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Etiologic Diagnosis of Ischemic Stroke Subtypes With Plasma Biomarkers

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Background and Purpose—Because there is no biologic marker offering precise information about stroke etiology, many patients receive a diagnosis of undetermined stroke even after all available diagnostic tests are done, precluding correct treatment.

Methods—To examine the diagnostic value of a panel of biochemical markers to differentiate stroke etiologies, consecutive acute stroke patients were prospectively evaluated. Brain computed tomography, ultrasonography, cardiac evaluations, and other tests were done to identify an etiologic diagnosis according to TOAST classification. Blood samples were drawn on Emergency Department arrival (<24 hours) to test selected biomarkers: C-reactive protein, D-dimer, soluble receptor for advanced glycation end products, matrix metalloproteinase-9, S-100b, brain natriuretic peptide (BNP), neurotrophin-3, caspase-3, chimerin, and secretagogin (assayed by ELISA).

Results—Of 707 ischemic stroke patients included, 36.6% were cardioembolic, 21.4% atherothrombotic, 18.1% lacunar, and 23.9% of undetermined origin. High levels of BNP, soluble receptor for advanced glycation end products, and D-dimer ($P<0.0001$) were observed in patients with cardioembolic stroke. Independent predictors (odds ratios with CIs are given) of cardioembolic stroke were as follows: atrial fibrillation 15.3 (8.4–27.7, $P<0.001$); other embolic cardiopathies 14.7 (4.7–46, $P<0.001$); total anterior circulation infarction 4 (2.3–6.8, $P<0.001$); BNP >76 pg/mL 2.3 (1.4–3.7, $P=0.001$); and D-dimer >0.96 $\mu\text{g/mL}$ 2.2 (1.4–3.7, $P=0.001$). Even among patients with transient symptoms ($n=155$), a high BNP level identified cardioembolic etiology (6.7, 2.4–18.9; $P<0.001$). A model combining clinical and biochemical data had a sensitivity of 66.5% and a specificity of 91.3% for predicting cardioembolism.

Conclusions—Using a combination of biomarkers may be a feasible strategy to improve the diagnosis of cardioembolic stroke in the acute phase, thus rapidly guiding other diagnostic tests and accelerating the start of optimal secondary prevention. (*Stroke*. 2008;39:2280-2287.)

Key Words: atherosclerosis ■ cerebral ischemia ■ natriuretic peptides ■ embolism ■ D-dimer ■ stroke

Stroke is the third leading cause of death in developed countries and the leading cause of long-term disability.¹ Each year, >5 million people die as a consequence of stroke, and at least 1 in 6 patients who survive a stroke will suffer another stroke within 5 years.² Because ischemic stroke is an etiologically heterogeneous disease, identification of the specific cause in every patient has important clinical implications, because prognosis, acute management, and long-term strategies to prevent recurrences may vary considerably for the different types of ischemic stroke.

Embolic or thrombotic occlusions of large arteries have been identified as the main cause of stroke, but other causes include abrupt occlusion of small penetrating arteries, arteritis, arterial dissection, venous occlusion, and profound anemia or hyperviscosity.³ Unfortunately, $\approx 35\%$ of all ischemic events remain classified as cryptogenic, mostly because no

potential etiologic mechanism is detected.⁴ In fact, many patients receive a diagnosis of undetermined stroke even after all available diagnostic tests are done, precluding optimal secondary prevention treatment.

Because the pathologic substrate is so different among stroke subtypes (ie, atherosclerosis versus cardiac embolism), we hypothesized that different stroke etiologies might be associated with a specific plasma protein expression pattern. Although at present there is no biologic marker offering precise information about stroke etiology, other authors have explored the role of different proteins, such as hemostatic markers, in different etiologic stroke subtypes.^{5–7} Moreover, several biomarkers have been recently related to cardiac disorders or thrombotic diseases.⁸ In this study, we tested the value of a wide panel of plasma biomarkers involved in several biochemical pathways to identify specific stroke etiologies.

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Subjects and Methods

Study Population

Patients with acute stroke admitted to the Emergency Department of a teaching hospital were prospectively recruited for 2 years (between 2002 and 2004) whether or not inclusion and exclusion criteria were fulfilled. Our target group consisted of patients who had had an acute ischemic stroke (established or transient) admitted within the first 24 hours after symptom onset. Patients with a known inflammatory or malignant disease were excluded from the final analysis. This study was approved by the ethics committee of the hospital, and all patients or relatives gave written, informed consent.

