Plasma Immunoproteasome Predicts Early Hemorrhagic Transformation in Acute Ischemic Stroke Patients

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Background and Purpose: Currently, blood biomarkers associated with an increased hemorrhagic transformation (HT) risk remain uncertain. We aimed to determine the significance of immunoproteasome as predictors of early HT in acute ischemic stroke patients. *Methods:* This study enrolled 316 patients with ischemic stroke. HT was assessed by computed tomography examination performed on day 5 ± 2 after stroke onset or immediately in case of clinical deterioration (CD). Plasma immunoproteasome subunits low molecular mass peptide 2 (LMP2), multicatalytic endopeptidase complex-like 1 (MECL-1), LMP7, interleukin-1β (IL1β), and high-sensitivity C-reactive protein (Hs-CRP) were measured with quantitative sandwich enzyme-linked immunosorbent assay kits. Factors associated with HT were analyzed using a multivariate logistic regression analysis. *Results:* There were 42 (13.3%, 42 of 316) patients who experienced HT. Compared with those patients without HT, plasma LMP2, MECL-1, LMP7, IL1β, and Hs-CRP concentrations on admission were significantly increased in patients with subsequent HT ($P < .001$). These protein concentrations increased with hemorrhage severity. Patients with CD caused by HT had the highest levels of LMP2 (1679.5 [1394.6-136.6] pg/mL), MECL-1 (992.5 [849.7-1075.8] pg/mL), LMP7 (822.6 [748.6-1009.5] pg/mL), IL1β (113.2 [90.6-194.5] pg/mL), and Hs-CRP (30.0 [12.8-75.6] mg/L) ($P < .05$). Logistic regression analysis showed cardioembolism, LMP2, MECL-1, and LMP7 as independent predictors of HT ($P < .05$). Receiver operating characteristic curve analysis demonstrated LMP2 ≥ 988.3 pg/mL, MECL-1 ≥ 584.7 pg/mL, and LMP7 ≥ 509.0 pg/mL as independent factors associated with HT ($P < .001$). *Conclusion:* Evaluation of plasma levels of immunoproteasome could be helpful in the early prediction of HT in acute ischemic stroke patients. **Key Words:** Immunoproteasome—hemorrhagic transformation—cerebral infarction—risk factors.

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Background

Hemorrhagic transformation (HT) is bleeding into an area of ischemic brain after stroke. It occurs in as many as 10%-40% of patients with ischemic stroke.[1,2] HT increases morbidity and mortality of acute ischemic stroke. It is difficult to predict a given patient's probability of experiencing HT. Currently, several clinical parameters and blood biomarkers are known to be associated with an increased HT risk. Factors such as age, hypertension, atrial fibrillation (AF), baseline National Institutes of Health Stroke Scale (NIHSS) score, treatment with antiplatelet or thrombolytic agents, the presence of early ischemic changes on cranial computed tomography (CT) on admission, high levels of plasma matrix metalloproteinase (MMPs), activated protein C, estimated glomerular filtration rate, tight-junction proteins, and S100B have been related to HT after ischemic event.[1-12] However, all studies in this field to date did not definitively elucidate the clinical relevance of any single marker or a panel of different markers.

Immunoproteasome is a subtype of proteasome that contains three major catalytic subunits: β1i (also known as low molecular mass peptide 2 [LMP2]; proteasome subunit beta 9 [PSMB9]), β2i (also known as multicatalytic endopeptidase complex-like 1 [MECL-1]; PSMB10), and β5i (also known as LMP7; PSMB8).[13] Immunoproteasome is present in several kinds of cells, including neurons, astrocytes, microglia, and endothelial cells in the brain areas.[14,15] Our previous study had confirmed that augmentation of both immunoproteasome LMP2 and LMP7 is potentially involved in the inflammatory pathophysiological mechanism of ischemia stroke, and selective inhibition of the immunoproteasome subunit LMP7 offers a strong neuroprotection in middle cerebral artery occlusion (MCAO) rats.[14] However, detailed knowledge on the relationship of immunoproteasome with human ischemia stroke is still not available. The aim of this prospective study in consecutive patients was therefore to assess (1) the rate of early HT in patients admitted for ischemic stroke, (2) the correlation between plasma immunoproteasome with early HT, and (3) the risk factors for early HT.

Subjects and Methods

Patients

We prospectively enrolled 316 patients with a first episode of ischemic stroke admitted within the first 72 hours of symptom onset to the neurology department of our hospital between March 2014 and September 2014. The study was approved by the local ethics committee and informed consent was obtained from each patient or a family member or relative. A diagnosis of acute ischemic stroke was made by stroke neurologists and was confirmed with CT or magnetic resonance imaging. A control group of 10 healthy subjects (men, 60%; mean age, 62.5 ± 11.9 years) was also studied. The exclusion criteria were patients who received oral anticoagulants or heparin, with previous stroke, transient ischemic attack, or intracerebral or subarachnoid hemorrhage, inflammatory or infectious diseases, epilepsies, hematologic diseases, cancer, and severe liver and renal failure. Those who were previously dependent were excluded from the present study. In each case, past and present clinical histories, including medication use, were obtained by interviewing the patient or a family member in cases where the patient was aphasic or unconscious. Each patient's hospital records were also reviewed for medical history. All patients underwent comprehensive clinical evaluation, blood pressure measurement, standard electrocardiogram (ECG), chest X-ray, blood routine, and fibrinogen and biochemical analyses. Stroke etiology was classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria[16]: large artery atherosclerosis, cardioembolic, small vessel disease, and other causes and uncertain causes. Stroke subtypes were also divided into groups according to the size of infarction observed on CT (the formula $\Pi/6 \times a \times b \times c$, where a and b are the largest perpendicular diameters measured on CT and c is the slice thickness): large infarction group (>10 cm^3), medium infarction group (4.1-10 cm^3), and small infarction group (<4 cm^3).[17]

Clinical evaluations were performed on admission and every day during patients' hospital stay. Stroke severity was assessed with the NIHSS. Symptomatic HT or clinical deterioration (CD) caused by HT was defined as an increase of ≥4 points in the NIHSS in combination with a visible HT on the head CT.[5,18] The infarct volume and the occurrence of early HT were investigated on repeated CT examination performed after 5 ± 2 days from stroke onset or immediately in case of clinical neurologic deterioration. The type of HT was classified according to the European Cooperative Acute Stroke Study-II criteria.[19] This classifies hemorrhagic infarction 1 (HI1) as small petechiae along the periphery of the infarct region; hemorrhagic infarction 2 (HI2) as more confluent petechiae within the infarct, without space-occupying effect; parenchymal hemorrhage 1 (PH1) as bleeding ≤30% of the infarcted area but with mild space-occupying effect; and parenchymal hemorrhage 2 (PH2) as bleeding >30% of the infarcted area with substantial space-occupying effect or as any hemorrhagic lesion outside the infarcted area. In cases of more than one hemorrhagic lesion on CT examination, the worst possible HT category was assumed. All CT evaluations were made by the same neuroradiologist who was blinded to the clinical and analytical results.

Laboratory Tests

Venous blood samples were collected on admission within 3 days of onset of acute ischemic stroke (blood samples from 292 patients were collected less than 24 hours after stroke onset, 17 less than 48 hours, and 7 less than 3 days after stroke onset). Ethylene Diamine Tetraacetic