Diagnostic Significance of Ischemia-Modified Albumin, S100b, and Neuron-Specific Enolase in Acute Ischemic Stroke

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Abstract

Objective: Use of biochemical markers of cerebral ischemia is a newly favored approach for early diagnosis of ischemic stroke. Our aim was to establish the sensitivity and specificity of ischemia-modified albumin (IMA) in acute ischemic stroke and to compare it with S100b and neuron-specific enolase (NSE). We also intended to investigate the usefulness of a panel composed of these three biomarkers in acute ischemic stroke.

Material and Methods: Consecutive adult patients who were admitted to the emergency department with focal neurological deficits were enrolled. Serum samples were obtained at the initial examination, and IMA, S100b, and NSE were measured. Receiver operating characteristics (ROC) curve analysis was used to determine the cut-off values of the biomarker and biomarker groups.

Results: Serum IMA, NSE, and S100b levels were significantly higher in ischemic stroke patients ($p<0.001$, $p=0.005$, $p=0.001$, respectively). The optimum diagnostic cutoff point for IMA was 0.31 ABSU with 90% sensitivity and 57% specificity; 18 µg/L for NSE with 61% sensitivity and 53% specificity; and 65 pcg/l for S100b with 87% sensitivity and 72% specificity. With a combination of IMA with either S100b or NSE, instead of IMA alone, the best results were obtained with IMA and S100b, at 97% sensitivity and 37% specificity.

Conclusion: This study indicates that IMA was a sensitive diagnostic biomarker in the acute phase of ischemic stroke, although its specificity is low. The combination of IMA with S100b and NSE did not add any beneficial effect to the specificity of IMA alone. *(JAEM 2014; 13: 112-7)*

Key words: Ischemic stroke, ischemic modified albumin, S100 calcium-binding protein beta subunit, neuron-specific enolase, biomarker

Introduction

Ischemic cerebrovascular disease is frequently encountered in emergency departments. Emergency physicians and general practitioners are usually on the front line in evaluating and performing the differential diagnosis and even in managing patients before referral to the neurologist. Diagnosis of ischemic cerebrovascular disease is not always straightforward and is particularly challenging with diseases mimicking stroke, such as migraine, postictal paresis, brain mass lesions, hypoglycemia, and conversion disorders. Radiological investigations are usually required for both diagnosis and exclusion of stroke mimics. Computed tomography (CT) remains the most accessible imaging modality, though this is insufficiently sensitive to detect ischemic stroke, especially in the early phase and in cases of lacunar or posterior fossa infarction (1, 2). Magnetic resonance imaging (MRI) has greater sensitivity for detecting ischemic stroke but can still appear normal in the first hours (3). It is also not available in every emergency department. Today, the presence of neurological deficit and absence of hemorrhage on CT images is the accepted way of diagnosing ischemic stroke, and confirmatory CT scanning is usually required for defining ischemia and lobar involvement.

Accurate and prompt evaluation of cerebrovascular disease requires new diagnostic approaches that can represent an alternative to neuroimaging techniques. Use of biochemical markers of cerebral ischemia is a newly favored approach for early diagnosis of ischemic stroke. Neuron-specific enolase (NSE), S100b, matrix metalloproteinase-9 (MMP-9), and myelin basic protein are some of these biomarkers that have been investigated. Blood levels of these have been shown to increase in ischemic stroke (4-6). However,

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none of these alone has been proven to be useful as a standard, sensitive, and specific diagnostic test for ischemic stroke. Biomarker panels composed of complementary multiple molecules have been reported to be more sensitive and specific for the diagnosis of acute ischemic stroke (7-9). Another biochemical marker of tissue ischemia, ischemia-modified albumin (IMA), has recently been added to the literature as a sensitive marker for acute stroke, both hemorrhagic and ischemic (10, 11). The purpose of this study was to determine the sensitivity and specificity of IMA in acute ischemic stroke and to compare these with S100b and NSE. We also intended to investigate the usefulness of a panel composed of these three biomarkers in acute ischemic stroke.

