Plasma Biomarkers in the Diagnosis of Acute Ischemic Stroke

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Abstract. Rapid diagnosis and timely treatment improves the outcome in patients with ischemic stroke, but a rapid and sensitive blood test for ischemic stroke does not exist. This study tested whether a panel of biomarkers might be useful in the diagnosis of acute ischemic stroke. Consecutive patients with suspected stroke presenting to the emergency department of a university hospital in Korea were enrolled. Plasma specimens were assayed for brain natriuretic peptide, D-dimer, matrix metalloproteinase-9, S100β, and a proprietary composite multimarker index (MMX). There were 139 patients in this study, 89 of whom were diagnosed with acute ischemic stroke, 11 with acute cerebral hemorrhage, and 39 with other brain disorders. The MMX value was significantly higher in the patients with acute ischemic stroke in comparison to 57 healthy controls (p <0.001), but there was no significant difference between the MMX value in patients with acute ischemic stroke vs those with acute cerebral hemorrhage (p = 0.884). The discriminatory capacity of MMX was modest, with an area under the receiver-operating-characteristic curve of 0.714 for acute stroke. Ischemic stroke was not diagnosed by any of the biochemical markers individually. Although the data suggest that MMX may be helpful to diagnose an acute stroke, it does not discriminate between acute ischemic stroke and acute hemorrhagic stroke.

Keywords: acute ischemic stroke, biochemical markers, multimarker index

Introduction

Stroke represents a leading cause of disability and mortality worldwide [1]. The timely administration of thrombolytics (tissue plasminogen activator [t-PA]) improves neurologic outcomes in patients with ischemic strokes [2], but the upper limit of the treatment window may be as short as 5 to 6 hr [3]. In fact, only a small proportion of ischemic stroke patients can be safely treated with thrombolytics [3-5]. Although a variety of obstacles limit tissue plasminogen activator use, diagnostic uncertainty is associated with underuse of fibrinolytic treatment [6]. The initial evaluation of the patient includes a history and physical examination, brain imaging, coagulation testing, and determination of serum glucose and electrolytes [3]. Computerized tomography (CT) is often normal after the onset of ischemia and may remain normal in patients with mild ischemic stroke [7]. Magnetic resonance imaging (MRI) is more sensitive in detecting ischemia than CT, but MRI may not be feasible in acutely ill patients because they are restless and because MRI is not immediately available in all facilities [7].

Many studies have evaluated biomarkers in cerebral ischemia. For example, acute stroke has been associated with elevation of serum inflammatory and anti-inflammatory mediators such as matrix metalloproteinase-9 (MMP-9) [8,9], markers of impaired hemostasis and thrombosis [10,11], and markers of glial activation, such as
S100β [12,13]. Recently, two studies examined a panel of markers that may be helpful in identifying patients with acute ischemic stroke in the emergency setting [14,15].

In the current study, we evaluated the clinical usefulness of four plasma biochemical markers for the diagnosis of acute ischemic stroke (D-dimer, MMP-9, B-type natriuretic peptide [BNP], and S100β) using the Triage® stroke panel (Biosite, Inc., San Diego, CA).

Materials and Methods

Study population. Consecutive adult patients (n = 139) with suspected stroke who presented between April and August 2007 to the Emergency Department of East-West Neo Medical Center in Korea were enrolled in the study. Clinical stroke was defined by focal neurologic signs or symptoms thought to be of vascular origin that persisted for >24 hr. Ischemic strokes were confirmed by MRI before discharge. Blood samples were obtained from the 139 patients with suspected stroke and 57 healthy controls. The subjects were divided into the following 4 groups: healthy controls with no history of brain disorders (group I); patients diagnosed with acute ischemic stroke by MRI (group II); patients diagnosed with acute cerebral hemorrhage (group III); and patients diagnosed with brain disorders other than ischemia or hemorrhage (group IV). All patients and healthy controls gave written consent for the use of their medical information for research purposes. The Institutional Review Board reviewed and approved the study protocol.

Biomarker analysis. Blood samples were obtained by venipuncture as soon as subjects arrived at the hospital. The samples were collected in EDTA-containing tubes and promptly centrifuged at 10,000 × g. The supernatant plasma was tested immediately. Biomarker analysis was performed using the Triage® stroke panel includes fluorescence immunoassays for BNP, D-dimer, MMP-9, and S100β in EDTA-anticoagulated whole blood or plasma specimens. The test procedure involves the addition of several drops of whole blood or plasma to the sample port on the test device. After addition of the sample, the cells are separated from the plasma by a filter contained in the device. The plasma reacts with fluorescent antibody conjugates within the reaction chamber and flows down the device detection lane by capillary action. Complexes of each fluorescent antibody conjugate are captured on discrete zones, resulting in binding assays that are specific for each analyte. The analytic ranges are as follows: MMP-9, 25-1300 ng/ml; D-dimer, 150-5000 ng/ml; S100β, 100-8000 pg/ml; and BNP, 10-5000 pg/ml.

