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NR2 antibodies: Risk assessment of transient ischemic attack (TIA)/stroke in patients with history of isolated and multiple cerebrovascular events

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ABSTRACT

Background and purpose: Predicting stroke using biomarkers would enable clinicians to help prevent stroke or mitigate damage. Several stroke biomarkers have been investigated but none has shown near term predictive value.
Methods: We studied patients presenting with a history of stroke or transient ischemic attack (TIA) to determine whether serum levels of autoantibodies to the NMDA receptor NR2 peptide (NR2Ab) reflected the presence of recent stroke compared with controls. Antibody levels were also correlated with clinical risk factors for stroke, including diabetes, hypertension, hyperlipidemia, and history of recent TIA or stroke.
Results: Of the 245 patients that presented with acute stroke or TIA, 130 consented to participate and results are available for the 120. Volunteers from the community were recruited as controls. Males and females with multiple recent strokes and females with acute strokes had elevated NR2Ab levels compared to non-stroke patients or controls. Using a multiple regression model, the predictive value for NR2Ab was compared to clinical risk factors. In men, the presence of stroke correlated with hypertension ($p<0.001$) and NR2Ab levels ($p<0.01$) and in women the presence of stroke correlated with hypertension ($p<0.001$), diabetes ($p<0.05$), atrial fibrillation ($p<0.05$) and NR2Ab ($p<0.01$).
Conclusion: These results suggest that NR2Ab levels reflect a history of multiple strokes and may serve as a predictive factor for stroke.

1. Introduction

During the past decade, several biomarkers for detection of stroke risk and/or acute stroke have been investigated. These include anti-phospholipid antibodies, anti-cardiolipin antibodies, von Willebrand factor, [1,2] C-reactive protein (CRP), phospholipase A2 (Pl-PLA_2), [3] matrix metalloproteinase-9 (MMP-9), [4] VCAM, [5] homocysteine, [6] glutamate, neuron-specific enolase, myelin basic protein, S-100b, [7,8] B-type neurotrophic growth factor and monocyte chemotactic protein-1 [1,2,9].

However, most of these biomarkers have low predictive value for stroke in the near term or reflect the severity of an established subacute or completed stroke. What would be more desirable is a biomarker with near term predictive value. This would be comparable to the stroke-predicting ability of a "crescendo" pattern of transient ischemic attack (TIA). *N*-methyl-D-aspartate (NMDA) receptor peptides and their autoantibodies (NR2Ab) have been proposed as biomarkers of the neurotoxicity underlying cerebral ischemia and stroke [10]. Anti-NMDA receptor IgG antibodies develop in response to the release of peptide fragments resulting from NMDA receptor turnover during excitotoxicity [10–12], and have been recognized in the serum of stroke patients since the late 1980s [13]. These antibodies were present in the aftermath of stroke and persisted for a period of many months [14]. These antibodies were not present in the serum of patients with Bell's palsy, meningitis or subarachnoid hemorrhage [15].

Preoperative serum concentrations of NR2Ab have been shown to be predictive of severe neurological adverse events after cardiac surgery [16]. Patients with a positive NR2Ab level >2.0 ng/ml prior to surgery were nearly 18 times more likely to experience a postoperative neurological event than patients with a negative test [16]. The NR2 peptide increases in association with microemboli during carotid endarterectomy and with postoperative neurological deficits following carotid endarterectomy [17,18].

The primary objective of this study was to identify NR2Ab concentrations in patients presenting with a history of multiple transient ischemic attacks (TIAs) or strokes or single or isolated strokes and

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compare these concentrations with controls. A secondary goal was to compare the relative predictive values of NR2Ab and clinical stroke risk factors. We studied patients presenting with acute stroke at DeKalb Medical Center, Decatur, GA, USA. To determine the presence of multiple recent strokes, we reviewed historical, clinical neurological, and neuroimaging data. For controls, we used healthy subjects of different ages without a history of stroke.

2. Materials and methods

2.1. Study design

This was a blinded, convenience cohort study involving a single measurement of NR2 antibody levels in patients with suspected stroke.

2.2. Subjects

Adults aged 18 years or older presenting within 72 h with suspected TIA (defined as a neurological deficit that resolved within 24 h), or suspected acute stroke were included in the study. Patients were excluded if they were pregnant or were transferred to another facility for inpatient care.

Patients were recruited on a convenience basis from January 2006 to January 2008. A number of patients with non-stroke diagnoses were also recruited, as were controls from community patient support groups and meetings of business people. The research protocol was approved by the DeKalb Medical Center Institutional Review Board. Written informed consent was obtained from each subject or a family member.