Materials and Methods

Patient Selection and Study Design

This prospective, case-control study was performed in our emergency department. The study protocol was approved by Karadeniz Teknik Üniversitesi Medical Faculty Ethics Committee, 2008/4/142. Consecutive adult patients who were admitted with focal neurological deficits developing within 24 h were enrolled, and blood samples were taken once written informed consent was obtained. Initial evaluation on admission was performed simultaneously by the emergency physician and a neurologist by means of a detailed physical and neurological examination. Demographic characteristics and vascular risk factors (hypertension, diabetes mellitus, hyperlipidemia, peripheral ischemic disease) were recorded in detail. Routine blood tests, including full blood count, coagulation tests, glucose level, renal and hepatic function tests, total protein, and albumin levels; chest radiography; electrocardiography; carotid ultrasonography; and echocardiography of all patients were performed in order to identify the potential mechanisms of cerebral infarct. The National Institutes of Health Stroke Scale (NIHSS) was used to determine stroke severity. All patients underwent brain computed tomography (CT). Magnetic resonance image (MRI), including diffusion-weighted images, was performed in negative CT cases. Ischemic stroke was defined as focal neurological deficit lasting longer than 24 h with ischemia in the appropriate brain area by CT or MRI. Cases with focal neurological deficit recovering within 24 h and with no ischemia in the appropriate region by MRI were regarded as transient ischemic attack (TIA). All study patients were hospitalized at least until the final diagnosis was established. Etiological subgroups were determined according to Trial of Org 10172 in acute stroke treatment (TOAST) criteria (12).

After a detailed investigation, patients diagnosed with intracranial hemorrhage, tumors, and other cerebrovascular diseases (sinus thrombosis, etc.); encephalitis; and recent and acute ischemic disease of other organs (such as acute myocardial infarction, peripheral artery disease, or pulmonary embolism) were excluded. Other exclusion criteria were recent trauma, chronic renal or hepatic failure, and presence of low serum albumin levels (less than 3.5 mg/dl). Emergency department patients without acute focal neurological deficit and normal MRI served as a control group and reference for biochemical parameters. Cases that described transient focal neurological deficit that recovered before admission to the hospital and with normal a neurological and radiological examination were not diagnosed as acute ischemic stroke but were suspected as having TIA and included in the control group. Inclusion of patients describing TIA symptoms into the control group is an accepTable methodology in the literature, because the evidence of brain ischemia is clinically and neuroradiologically absent (7, 13, 14). Other diagnoses in the control group patients were hypertensive attack, migraine, benign positional vertigo, and conversion disorder. The exclusion criteria for the control group were the same as those for the patient group. Informed consent was taken from all study participants or their legally authorized representatives.

Biochemical Assessments

Blood samples were placed in plain tubes containing separation gels and allowed to clot for 30 min. They were centrifuged before separating the serum. Samples were immediately frozen and stored at –80°C for the IMA, S-100b, and NSE assays.

2.2.1. Measurement of IMA levels: Reduced cobalt-to-albumin binding capacity (IMA level) was analyzed using the rapid and colorimetric method described by Bar-Or et al. (15). Two hundred microliters of patient serum was placed into glass tubes, and 50 mL of 0.1% cobalt chloride (Sigma, CoCl2.6H2O) in H2O was added. After gentle shaking, the solution was left for 10 min in order to ensure sufficient cobalt-albumin binding. Fifty microliters of dithiothreitol (DTT) (Sigma, 1.5 mg/ml H2O) was added as a colorizing agent, and the reaction was quenched after 2 min by adding 1.0 mL of 0.9% NaCl. A colorimetric control was prepared for preoperative and postoperative serum samples. For the colorimetric control samples, 50 mL of distilled water was substituted for 50 mL of 1.5 mg/mL DTT. Specimen absorbencies were analyzed at 470 nm using a spectrophotometer (Shimadzu UV1601, Australia). The color of the DTT-containing specimens was compared with that of the colorimetric control tubes. The results were reported as absorbance units (ABSUs).

2.2.2. Measurement of S100B protein levels: Levels of S100B were determined by enzyme-linked immunosorbent assay kit (BioVendor-Laboratorni Medicina, Czech Republic), according to the manufacturer's instructions. The absorbance of samples was measured at 450 nm using a VERSA max tunable microplate reader (Molecular Devices, California, USA). The results were expressed as picograms per milliliter.