Multimarker index. A proprietary Multimarker Index (MMX) value was automatically calculated by the Triage Meter from the individual biomarker values. The MMX value is computed by an algorithm derived from the manufacturer's study of individual BNP, D-dimer, MMP-9, and S100β results. The algorithm is based on the concentrations of each analyte and incorporates weighting factors for each analyte. The test kit includes two control materials of different concentrations that are run automatically with every sample. If the automatic check shows that the control results fall within specified limits, the instrument reports the MMX result for the specimen being tested. If the control results exceed the limits, the MMX result is not reported and the instrument displays an error message. The samples are reanalyzed with another test kit. According to the manufacturer, the CVs of the MMX value and each individual analyte are ≤6%. The MMX result has a reportable range of 0-10. The manufacturer's recommended cutoff values are 1.3 and 5.9; thus, ≤1.3 represents a low probability of stroke, >1.3 is considered abnormal and suggests further evaluation, and >5.9 represents a high probability of stroke.

Statistical analysis. Data were expressed as mean ± SD. The Student t-test was used to compare test results between groups and p values <0.05 were deemed significant. Fisher's exact test was used for comparison of noncontinuous variables. To measure and compare the predictive accuracy and performance of the Triage stroke panel, receiver-operator-characteristic (ROC) curves, in which sensitivity is plotted as a function of (1-specificity), were generated. Calculations were performed with the SPSS statistical program (v 15.0, SPSS, Inc., Chicago, IL).

Results

The 196 subjects in this study comprised the following groups: healthy controls, 57 (group I); ischemic stroke, 89 (group II); cerebral hemorrhage, 11 (group III); other brain disorders, 39 (group IV; Table 1). In patients with acute ischemic stroke, the median time from symptom onset to blood collection was 6 hr (range, 0.2-120 hr); 43.8% of the patients arrived at the hospital within 3 hr.

The distribution of values of the Triage stroke panel is shown in Table 2. The MMX value was significantly higher in patients with acute ischemic stroke in comparison to healthy controls (p <0.001), but there was no significant difference between the MMX value in patients with acute ischemic stroke vs the patients with acute cerebral hemorrhage (p = 0.884). Of the test results for the individual markers, BNP, D-dimer, and S100β values were significantly higher in group II than in group I (p <0.001, p <0.001, and p <0.001, respectively). Only the BNP value was significantly higher in group II vs group III (p = 0.008). However, the MMX and the 4 individual biomarkers were significantly
different in the patients with acute stroke (groups II and III) compared with the non-stroke subjects (groups I and IV; Fig. 1).

The MMX result gave an area under the ROC curve of 0.714 for the diagnosis of acute stroke (Fig. 2A). Although the MMX result could discriminate patients with acute stroke from the healthy control group, the MMX result did not discriminate patients with ischemic stroke from patients with cerebral hemorrhage (Fig. 2B). BNP gave an area under the ROC curve of 0.661 for discriminating acute ischemic stroke from acute cerebral hemorrhage (Fig. 2B).

The diagnostic sensitivities and specificities of the MMX result for ischemic stroke were 91.0% and 21.5% at the lower cut-off value of 1.3 and 20.2% and 93.5% at the upper cut-off value of 5.9, respectively. The individual biomarker and MMX values showed no significant differences, based on the duration after symptom onset (p = 0.56).
Table 1. Characteristics of control subjects and patients.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=57)</th>
<th>Group II (n=89)</th>
<th>Group III (n=11)</th>
<th>Group IV (n=39)</th>
<th>p valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control subjects</td>
<td>Ischemic stroke</td>
<td>Cerebral hemorrhage</td>
<td>Other brain disorders</td>
<td></td>
</tr>
<tr>
<td>Age (yr)b</td>
<td>43.8±12.0</td>
<td>66.6±11.8</td>
<td>56.4±14.5</td>
<td>65.2±15.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male:female</td>
<td>25:32</td>
<td>50:39</td>
<td>5:6</td>
<td>14:25</td>
<td>0.566</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6 (10.5%)</td>
<td>50 (56.2%)</td>
<td>8 (72.7%)</td>
<td>15 (38.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (7.0%)</td>
<td>21 (23.6%)</td>
<td>1 (9.1%)</td>
<td>4 (10.3%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>0 (0.0%)</td>
<td>10 (11.2%)</td>
<td>2 (18.2%)</td>
<td>3 (7.7%)</td>
<td>0.067</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>0 (0.0%)</td>
<td>2 (2.2%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0.092</td>
</tr>
<tr>
<td>Durationc (hr)</td>
<td>-</td>
<td>0.2~120 (6.0)</td>
<td>0.5~72 (3.0)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

a Group II vs Group I, III, and IV.

b Values are reported as mean ± SD.

c Time between onset of stroke symptoms and blood collection (median time)

Table 2. Results of the Multimarker Index (MMX) and single plasma biomarkers included in the Triage stroke panel.a