Clinical Protocol

A detailed history of vascular risk factors was obtained for each patient. Stroke severity was assessed by the National Institutes of Health Stroke Scale (NIHSS).³ To identify the potential mechanism of cerebral infarction, ECG, chest radiography, carotid ultrasonography, complete blood count and leukocyte differential, and blood biochemistry were performed in all patients; when indicated, some patients also underwent special coagulation tests (young patients without cause of stroke determined, familial or personal history of thrombosis, suspicion of antiphospholipid syndrome, or analytic disorders) or transthoracic/transesophageal echocardiography and Holter monitoring (all patients with clinical or neuroimaging findings presumably due to an embolus arising from the heart, young patients without identified etiology, or suspicion or history of cardiopathy). With this information and the neuroimaging data, previously defined etiologic subgroups were determined according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.⁹ This classification defines 5 etiologic subtypes of stroke: large-artery atherosclerosis or atherothrombotic, cardioembolic, small-vessel occlusion or lacunar, stroke of undetermined etiology or cryptogenic, and stroke of other determined etiology. Atherothrombotic and cardioembolic subtypes include patients with clinical (cortical impairment or brain stem or cerebellar dysfunction) and brain imaging findings (infarcts >1.5 cm). In the "atherothrombotic subtype," a stenosis >50% of an appropriate intracranial or extracranial artery should be demonstrated. In the "cardioembolic subtype," the presence of a high- or medium-risk cardiac source of embolism is necessary. "Lacunar subtype" requires a traditional clinical lacunar syndrome without evidence of cerebral cortical dysfunction and normal computed tomography/magnetic resonance imaging findings or a relevant brain stem or subcortical hemispheric lesion with a diameter <1.5 cm. Strokes of "undetermined etiology" include patients with 2 or more causes identified, those patients with a negative evaluation, and patients with incomplete evaluation. Stroke of "other determined etiology" includes patients with rare causes of stroke: nonatherosclerotic vasculopathies, hypercoagulable states, or hematologic disorders. Because this last group includes very rare and heterogeneous etiologies representing an anecdotal number of patients, we excluded those subjects (n=8) for the final analysis. Stroke was also classified according to the Oxfordshire Community Stroke Project criteria, based on clinical symptoms, location, and extent of cerebral infarction.¹⁰

Undetermined Stroke Follow-Up

At discharge from the hospital, each patient received an etiologic diagnosis of stroke. Those patients belonging to the undetermined subtype were followed up in the outpatient clinic until an etiology of stroke was reached or the undetermined origin was confirmed. For that purpose, diagnostic tests were completed or performed once again, depending on clinical suspicion (ie, in those cases with a high suspicion of cardioembolic etiology, Holter monitoring or echocardiographic studies were repeated or expanded). The study investigators were blinded to the laboratory results when diagnosing the etiologic subtype.

Neuroimaging Protocol

An experienced neurologist performed all transcranial Doppler examinations with a Multi-Dop X/TCD (DWL Elektronische Systeme GmbH) device, and vessel occlusions or stenoses were recorded as previously described.¹¹ Patients received a carotid artery ultrasound (5- and 10-MHz linear probes; Aplio-80, Toshiba) examination to assess the presence and severity of stenosis or occlusion in the extracranial internal carotid artery.¹² On admission, all patients underwent brain computed tomography that was reviewed by a neuroradiologist with extensive experience in acute stroke who was blinded to the clinical details and biomarker results.

Treatment Protocol

Standard therapies were given on the basis of stroke characteristics and arrival times. Generally speaking, patients received thrombolytics, antiplatelet agents, or anticoagulants, or they were recruited into neuroprotection clinical trials.

Immunoassays

Blood samples were drawn at Emergency Department arrival to test a panel of biomarkers that included C-reactive protein (CRP), D-dimer (DD), soluble receptor for advanced glycation end products (sRAGE), matrix metalloproteinase-9 (MMP-9), S-100b, brain natriuretic peptide (BNP), neurotrophin-3, caspase-3, chimerin, and secretagogin (Table 1). Blood testing for biomarkers was performed within 24 hours of stroke onset and before any treatment was administered to avoid drug-biomarker interference. Blood was drawn into EDTA tubes centrifuged at 3000 rpm for 15 minutes, and plasma was frozen at -80°C until analysis.

All biomarkers were assayed by ELISA. Immunoassays were forward immunometric (sandwich) assays performed in 384-well microtiter plates with a Tecan Genesis RSP 200/8 Workstation (Tecan). Each sample was tested twice with biotinylated antibodies (Biosite Inc, San Diego, Calif).

Statistical Analyses

Descriptive and frequency statistical analyses were obtained and comparisons were performed with SPSS for Windows, version 12.0. Biomarkers were not normally distributed (P-P plot), and values are expressed as median (interquartile range). Statistical significance for intergroup differences was assessed by Pearson's χ^2 for categorical variables and the Student's *t* or Mann-Whitney *U* test for continuous variables. To study the correlation between quantitative variables, Pearson or Spearman tests were used. To calculate the sensitivity and specificity for biomarker cutoff values to predict a specific stroke etiology, a receiver operator characteristic (ROC) curve was configured. Variables associated in the univariate analysis were entered into a forward stepwise logistic-regression model to identify variables independently associated with cardioembolic etiology (odds ratios [OR] with their 95% CIs are given). Selected biomarkers were modeled as dichotomous variables (with the cutoff values obtained from the ROC curves), and other variables such as NIHSS score and age were included as continuous variables in the model. Goodness of fit of the multivariable logistic model was tested with the Hosmer-Lemeshow test. An equation from the regression model was performed to calculate the predictive probability of cardioembolic etiology. Model discrimination was assessed by an ROC curve. A probability value <0.05 was considered statistically significant.