Measurement of NSE levels: Levels of NSE were determined using an enzyme-linked immunosorbent assay kit (DRG International Inc., USA), according to the manufacturer's recommendations. The absorbance of samples was measured at 450 nm using a VERSA max tunable microplate reader (Molecular Devices, California, USA). The results were expressed as micrograms per liter.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences 13.0 (SPSS Inc., Chicago, IL, USA). Receiver operating characteristics (ROC) analyses were performed using the Analyse It v2.12 (Analyse-it Software, Ltd. Leeds, LS3 1HS, United Kingdom) program. Descriptive statistics (mean and standard deviation) were determined for each biomarker. Student's t-test was used to test the differences in biomarkers. Statistical significance was set at a p value of 0.05. Demographic variables of patients and controls were compared by using student's t-test for continuous variables and the chi-square test (or Fisher's exact when required) for categorical variables. ROC curve analysis was used to determine the cut-off values of biomarkers and biomarker groups. The sensitivities and specificities

of the tests were used to compare the diagnostic accuracy of each biomarker and biomarker group.

Results

Forty-five patients with suspected stroke were evaluated. Four patients who described questionable focal neurological deficits (tingling in one hand and loss of fluency in speaking) lasting less than an hour and with normal neurological examination at admission and normal investigation were not diagnosed as acute ischemic stroke but were assumed to have TIA and included in the control group. There was no TIA patient that had neurological deficits at the time of admission and during hospitalization. Of the remaining 41 patients, three were excluded due to low albumin levels (less than 3.5 mg/dl). Demographic characteristics of the patient and control groups are presented in Table 1.

Mean serum IMA level was 0.539±0.187 ABSU for patients and 0.305±0.082 ABSU for the control group. Mean serum NSE level was 32.671±30.424 µg/l for patients and 17.417±7.085 µg/l for the control group. Mean serum S100b level was 197.50±242.112 pg/ml for patients and 62.27±11.876 pg/ml for the control group. Serum IMA, NSE, and S100b levels were significantly higher in ischemic stroke patients (for IMA p<0.001, for NSE p=0.005, for S100b p=0.001) (Table 2).

ROC curve analysis revealed an area under the curve value of 0.87 (bootstrap 95%, confidence interval [CI] 0.78-0.96) for IMA, 0.89 (bootstrap 95%, CI 0.81-0.96) for S100b, and 0.67 (bootstrap 95%, CI 0.55-0.80) for NSE (Figure 1). The optimum diagnostic cutoff point maximizing sensitivity and specificity for IMA was 0.31 ABSU, with 90% sensitivity, 57% specificity, 81% negative predictive value (NPV), and 72% positive predictive value (PPV). The optimum diagnostic cutoff point for NSE was 18 µg/l, with 61% sensitivity, 53% specificity, 52% NPV, and 62% PPV. The optimum diagnostic cutoff point for S100b was 65 pg/l, with 87% sensitivity, 72% specificity, and 81% NPV and PPV. With a three-variable panel composed of IMA, NSE, and S100b, the sensitivity rose to 97%, though the specificity declined to 23%, with 88% NPV and 62% PPV. In the case of two-variable biomarker panels, a combination of IMA with either S100b or NSE increased the sensitivity of NSE and S100b alone but reduced the specificity of both. With a combination of IMA with either S100b or NSE, instead of IMA alone, the best results were obtained with IMA

Table 1. Demographic features of patients and controls

	Patients (n=38)	Controls (n=30)	p
Age (y)	66.1±12.8	64.6±12.4	0.622
Sex			
Female	18	10	0.358
Male	20	20	
Hypertension	33	12	<0.001
Diabetes mellitus	6	2	0.288
Hyperlipidemia	9	9	0.757
Previous stroke	2	-	0.500
Coronary artery disease	8	5 (%17)	0.884
Serum albumin g/dL),mean	3.7±0.6	3.9±0.6	0.134
NIHSS, mean	13±8.2	-	
Time for blood draw (hours), mean	9.2±3.9		
Type of stroke			
Large-artery atherosclerosis	21		
Cardioembolism	12		
Undetermined etiology	5		

Table 2. Mean serum levels of the biomarkers

	Patients (n=38)	Controls (n=30)	p
IMA (ABSUs)	0.539±0.18	0.305±0.08	<0.001
S100b (pg/mL)	197.50±242.11	62.27±11.87	0.001
NSE (mg/L)	32.671±30.42	17.417±7.08	0.005
p<0.05			

Table 3. Sensitivity and specificity of biomarker panels

	Sensitivity	Specificity	PPV	NPV
IMA, NSE	0.95	0.30	0.63	0.82
IMA, S100b	0.97	0.37	0.66	0.92
NSE, S100b	0.92	0.47	0.69	0.82
IMA, NSE, S100	0.97	0.23	0.62	0.88
PPV: Positive predictive value, NPV: Negative predictive value				

