Stroke

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Multiple Panel of Biomarkers for TIA/Stroke Evaluation

[Letters to the Editor]

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To the Editor:

Brey et al [1] reported what is to their knowledge the first study to demonstrate a prospective association between sera cofactor-dependent anticardiolipin antibodies and stroke independent of other risk factors as well as myocardial infarction (MI). In addition to lending support to basic research that has shown the pathogenicity of antiphospholipid-protein antibodies (aPL) in thrombosis, [2] this well-conducted epidemiological study of Japanese-American men enrolled in the Honolulu Heart Program and followed for up to 20 years provides evidence for the role of aPL as potentially important markers and/or causes of increased vascular risk associated with ischemic stroke and MI.

It is known that stroke is a multisystemic disorder involving mechanisms of thrombotic and neurotoxic coupling. [3] Biochemical markers including glutamate, homocysteine (a sulfinic analog of aspartate [4]), and *N*-methyl-d-aspartate (NMDA) receptor autoantibodies (aAb) are independently associated with neurotoxicity and can be measured in blood. [5] The aPLs are a part of the structural components of excitatory membranes containing glutamate receptors and may be involved in the neurotoxicity process as well. [3] Consequently, the appearance of elevated levels of aPL in blood represents an additional indicator of NMDA neuroreceptor damage under ischemic conditions.

The development of a multiple panel of biomarkers for stroke analogous to that now in use for MI would be beneficial for the emergency bedside diagnosis of stroke and may help differentiate ischemic from hemorrhagic stroke. We assessed 3 proposed biomarkers [5]: glutamate and homocysteine as correlates of large and middle artery dysfunction, and NR2A aAb as a criterion of microvascular damage

independently associated with neurotoxicity and thrombosis in patients with transient ischemic attack (TIA)/stroke. We studied 92 patients with high blood pressure, prestroke, and TIA, subdivided according to symptom severity, including patients with left hemispheric stroke admitted within 6 hours of stroke onset (30.4+/-3.2 score on the Orgogozo Stroke Scale) and patients with intracerebral hemorrhage located in the left hemisphere (Table 1). Patients underwent neurological examination and neuroimaging (computed tomography, T2-weighted MRI, diffusion-weighted imaging , and Doppler angiography). 6,7 After informed consent, blood samples were collected on the day of admission from all subjects. Plasma levels of glutamate and homocysteine and serum levels of the NMDA receptor NR2A subtype were assessed by high-performance liquid chromatography and enzyme-linked immunosorbent assay. 6

TABLE 1. Plasma Concentrations of Glutamate and Homocysteine and Serum Concentrations of NR2A Autoantibodies in Patients and Control Subjects

			Gender		Concentration		
Subjects	n	Age, y	M	F	Glutamate*	Homocysteine*	NR2A aAb†
Control	30	51.6±4.6	12	18	32.0±3.8	8.3±0.5	1.4±0.3
High blood pressure	25	36.9±2.3	8	17	32.8±0.9	11.5‡±0.4	1.9±0.1
Prestroke	12	59.9±4.7	5	7	37.5±1.2	12.3‡±0.6	2.5‡±0.1
TIA	14	58.9±1.7	7	7	40.4‡±1.1	12.9‡±0.5	4.2‡±0.2
Ischemic stroke	23	54.7±1.4	14	9	30.8±1.2	13.0±0.7	4.9§±0.8
Hemorrhage	18	53.0±4.4	12	6	33.3±3.5	9.2±0.3	1.7±0.1

aAb indicates autoantibodies.

Data are mean±SEM, *expressed in micromoles per liter; †expressed in nanogram per milliliter; ‡$P<0.05$ and §$P<0.01$ compared with controls.

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Plasma glutamate concentrations were highest in patients with TIA (Table 1). Homocysteine levels increased in patients with cerebrovascular abnormalities, prestroke, TIA, and ischemic stroke (in that order) and depended on stroke severity, whereas amino acid contents did not show such a correlation (Table 1). We did not observe any changes in the dynamic of glutamate and homocysteine for patients with hemorrhage during the first 3 hours of hospitalization (Table 1). Detailed T2-weighted MRI and DWI in 9 patients with TIA were analyzed on the third hour of admission. Regional ischemia was clearly depicted as hyperintensity on DWI, while T2-weighted imaging showed no changes. T2-weighted MRI showed an area of infarction in 4 patients that developed to day 7 of observation and was accompanied by neurological worsening.

Excessive activation of NMDA receptors is the result of glutamate and homocysteine neurotoxicity. [4] Level of NR2A aAb in the blood of healthy controls was 1.4+/-0.25 ng/mL, whereas for patients with cerebrovascular abnormalities, it began to increase and achieved the highest levels in those with prestroke and TIA/stroke (Table 1). Different profiles of elevated NR2A aAb were revealed in the blood of patients with ischemic stroke, while no changes in sera of patients with hemorrhage were detected [7] (Table 2). The appearance of NR2A autoantibodies elevated above control depended on the severity of disorder, with the same tendency as homocysteine. The correlation between infarct volume and the level of NR2A aAb was demonstrated by CT and MRI. Concentration of aAb was lower in patients with infarcts localized in the posterior region (4 to 5 cm^3) and significantly higher in infarcts with a cortical topography (>25 cm^3). When neuroprotective glycine was administered, we observed NR2A aAb reduction in patients with acute stroke that was accompanied by an improvement in neurological function. [8]

TABLE 2. NR2A Autoantibody Monitoring in Patients with Ischemic and Hemorrhagic Stroke

	NR2A Autoantibody, ng/ml	
Time, h	Ischemic Stroke*	Hemorrhage
0 (on admission)	5.04±0.91	1.72±0.12
3	4.96±0.32	1.68±0.21
6	5.10±0.71	1.70±0.13
9	7.90±1.23	1.71±0.09
12	7.30±1.53	1.64±0.20
24	3.20±0.62	1.65±0.15
72	3.50±0.50	1.70±0.14

*$P<0.001$, versus control (1.4±0.3 ng/ml).

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Our experimental [9] and clinical research data have demonstrated that simultaneous assessment of these 3 biomarkers allows neurotoxicity and

thrombosis to be correlated with severity of cerebral ischemia and as such represents a promising additional tool for use with neuroimaging for the diagnosis of TIA/stroke. Development of a blood test that would also detect the thrombotic marker (anticardiolipin antibodies) observed by Brey et al 1 would, when used in conjunction with our proposed biomarkers, help evaluate the thrombotic and neurotoxic contributions in stroke; guide antiplatelet, antithrombotic, and neuroprotective therapy; and assess patient follow-up and recovery after ischemic events.

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