Results

A total of 964 consecutive patients with a suspicion of stroke were evaluated. Of these, 149 patients had brain hemorrhages on the computed tomography scan performed in the Emergency Department, 95 had other pathologic processes mimicking stroke, and 13 ischemic strokes fulfilled some exclusion criteria. Finally, 707 patients who had had an acute ischemic stroke were included in the study.

Table 1. Characteristics of Biomarkers

Biomarkers	General Features	Ischemic Stroke Studies
BNP	Vasoactive peptide hormone	↑ BNP in acute phase
	Natriuretic, diuretic, and vasodilator activity	↑ BNP predicts poststroke mortality
	Cardiac and cerebral origin	
DD	Products of degradation of fibrin	↑ DD in acute, subacute, and chronic phases
	Marker of hemostatic imbalance	↑ DD in cardioembolic stroke
		Predictor of new vascular events and prognostic role
S100b	Calcium-binding protein	↑ S100b in acute phase
	Synthesized in astroglial cells	S100b associated with clinical deficit, infarct volume, and functional disability
	Marker of brain damage	
RAGE	Transmembrane receptor	No studies
	Immunoglobulin superfamily	
	Expressed by endothelial cells, mononuclear cells, and neurons	
	Overexpressed at sites of vascular damage sRAGE have antiatherogenic effects	
CRP	Pentraxin	↑ CRP in acute phase
	Acute-phase protein; participates in the systemic response to inflammation	Predictor of risk of cerebrovascular events
	Hepatic and extrahepatic synthesis	Prognostic value after stroke
MMP-9	Proteolytic enzyme	↑ MMP-9 in acute phase
	Involved in tissue remodeling	↑ MMP-9 associated with hemorrhagic transformation
	Important role in neuroinflammation	Correlated with infarct size and clinical deficit
Chimerin	Nonprotein kinase C (GAP family)	No studies
	Synthesized by neurons (α 1 isoform)	
	Regulatory function in actin repolymerization (neurocytoskeleton)	
Secretagoin	Calcium-binding protein	↑ Secretagoin in acute phase
	Expressed in neuroendocrine cells	
Neurotrophin-3	Marker of neuronal death	
	"Neuronal survival factor" (neurotrophin family of growth factors)	Endogenous neurotrophin-3 enhances neuronal injury during acute stroke
Caspase-3	Cysteine protease	↓ Neurotrophin-3 synthesis has neuroprotective role
		Activation of caspase-3 in permanent and transient brain ischemia
	"Executioner" in apoptosis	Caspase activation after ischemia-induced brain damage

Baseline Demographics and Clinical Presentation of the Stroke Cohort

Mean patient age was 72 ± 12 years; 355 were men (50.3%). On admission, 60.7% of patients had a slight neurologic deficit (NIHSS score <8); 33.7%, a moderate neurologic deficit (NIHSS score 8 to 20); and 5.6%, a severe neurologic deficit (NIHSS score >20). Regarding symptom duration, 22% were transient ischemic attacks (TIAs). There were no differences in the distribution of risk factors and previous treatment between infarcts and TIAs (data not shown). According to the Oxfordshire Community Stroke Project classification, 40.2% had a partial anterior circulation infarction; 23.4%, a total anterior circulation infarction; 23.2%, a lacunar circulation infarction; and 13.2%, a posterior circulation infarction.

Stroke Etiologic Subtypes

The etiologic diagnosis for each ischemic stroke was assigned at discharge: 259 were cardioembolic (36.6%), 151 (21.4%) were atherothrombotic, 128 were lacunar (18.1%), and 169 had an undetermined etiology (23.9%). The distribution of risk factors and clinical features among different etiologic subtypes is shown in Table 2. Undetermined etiology was more frequent among TIA patients (Table 2).

Biomarker Profile With Respect to Vascular Risk Factors and Stroke Severity

Mean time of blood sampling from stroke onset was 8.6 ± 14.5 hours; 54.5% of blood samples were collected within 6 hours after stroke onset. The influence of risk factors and previous treatments on the level of biomarkers studied

Table 2. Risk Factors and Clinical Features Regarding Stroke Etiology

	Cardioembolic, n=259	Atherothrombotic, n=151	Lacunar, n=128	Undetermined, n=169	P Value
Sex (female)	62.5	37.1	37.5	50.6	<0.001
Age, mean±SD, y	75±13	69±11	72±11	72±13	<0.001
Previous stroke	20.5	26.7	25.6	23.4	0.494
Claudication	6.6	14.2	5.6	10.3	0.036
Ischemic cardiopathy	20.2	14.2	17.6	7.9	0.006
Atrial fibrillation	59.9	4.1	5.6	9.1	0.001
Other embolic cardiopathies	14	0	0.9	3	0.001
Hypertension	55.3	60.8	68.5	5.1	0.037
Diabetes mellitus	23.7	34.5	36.1	21.2	0.005
Smoking	8.6	25	22.2	15.8	0.001
Enolism	2.3	6.8	8.3	5.5	0.061
Dyslipidemia	22.2	29.1	27.8	23	0.365
Baseline NIHSS score, median (interquartile range)	10 (4–17)	5 (2–10)	4 (2–6)	4 (0–11)	0.001
TIA/infarct, %/%	28.4/39	25.2/20.3	7.7/21	38.7/19.7	<0.001