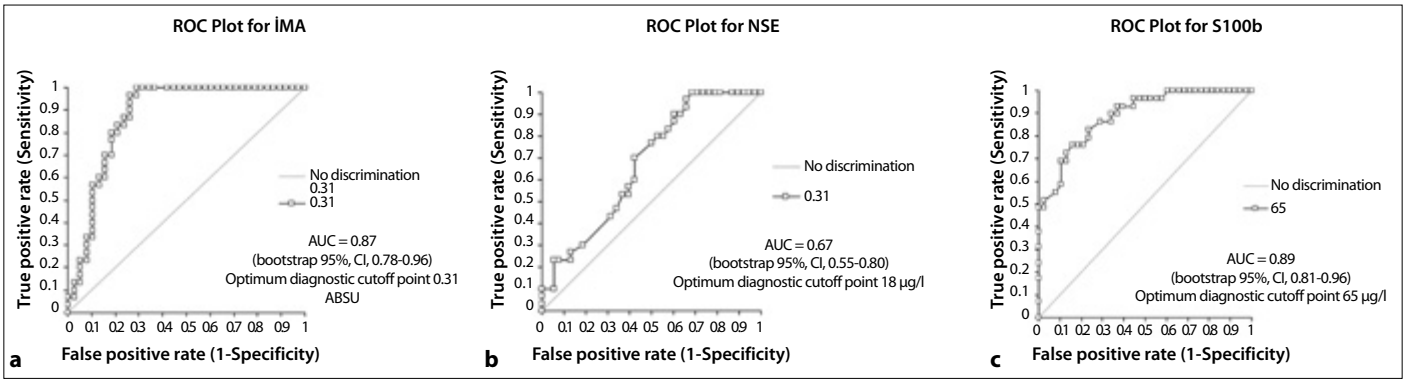

Figure 1 a-c. ROC of IMA (a), NSE (b), and S100b (c)

and S100b, at 97% sensitivity, 37% specificity, 92% NPV, and 66% PPV (Table 3).

Discussion

Acute ischemic stroke is an emergency situation seen in many emergency departments. The early diagnosis and management of acute ischemic stroke require a specialist approach and advanced neuroradiological investigations. The very narrow time interval for thrombolytic therapy in the treatment of ischemic stroke increases the importance of early diagnosis (16). The importance of early diagnosis persists in cases in which thrombolytic treatment cannot be performed. Blood pressure, blood sugar, and the establishment of body temperature regulation in the early period are known to influence the prognosis in ischemic stroke patients (17-21). In addition, treatments aimed at preventing early neurological complications that may develop in ischemic stroke patients, such as cerebral edema and hemorrhagic transformation, and deep vein thrombosis and pulmonary embolism in immobile patients are recommended (21). The diagnosis and management of ischemic stroke are difficult in hospitals in rural areas. Patients undergoing a stroke who are first seen by a general practitioner or family doctor and failure to perform transfer procedures to the relevant health institution in the event of a high likelihood of ischemic stroke once the brain CT has been performed for excluding hemorrhage are the main obstacles to these patients receiving the requisite acute treatments (22). This has led to the investigation of diagnostic techniques other than radiological ones, assisting the diagnosis of acute ischemic stroke in pre-hospital situations.

Biochemical diagnostic techniques are easy and rapid and can be performed in many emergency departments. They are frequently and widely used by cardiologists and non-cardiologist physicians in identifying diseases requiring urgent intervention, such as acute coronary artery syndrome and pulmonary embolism. In this way, these patient groups can be distinguished easily from patients with other diseases that may present a similar picture, and the appropriate procedures and treatment can be administered very rapidly. Similarly, the validity of biochemical diagnostic techniques in ischemic cerebrovascular events has recently been widely investigated. Research has particularly concentrated on glial activation (S100b, myelin basic protein), neuronal injury (NSE, NMDA antibodies), and biomarkers showing inflammation and tissue damage (CRP, cytokines, MMP-9) (23, 24). Many studies have determined a powerful correlation between stroke diagnosis and increased serum levels of these markers by examining their serum levels in cerebral ischemia, but no biomarker capable of being used in a sensitive and reliable manner in diagnosing acute ischemic stroke has been identified. This may be attributed to cell groups in the brain tissue having different distributions and ischemia tolerances, the rather complex nature of the ischemic process, and the presence of the blood-brain barrier (25).