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=57)</th>
<th>Group II (n=89)</th>
<th>Group III (n=11)</th>
<th>Group IV (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMX</td>
<td>2.1±1.1</td>
<td>4.0±1.9</td>
<td>4.3±1.9</td>
<td>3.1±2.1</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>11.3±6.1</td>
<td>90.8±156.4</td>
<td>16.3±10.8</td>
<td>34.2±39.0</td>
</tr>
<tr>
<td>D-dimer (ng/ml)</td>
<td>188.6±113.8</td>
<td>888.1±1289.0</td>
<td>3312.7±2104.8</td>
<td>788.8±1055.6</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>211.2±184.8</td>
<td>242.1±242.6</td>
<td>713.6±562.6</td>
<td>175.1±262.2</td>
</tr>
<tr>
<td>S100β (pg/ml)</td>
<td>188.6±147.1</td>
<td>103.1±13.6</td>
<td>&lt;100 b</td>
<td>109.5±33.6</td>
</tr>
</tbody>
</table>

a Values are reported as mean ± SD. b All patients in group III had S100β concentrations <100 pg/ml.

MMX = Multimarker Index; BNP = B-type natriuretic peptide; MMP-9 = matrix metalloproteinase-9.

Fig. 2. Panel A: Receiver-operator-characteristic (ROC) curves demonstrating sensitivity as a function of 1-specificity for discriminating patients with acute infarction from the other groups, based on the MMX incorporating all 4 biomarkers and each biomarker alone. MMX had an area under the ROC curve of 0.714. BNP, D-dimer, MMP-9, and S100β had areas under the ROC curve of 0.686, 0.613, 0.547, and 0.441, respectively. Panel B: ROC curves demonstrating sensitivity as a function of 1-specificity for discriminating patients with ischemic stroke from patients with cerebral hemorrhage based on the MMX incorporating all 4 biomarkers and each biomarker alone. MMX had an area under the ROC curve of 0.479; BNP, D-dimer, MMP-9, and S100β had areas under the ROC curve of 0.661, 0.168, 0.293, and 0.534, respectively.
Discussion

The t-PA therapy for patients with ischemic stroke is most effective and must be administrated within 5 to 6 hr after symptom onset [3]. Regretably, rapid and accurate diagnostic tests for ischemic stroke are not currently available [3]. Radiographic studies (CT or MRI) to confirm the ischemic stroke are less sensitive in patients with mild ischemic stroke and may not be immediately available [7]. There are reports that a panel of blood biochemical markers may be helpful in identifying patients with acute cerebral ischemia who would benefit in the emergency setting [14,15].

In the current study, we evaluated the potential diagnostic usefulness of a panel consisting of 4 plasma biochemical markers (BNP, D-dimer, MMP-9, and S100β) in predicting acute stroke. BNP supports the growth and differentiation of central and peripheral neurons [7]. Elevation of BNP has been reported to occur within the first 24 hr after ischemic stroke, although the mechanisms by which this occurs remain unknown [14,16,17]. D-dimer is a breakdown product of fibrin mesh after factor XIII stabilization, and indicates thrombus formation [7]. D-dimer is elevated in any clinical circumstance in which both clot formation and fibrinolysis are increased, such as venous thrombosis, pulmonary embolism, and disseminated intravascular coagulation. Therefore, it has been demonstrated that D-dimer is elevated after cerebral ischemia or subarachnoidal hemorrhage [10,11,18,19]. MMP-9 is a collagenase that is associated with destruction of plaque matrix and endothelial damage. MMP-9 is elevated in various pathologic conditions, including inflammation, atherosclerosis, tumor, metastasis, and ischemic stroke [20-22]. Although these biomarkers are not specific to central nervous system tissues, they were shown to be significantly elevated in patients with acute ischemic stroke and acute cerebral hemorrhage patients in the current study. However, only BNP was significantly higher in patients with acute ischemic stroke than in patients with acute cerebral hemorrhage. Furthermore, the MMX result did not show significant difference between patients with acute ischemic stroke and acute cerebral hemorrhage. So, only BNP was capable of distinguishing between these two disorders in this study. S100β is an acidic calcium binding protein found in glia and Schwann cells [7] and reflects the role of astrocyte activation. S100β is elevated after stroke and brain injury [14], but this marker did not contribute to the diagnosis of ischemic stroke in the current study.

The predictive accuracy of the MMX result for the diagnosis of acute stroke was demonstrated by the ROC curve. Our data support the notion that the MMX value may help to diagnose acute stroke because MMX was significantly elevated in the acute stroke group (group II and III) compared to the other brain disease and healthy control groups. However, the value of MMX was not significantly different between the patients with acute ischemic stroke and acute cerebral hemorrhage. This is a major problem since t-PA is contraindicated in acute cerebral hemorrhage [2,3]. It is important to distinguish patients with acute ischemic stroke from acute cerebral hemorrhage as early as possible. However, the MMX was not useful for this purpose in our study.

The limitations of this study include the following: the mean age was different in the patients with acute ischemic stroke compared to the other patient groups and the number of patients with acute cerebral hemorrhage was smaller than the other groups. In conclusion, our data suggest that MMX, which incorporates four biochemical markers, may be helpful to diagnose acute stroke, but it does not discriminate acute ischemic stroke from acute hemorrhagic stroke. Additional studies are needed to refine the cut-off values of MMX in control populations and patients.

Acknowledgement

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References