All values are percentages unless indicated otherwise.

was analyzed (data not shown). Some of those risk factors showed a significant correlation with higher concentrations of several biomarkers: previous stroke increased DD levels; ischemic cardiopathy increased BNP levels; atrial fibrillation increased BNP, DD, RAGE, and caspase-3 levels; hypertension increased CRP and MMP-9 levels, and diabetes increased secretagogin levels. Moreover, patients who were taking anticoagulants had higher levels of BNP and RAGE.

Regarding stroke extent, BNP, DD, and RAGE were significantly higher among patients with total anterior cerebral infarction. In fact, we found slight correlations between the severity of neurologic deficit (NIHSS score) on admission and the following biomarkers: BNP ($r=0.11$, $P=0.005$), CRP ($r=0.14$, $P<0.001$), DD ($r=0.27$, $P<0.001$), MMP-9 ($r=0.12$, $P=0.002$), and RAGE ($r=0.20$, $P<0.001$).

Biomarkers Profile by Stroke Etiology

Biomarker distribution among the 4 etiologic stroke subtypes is shown in Table 3. Levels of BNP, DD, and sRAGE were significantly higher in the group of patients with cardioem-

bolic stroke. BNP was the biomarker that best differentiated cardioembolic stroke from any other etiology (Figure 1a), even among TIA patients (Figure 1b). The ROC curves obtained to select the cutoff values that offered the best sensitivity and specificity for differentiating cardioembolic stroke from other etiologies identified the following values: BNP=76 pg/mL, DD=0.96 μg/mL, and RAGE=1.2 ng/mL, which were used for inclusion in the multivariable analysis.

Logistic-Regression Analysis for Cardioembolic Stroke

BNP and DD appeared to be independent predictive factors of cardioembolic stroke (BNP >76 pg/mL OR=2.3 [1.4 to 3.7], $P=0.001$; and DD >0.96 μg/mL OR=2.2 [1.4 to 3.7], $P=0.001$). Atrial fibrillation OR=15.3 (8.4 to 27.7, $P<0.001$), other embolic cardiopathies OR=14.7 (4.7 to 46, $P<0.001$), and total anterior cerebral infarction OR=4 (2.3 to 6.8, $P<0.001$) were the remaining independent predictors.

Because the diagnosis of cardioembolic stroke is often directly derived from atrial fibrillation, atrial fibrillation is

Table 3. Biomarker Distribution Regarding Etiology

	All Strokes, n=707	Cardioembolic, n=259	Atherothrombotic, n=151	Lacunar, n=128	Undetermined, n=169	P Value
BNP, pg/mL	66.5 (21.5–160.4)	143.9 (63.3–237.3)	44.3 (0.0–106.5)	37.2 (0.0–84.4)	45.8 (13.2–108.2)	$P<0.001$
DD, μg/mL	0.8 (0.3–1.9)	1.1 (0.5–2.3)	0.5 (0.2–1.7)	0.6 (0.3–1.3)	0.8 (0.3–1.9)	$P<0.001$
CRP, μg/mL	10.6 (4.7–25.3)	11.7 (4.6–28.6)	8.7 (4.1–21.7)	9.8 (4.6–23.7)	11.8 (5.2–27.7)	$P=0.52$
S100b, pg/mL	58.65 (31.1–123.2)	62.3 (33.9–125.7)	50.5 (25.7–113)	51 (22.9–123)	66.5 (34.5–138.7)	$P=0.07$
RAGE, ng/mL	0.9 (0.6–1.5)	1.1 (0.7–1.9)	0.8 (0.5–1.3)	0.8 (0.6–1.1)	0.9 (0.5–1.4)	$P<0.001$
Chimerin, ng/mL	0 (0–0.8)	0.0 (0.0–0.5)	0.0 (0.0–1.1)	0.0 (0.0–0.7)	0.0 (0.0–0.9)	$P=0.15$
Neurotrophin-3, ng/mL	0 (0–0.7)	0.0 (0.0–0.8)	0.1 (0.0–0.7)	0.0 (0.0–0.7)	0.0 (0.0–0.7)	$P=0.75$
Secretagogin, ng/mL	0.2 (0–0.3)	0.1 (0.1–0.3)	0.2 (0.1–0.3)	0.1 (0.1–0.3)	0.2 (0.1–0.4)	$P=0.23$
Caspase-3, ng/mL	2.1 (0.9–5)	2.3 (1.1–4.9)	2 (0.8–4.7)	2.2 (0.9–6)	1.7 (0.8–4.7)	$P=0.30$
MMP-9, ng/mL	257.5 (145.5–462.5)	258.2 (151.9–476.5)	252.9 (170.6–433.8)	242.7 (142.6–460.5)	279.6 (112.8–493.5)	$P=0.79$

Medians and interquartile ranges are shown.

