariate logistic regression model to identify independent predictors of hemorrhagic versus ischemic stroke. Three models were developed regarding the blood sample was drawn within the first 3, 6 or 24 h. Goodness of fit of the multivariate logistic models was tested with the Hosmer–Lemeshow test. Discrimination capacity of the models was assessed by ROC curves. The area under the ROC curve (AUC) showed the discriminating ability of the model; an AUC of 0.5 indicates no discrimination, whereas an AUC of 1.0 indicates perfect discrimination. Comparisons between two different AUCs were done by Medcalc statistical software.

A classification and regression tree (CART; CART Pro v6.0 software, Salford Systems, San Diego, CA, USA) analysis [10] to provide a predictive model of stroke subtypes was also performed by the Gini method. The same factors included in the multivariate logistic regression were included in CART analysis. The thresholds in the minimum number of patients used for the nodes were 100 for parent nodes and 50 for terminal nodes in the analysis at 6 h and 50 for parent nodes and 20 for terminal nodes at 3 h (since there were fewer cases).

3. Results

Regarding stroke subtypes, 776 (84.8%) were ischemic and 139 (15.2%) were hemorrhagic strokes. Patients that had a hemorrhagic stroke were younger ($p=0.019$), had more previous hemorrhagic stroke ($p<0.001$), drunk more alcohol ($p=0.019$), had less dyslipidemia ($p=0.008$), less previous peripheral arteriopathy ($p=0.020$), less previous coronary disease