Blood samples (5 ml) were withdrawn by venipuncture into standard evacuated collection tubes with a serum separator and centrifuged at 4000 RPM for 4 min. Samples were then transferred to 1 ml Eppendorf tubes and frozen at −80 °C.

2.2.1. ELISA procedure

Antibody concentrations in the sera were assessed using the Gold Dot NR2 Antibody Test (CIS Biotech, Inc., Atlanta, GA) according to the manufacturer's procedure. Briefly, 100 μl of diluted sera (1:50; 20 μl of serum sample + 980 μl of diluent) and sets of calibrators were added to NR2 peptide-coated wells of microtiter plates and were incubated for 30 min on a shaker at 37 °C. After the wells were washed with buffer, 100 μl of Protein A-HRP labeled antibodies was added and incubated for 30 min on a shaker at 37 °C. After additional washing, 100 μl of TMB ready-to use substrate was added. The color reaction was developed for 10 min, stopped with stop reagent (100 μl), and measured at 450/630 nm on a microplate reader (ELx800™, Biotek® Instruments, Inc., USA). The NR2Ab titer in each sample was calculated using calibration from the standards provided with Gold Dot NR2 Antibody Test kit.

2.3. Clinical evaluation

All patients had a standard clinical neurological stroke evaluation, including screening for recombinant tissue plasminogen activator (rTPA) when appropriate, standard stroke history, general medical examination and neurological examination. All stroke patients underwent emergent CT scanning and 90% had MRI scans (to be discussed in the later part) performed within 24 h. Each patient was seen and followed during hospitalization by an experienced stroke neurologist. Hospital course and discharge examination data were also noted. Evidence of recent prior strokes was based upon history, available recent records, comparison with previous MRI and CT scans, and the examination of DWI, ADC, T2-weighted and T1-weighted scans for evidence of recent multiple strokes according to the method of Coutts et al. [19]. Clinical determinations were made prior to NR2Ab determinations. All controls completed a standard questionnaire detailing medical history, stroke risk factors, medications, and details of any prior strokes.

2.4. Magnetic resonance imaging and analysis

Images were obtained on a standard clinical MRI scanner (Siemens 1.5 Tesla Avanto) operating with single-shot echo planar-capable gradients. The standard imaging data set comprised T1-weighted sagittal and diffusion-weighted, apparent diffusion coefficient, FLAIR, and T2-weighted axial sequences. Images were reviewed on GE Centricity software.

2.5. Classification of patients and controls

Controls were classified into the "Prior Only" group if they reported a history of stroke. Otherwise, controls were classified in the "No Stroke" group. Patients were classified into one of four groups.

2.5.1. No stroke

Patients admitted with stroke in the differential diagnosis but who had a non-stroke discharge diagnosis or controls without history of stroke. Patients recruited with non-stroke diagnoses were included in this group.

2.5.2. Prior only

Patients admitted with acute stroke in the differential diagnosis but diagnosed with a history of remote stroke or controls with a history of prior stroke or TIA (more than 6 months prior).

2.5.3. Acute only

Patients admitted with acute stroke in the differential diagnosis and discharged with acute stroke as the diagnosis.

2.5.4. Multiple recent

Patients discharged with a diagnosis of acute stroke or TIA and evidence of prior stroke or TIA within 6 months.

2.6. Statistical analyses

Patient data were entered into a Microsoft Access database and exported as spreadsheets. Statistical analysis was performed using R Statistical package (http://www.r-project.org/). Standard descriptive statistics were calculated in reporting patient characteristics. A multiple logistic regression analysis, analysis of variance, and Welch two-sample t-test were applied using R.

3. Results

3.1. Patient and control group characteristics

Patient enrollment and disposition are summarized in Fig. 1. The average age, sex distribution, and prevalence of stroke risk factors are shown in Tables 1A and 1B for each of the aforementioned groups. The average age in each of the four groups was 60 ± 5 years.

3.2. NR2 antibody results

Male and female groups were analyzed separately. The mean and standard deviation of NR2Ab levels in each group were determined with descriptive statistics and the results are shown in Tables 1A and 1B. The multiple recent groups had the highest levels of NR2Ab, followed by patients with prior stroke and acute stroke. In all of the female stroke groups, including the no stroke group, NR2Ab levels were higher than in the male groups. The results for each group were compared to the other groups using the Welch two-sample t-test