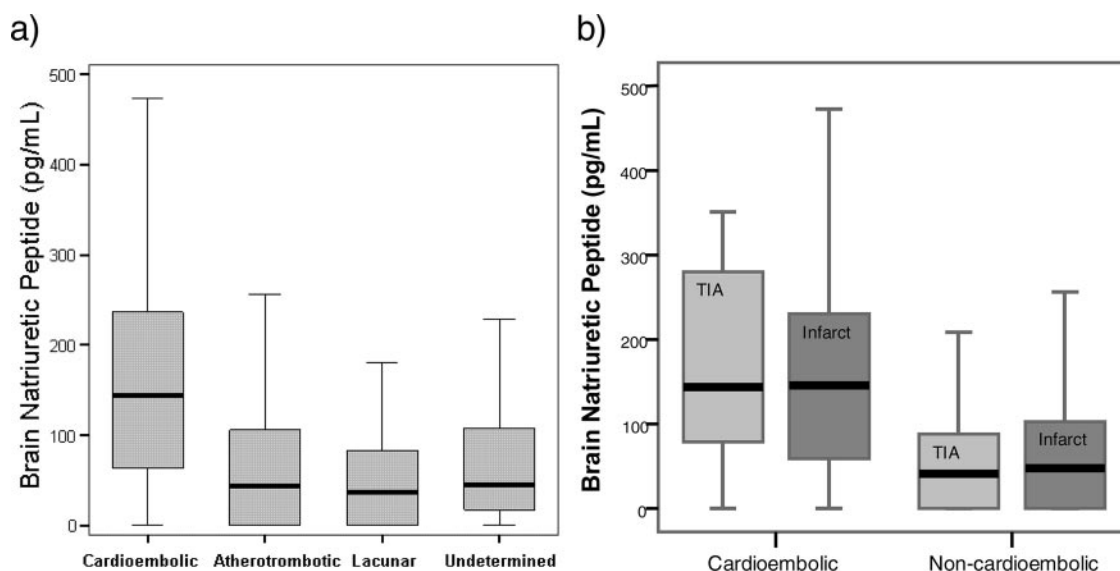


Figure 1. BNP was the test biomarker that best distinguished stroke etiologies, with highest plasma levels among cardioembolic stroke (a) including cardioembolic TIA (b), and offered the best sensitivity and specificity to discriminate between cardioembolic stroke and the remaining etiologies.

unlikely to be independent of the dependent variable “cardioembolic stroke”; therefore, we repeated the analysis after excluding atrial fibrillation and “other embolic cardiopathies.” ORs for the biomarkers according to this approach were as follows: BNP OR=4.28 (2.83 to 6.49, $P<0.0001$) and DD OR=1.77 (1.16 to 2.69, $P=0.008$). In many cases, we could not determine for sure whether a patient had (paroxysmal) atrial fibrillation or not within the first days after stroke; therefore, this final model might be more realistic in daily practice.

The results of the multiple logistic regression were similar when we took into account only those patients whose blood samples were obtained within 6 hours of symptoms onset (BNP OR=3.29 [1.85 to 5.84, $P<0.001$], DD OR=2.37 [1.36 to 4.13, $P=0.002$], and RAGE OR=1.89 [1.07 to 3.38, $P=0.030$], when only biomarkers were included in the model, and BNP OR=4.32 [2.4 to 7.77, $P<0.001$], when all variables except atrial fibrillation and “other embolic cardiopathies” were included). Among patients with transient symptoms ($n=155$), a high BNP level (>120 pg/mL) also identified cardioembolic TIA (OR=6.7 [2.4 to 18.9], $P<0.001$), together with atrial fibrillation (OR=26.4 [8.4 to 82.6], $P<0.001$).

Combining Biomarkers: Predictive Value Tests

The optimal biomarker cutoff point for discriminating the presence or absence of a cardioembolic source was determined to be BNP >76 pg/mL with a specificity of 68% (95% CI, 64 to 72), a sensitivity of 72% (95% CI, 66 to 73), a positive predictive value of 55% (95% CI, 49 to 60), and a negative predictive value of 82% (95% CI, 78 to 86); and DD >0.96 $\mu\text{g/mL}$ with a specificity of 64% (95% CI, 60 to 69), a sensitivity of 56% (95% CI, 50 to 63), a positive predictive value of 46% (95% CI, 40 to 52), and a negative predictive value of 73% (95% CI, 69 to 78).