In order to resolve this dilemma, the combined use of multiple biomarkers capable of being correlated with different aspects of cerebral ischemia has been proposed, and in recent years, the validity in ischemic stroke diagnosis of panels including multiple biomarkers has been investigated. Reynould et al. examined more than 50 biomarker plasma levels in 223 stroke patients. S110b, B-type neurotrophic growth factor, von Willebrand factor, MMP-9, and monocyte chemotactic protein-1 were found to be correlated with stroke (9). The specificity and sensitivity of these four markers in ischemic stroke diagnosis at 12 h were 98% and 89%, respectively. Lynch et al. reported that a panel of MMP-9, vascular cell adhesion molecule (VCAM), and vWF and a panel containing S100b, VCAM, and vWF were 90% sensitive and specific in acute ischemic stroke patients in the first 6 hours (7). Laskowitz et al. determined that a panel of MMP-9, brain natriuretic factor, D-dimer, and S100b was 85% sensitive and 34% specific in the first 24 h in ischemic stroke patients (25).

We selected IMA, S100b, and NSE as biomarkers for our study. The S100b isoform is present in high concentrations in glial and Schwann cells. NSE is an isoenzyme of the glycolytic enzyme enolase and is mainly found in neurons and neuroendocrine cells. Increased levels of serum S100b and NSE in cerebrovascular events have been reported in several studies, and serum levels have been shown to be correlated with cerebral infarct volume and clinical outcome (4, 26). However, the fact that significant blood levels of these biomarkers emerge more in the second and third days reduces their value in early diagnosis and restricts their clinical use. IMA is a metabolic variant of albumin that is produced when the N-terminus of the albumin undergoes modification during ischemia and IMA is therefore unable to bind to cobalt and copper ions. This modified albumin can be identified using the cobalt binding test (15). IMA levels rise in the early stage of ischemia, and serum levels can easily be measured. However, IMA is not specific to brain ischemia and also increases in other ischemic diseases, such as myocardial infarct, pulmonary embolism, and lower limb ischemia (27-30). The combined use of IMA with cerebral tissue biomarkers, such as s100b and NSE, might be suggested to contribute to clinical applications, since IMA rises during the early period of ischemia and is sensitive for ischemia.

In our study, IMA was found to be more sensitive in diagnosing ischemic stroke compared to NSE and S100b. Its NPV was similar to that of S100b but higher than that of NSE. The specificity of each biomarker was considerably low. Although IMA is non-specific to brain ischemia, the fact that it is more sensitive than S100b and NSE in ischemic stroke may be attributed to ischemia-associated processes at the beginning of a cerebral infarct being more prominent compared to neuronal damage. In addition, the difference between the kinetic properties of these markers may affect the sensitivity, since detection of S100b and NSE in the systemic circulation occurs relatively later than IMA. IMA has a small molecular structure and is found in high levels in the circulation, also making it an advantageous biomarker. A combination of IMA with S100b, which has the highest specificity, exhibited a test sensitivity of 97%, although the test specificity decreased. Adding NSE to this combination had no additional effect. With high sensitivity and NPV, a combination of IMA and S100b may be a useful diagnostic panel in the diagnosis of ischemic stroke. However, the most significant problem with these three markers and combinations thereof is that their specificities are lower than would be expected of a good marker or panel, although the optimum diagnostic cutoff point was chosen to maximize sensitivity while not compromising specificity.

Study Limitations

The most significant limitation in this study is the low number of patients. Another limitation of the study is the control group reflecting stroke mimics that constitute a problem in stroke diagnosis. A study examining sensitivity and specificity in which conditions that can imitate an ischemic stroke picture with focal neurological deficit (such as hemorrhage, tumor, encephalitis, etc) are adopted as the control group might be more appropriate.

Conclusion

In conclusion, this study showed that IMA, a non-specific biomarker for cerebral ischemia, was more sensitive in diagnosing acute ischemic stroke compared to NSE and S100b. Each of the biomarkers investigated had low specificity. The combined use of IMA, NSE, and S100b did not increase the specificity of the diagnostic panels. With its high sensitivity, IMA is an excellent biomarker and can be employed in a sensitive manner in another diagnostic panel in the diagnosis of acute ischemic stroke.

Ethics Committee Approval: The study protocol was approved by Karadeniz Teknik Üniversitesi Medical Faculty Ethics Committee, 2008/4/142.

Informed Consent: Informed consent was obtained from the patient.

Peer-review: Externally peer-reviewed.

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