Measuring both biomarkers might improve the etiologic diagnosis of stroke (Figure 2). In fact, the number of cases

with a diagnosis of cardioembolic stroke increased parallel to BNP and DD deciles (Figure 2A). Moreover, if we consider that a negative test yields BNP and DD levels below the cutoff, we observe that the sensitivity and negative predictive value increase (sensitivity=87%, Figure 2B). In contrast, if we consider that a positive test yields both values above the cutoff, the specificity and positive predictive value rise (specificity=85%, Figure 2B).

Undetermined Stroke Follow-Up and Biomarker Subanalysis

The undetermined etiologic subtype included 169 patients: in 45 patients (27%) we found no cause despite extensive evaluation; 13 patients (8%) had 2 or more potential causes of stroke; and in 71 (42%), the evaluation was incomplete (9 deaths, 17 transfers to other hospitals, and 45 due to patient characteristics precluding full evaluation). During follow-up, we identified the stroke etiology in 40 patients (23%): 10 had a cardioembolic source; 9, atherothrombotic; 14, lacunar; and 7, other determined etiologies. A review of biomarker data among the 10 patients reclassified with cardioembolic stroke (Figure 3) showed that 30% of them had BNP values >76 pg/mL and DD >0.96 $\mu\text{g/mL}$, and 70% had BNP or DD values above those cutoff points. In contrast, among atherothrombotic and lacunar strokes, we found no patients with increases in both biomarkers.

Cardioembolic Stroke Prediction Model

With these new data, a logistic-regression analysis showed that atrial fibrillation (OR=17.2 [9.4 to 31.6], $P<0.001$), baseline NIHSS score (OR=1.1 [1 to 1.1], $P<0.001$), other embolic cardiopathy (OR=9.8 [3 to 31.9], $P<0.001$), ischemic cardiopathy (OR=1.9 [1 to 3.6], $P=0.05$), female sex (OR=1.7 [1 to 2.8], $P=0.032$), age (OR=1 [0.9 to 1], $P=0.016$), BNP >75.6 pg/mL (OR=3 [1.8 to 4.9], $P<0.001$), and DD >0.96 $\mu\text{g/mL}$ (OR=2 [1.2 to 3.4], $P=0.006$) were independently associated with cardioembolic etiology.

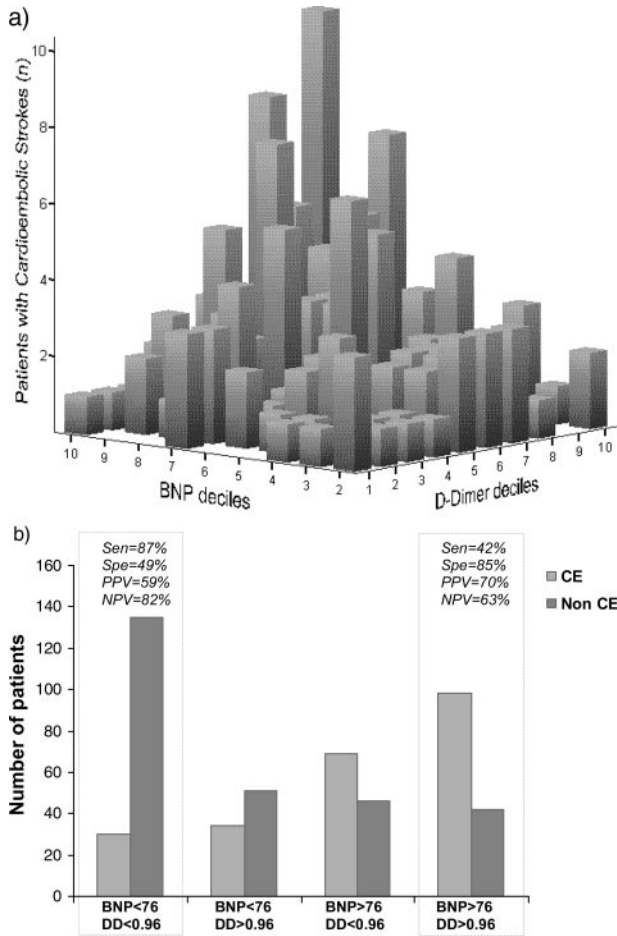


Figure 2. Cardioembolic stroke showed high levels of BNP and DD, with most of those patients located in the upper deciles for both biomarkers (a). The number of patients with cardioembolic stroke according to BNP and DD levels is shown; sensitivity, specificity, and positive and negative predictive values are shown when the test was considered negative if both biomarkers were below the cutoff point or positive when both were above the cutoff point (b).

This multivariable model showed a good fit (Hosmer-Lemeshow test $P=0.33$). The model showed a sensitivity of 66.5% and specificity of 91.3%, with an overall accuracy of 82.6%. The probability predicted for cardioembolic etiology was obtained from the following equation:

$$1/[1 + e^{2.07 - (0.531 \times \text{female}) + (0.025 \times \text{age}) - (0.636 \times \text{ischemic cardiopathy}) - (2.845 \times \text{atrial fibrillation}) - (2.283 \times \text{other embolic cardiopathy}) - (0.077 \times \text{baseline NIHSS score}) - (1.093 \times \text{BNP} > 75.6 \text{ pg/mL}) - (0.712 \times \text{DD} > 0.96 \text{ } \mu\text{g/mL})].$$

The discriminating ability of the model was very good as shown by the ROC curve, with an area under the curve of 0.89 (95% CI, 0.863 to 0.917; see Figure 4).

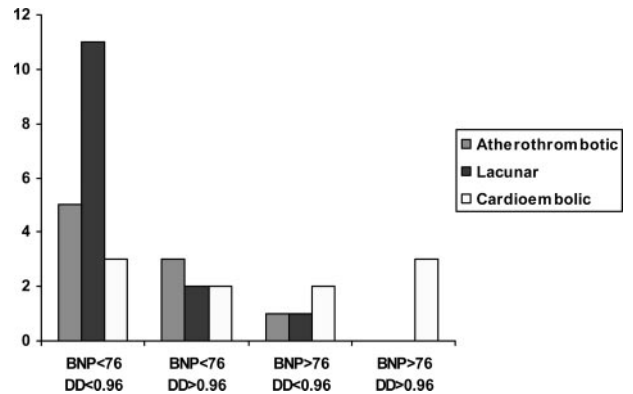


Figure 3. Relation between BNP and DD level at Emergency Department arrival with final etiology identified during outpatient clinic follow-up among patients diagnosed with undetermined stroke at hospital discharge.

Discussion

The results of our study indicate that differences in plasma biomarkers might be useful for distinguishing cardioembolic stroke from other stroke subtypes. Although diagnostic tests based on blood markers are commonly used for cardiogenic disorders (BNP for the diagnosis of heart failure and troponin I for myocardial infarction⁸), the use of biomarkers is still incipient in the stroke field.^{13,14}

Other studies have previously addressed the importance of a unique biomarker in the etiologic diagnosis of stroke; for instance, Ageno et al⁵ reported an optimal DD cutoff value of 2 $\mu\text{g/mL}$ to predict cardioembolic stroke, with a sensitivity of 59.3% and a specificity of 93.2%. However, that study did not take into account confounding factors such as atrial fibrillation and did not perform any kind of multivariate analysis.

Combining Biomarkers to Diagnose Stroke Etiology

BNP >76 pg/mL and DD >0.96 $\mu\text{g/mL}$ indicated a cardioembolic etiology with a sensitivity of 72% and 56%, respectively, and a specificity of 69% and 64%, respectively. In fact, the most useful property of these markers is their negative predictive value, meaning that in 82% of patients with BNP <76 $\mu\text{g/mL}$, the etiologic diagnosis will not be cardioembolic stroke. Determination of both biomarkers jointly to classify an acute ischemic stroke in an etiologic subtype increases the probability of diagnosing a stroke as cardioembolic when it is actually cardioembolic; ie, if a patient has high levels of both (BNP >76 pg/mL and DD >0.96 $\mu\text{g/mL}$), the probability that that stroke is cardioembolic is 70% (positive predictive value).

We hypothesize that if we were able to identify a third or even more biomarkers with properties similar to those of BNP and DD, we could approach a very accurate etiologic diagnosis. Interestingly, the combination of clinical and biochemical parameters yielded the most accurate prediction of cardioembolism probability according to a simple equation that included sex, age, presence of main cardiac embolic diseases, stroke severity, and both selected biomarkers.

BNP and DD for Cardioembolic Stroke

BNP is secreted mainly by the myocardium, with some evidence that also suggests a cerebral origin.¹⁵ This peptide does not cross

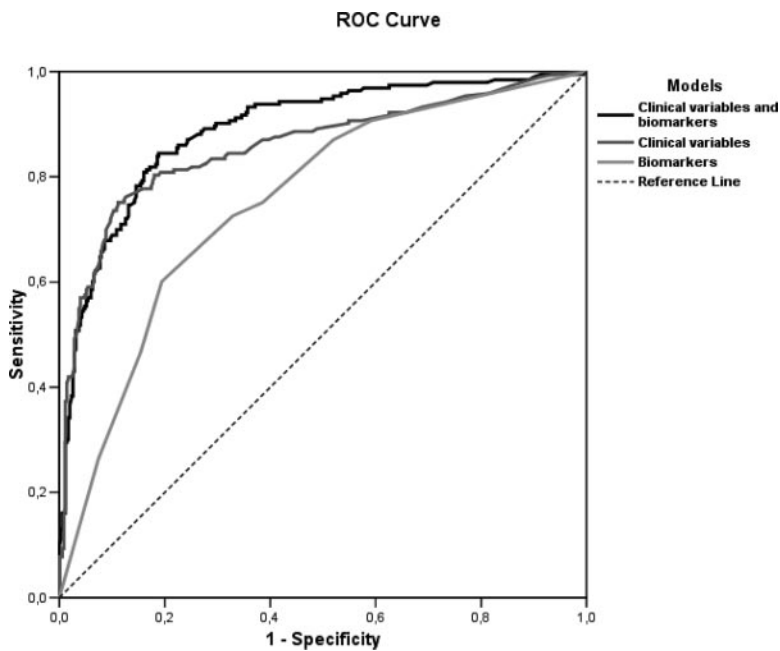


Figure 4. Best model to predict cardioembolic stroke etiology, combining clinical and biomarker data (area under the curve=0.89) compared with isolated clinical (area under the curve=0.86) or biochemical (area under the curve=0.75) data.

the blood-brain barrier, although during cerebral ischemia, the blood-brain barrier is compromised and different molecules released from injured brain tissue have the potential to cross it. Therefore, BNP release has been claimed to be associated with the severity of brain ischemia, reflecting increased biosynthesis and secretion from ischemic brain tissue.

Plasma levels of BNP have previously been described as elevated in the acute phase of stroke,^{16,17} and some authors have suggested that BNP levels are correlated with neurologic deficits.^{17,18} However, although in our study BNP also showed a slight correlation with the NIHSS score, it remained an independent predictor of cardioembolic etiology after correcting for stroke severity and extent. Moreover, BNP is also an independent predictor of cardioembolic TIA, thus supporting the concept that infarct size is not the cause of excessive BNP production. BNP concentrations are increased when systolic or diastolic ventricular dysfunction exists¹⁹; therefore, among our stroke patients, high BNP levels may indicate a cardioembolic source, ie, patients with “optimal” cardiac conditions to develop thrombi.

The left atrium is the main source of BNP in patients with atrial fibrillation.^{20,21} Such patients show higher plasma BNP levels than control subjects, and patients with atrial fibrillation plus clinical evidence of thromboembolism have higher BNP levels than those patients with atrial fibrillation alone.²²

High levels of DD, products of degradation of cross-linked fibrin by plasmin, are also an independent predictor of cardioembolic stroke, thus confirming the results of previous reports. Various studies have shown that the coagulation and fibrinolytic systems are activated in cerebral ischemia, and DD is the most frequently used indicator of blood coagulation activation.²³ After stroke, enhancement of the coagulation system reflects the mechanism of thrombus formation and vessel occlusion. In fact, thrombus formation in the cardiac chambers is mainly due to blood stasis, leading to a fibrin-rich clot very similar to venous thrombi. Conversely, thrombi

originating in the large arteries are mostly platelet rich, and fibrin formation is secondary to platelet activation.²⁴

Although the high DD and BNP concentrations in the cardioembolic group may be partially explained by the high prevalence of atrial fibrillation, our results confirm that these biomarkers are independent predictors after adjustment for age, sex, atrial fibrillation, cardiovascular risk factors, treatment, and neurologic deficit.

Biomarkers for Noncardioembolic Stroke Etiologies

We failed to identify biomarkers specific for other etiologies such as atherothrombotic or lacunar strokes. However, lower sRAGE levels appeared in lacunar and atherothrombotic strokes. This association may be explained by that fact that sRAGE has an antiatherogenic role and is decreased in diabetes and hypertension, both risk factors for large- or small-vessel atherothrombotic disease.²⁵

Advantages of Rapid Stroke Etiology Identification

A strength of our study is that the median time between stroke onset and sample collection for biomarker determination was only few hours. Therefore, etiologic information offered by these markers would be available much earlier than usual for etiologic diagnosis.

Because recurrent embolism to the brain occurs within 2 weeks in 6% to 12% of patients who initially experience embolic stroke from cardiac sources,²⁶ it is critical to identify this etiologic subtype. However, in many cases, echocardiographic techniques are delayed or the rhythm abnormality responsible for the index stroke is no longer present when the patient is examined, sometimes making it difficult to initiate anticoagulant therapies, even when the neurologist suspects a cardiac source for the brain embolus in the absence of objective proof.

Study Limitations

Our study had several practical limitations. One inherent weakness of any classification system is the lack of a “gold

standard,” such as pathologic confirmation, to define the exact mechanism of stroke. Pathologic verification of the suspected mechanism is often not feasible in stroke because most victims survive. Our results are based on etiologic classifications, such as TOAST, which is not 100% reliable.

Because the stroke recurrence rate of patients with a diagnosis of “stroke of undetermined origin” after a routine diagnostic work-up was not high,²⁷ it remains to be demonstrated whether a biomarker-enhanced etiologic classification would significantly influence this low recurrence rate.

Regarding biomarker measurement, because variability exists among different assays used to determine DD and BNP levels, these assays should be compared and validated in the future if any of them are to be used in clinical practice in stroke, as is being done for other indications.^{28,29}

Conclusions

Our results suggest that using a combination of plasma biomarkers (including BNP and DD) may be a feasible strategy to improve cardioembolic stroke diagnosis in the acute phase of stroke, thus rapidly guiding other diagnostic tests in the acute setting, reducing the huge number of strokes of undetermined etiology, and accelerating the start of optimal pharmacologic secondary prevention.

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