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Relationship between plasma high-mobility group box-1 levels and clinical outcomes of ischemic stroke☆,☆☆

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Abstract

Purpose: High-mobility group box-1 (HMGB1) is regarded as a central mediator of inflammation and involved in many inflammatory diseases. This study aimed to investigate impact of plasma HMGB1 level on 1-year clinical outcomes of ischemic stroke.

Methods: Plasma HMGB1 levels of 338 patients were quantified by enzyme-linked immunosorbent assay. The end points were mortality and unfavorable outcome (modified Rankin Scale score>2) after 1 year.

Results: Plasma HMGB1 level emerged as an independent predictor of 1-year clinical outcomes. Its prognostic value was similar to National Institutes of Health Stroke Scale score's. It improved prognostic value of National Institutes of Health Stroke Scale score.

Conclusion: Plasma HMGB1 level represents a novel biomarker for predicting 1-year clinical outcomes of ischemic stroke.

1. Introduction

High mobility group box 1 (HMGB1) belongs to a family of molecules called alarmins [1]. Passive release of HMGB1 from dying cells is a sign of necrotic damage [2]. In addition to passive release, actively-regulated secretion of HMGB1 may also occur, but primarily in immune cells [3]. Extracellular HMGB1 binds to its receptors, including receptors for advanced glycation end-products and Toll-like receptors, and augments inflammation [4–6]. Released HMGB1 from damaged neurons can be detected in mouse models as early as 30 minutes after middle cerebral artery occlusion [7]. Inhibition of HMGB1 by either siRNA or neutralizing antibodies is neuroprotective in ischemic stroke [8,9]. Moreover, HMGB1 is increased in the cerebrospinal fluid from meningitis patients [10] and in the serum of cerebral ischemia patients [11,12]. Recent data have identified HMGB1 in the cerebrospinal fluid of subarachnoid hemorrhage [13,14] and in the serum of intracerebral hemorrhage [15] as a potential biomarker of neurological outcome, suggesting HMGB1 may represent a marker of neurological injury. However, to our best knowledge, no published information exists to date about the association of HMGB1 in the peripheral blood with disease outcome of ischemic stroke. The present study aimed to investigate the

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ability of plasma HMGB1 to predict the long-term disease outcome in the patients with ischemic stroke.

2. Methods

2.1. Study population

Between January 2008 and July 2010, all patients with first-ever ischemic stroke confirmed by brain magnetic resonance imaging who were admitted to Department of Neurology, The First Hospital of Jia Xing were initially evaluated in the study. Exclusion criteria from the study were concurrent renal or hepatic insufficiency, malignancy and recent infection, surgery, or major trauma. The study protocol and informed consent approach were approved by the Ethics Committee of The First Hospital of Jia Xing before implementation. The study individuals or their relatives provided written informed consent to participate in this trial.

2.2. Clinical protocol

Initial stroke severity was assessed by the National Institutes of Health Stroke Scale (NIHSS). Participants were followed up until death or completion of one year after stroke. Long-term outcome was assessed by means of the modified Rankin Scale score at 1 year. An unfavorable outcome was defined as a modified Rankin Scale score >2. For follow-up, we used structure telephone interviews performed by 1 doctor, blinded to clinical information and HMGB1 levels.

2.3. Immunoassay methods

The informed consents were obtained from patients or family members in all cases before the blood were collected. Venous blood was drawn on admission. The blood samples were immediately placed into sterile EDTA test tubes and centrifuged at 1500g for 20 minutes at 4°C to collect plasma. Plasma was stored at −70°C until assayed. The concentration of HMGB1 in plasma was analyzed by enzyme-linked immunosorbent assay using commercial kits (R&D Systems Inc, Minneapolis, MN) in accordance with the manufactures' instructions. The detection threshold of this assay is <1 ng/mL. The blood samples were run in duplicate. Researchers running enzyme-linked immunosorbent assays were blinded to all patient details.

2.4. Statistical analysis

Statistical analysis was performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and MedCalc 9.6.4.0 (MedCalc Software, Mariakerke, Belgium). The normality of data distribution was assessed by the Kolmogorov-Smirnov test or Shapiro-Wilk test. The categorical variables are presented as percentages, and the continuous variables are presented as mean ± SD if normally distributed or median (interquartile range) if not normally distributed. Comparisons were made by using (1) χ^2 test or Fisher exact test for categorical data, (2) unpaired Student t test for continuous normally distributed variables, and (3) the Mann–Whitney U test for continuous non-normally distributed variables. The relations of HMGB1 to 1-year mortality and unfavorable outcome were assessed in a logistic-regression model with odds ratio (OR) and 95% confidence interval (CI). Variables showing $P < .1$ in univariate analysis were included in the multivariate model. The receiver operating characteristic (ROC) curves were used to determine the best threshold for on admission values of HMGB1 to predict 1-year mortality and unfavorable outcome. Assessment of the predictive performance of on admission values of HMGB1 was analyzed by calculating the sensitivity and specificity. The area under curve (AUC) was calculated based on the ROC curves. AUC ranges from 0.5 to 1.0. An AUC closer to 1 indicates a higher predictive power. In a combined logistic-regression model, we estimated the additive benefit of HMGB1 to NIHSS score. A P value of less than .05 was considered statistically significant.

3. Results

3.1. Patients characteristics

During the recruitment period, 382 patients were admitted with an initial diagnosis of first-ever ischemic stroke, 351 (91.9%) patients fulfilled the inclusion criteria, and adequate data on admission and follow-up were available for 338 individuals (88.5%) who were finally included in the analysis. Table 1 summarizes the baseline demographic data, cardiovascular risk factors, and stroke characteristics of study participants. A significant correlation emerged between baseline plasma HMGB1 level and NIHSS score ($r = 0.647$, $P < .001$), as well as between baseline plasma HMGB1 and C-reactive protein level ($r = 0.538$, $P < .001$).

3.2. The relationship between plasma HMGB1 concentration and 1-year mortality

The 1-year mortality rate was 20.1% (68/338). Table 2 showed that 1-year mortality was associated with older age, higher NIHSS score, body mass index, HMGB1, glucose and C-reactive protein, diabetes mellitus, coronary heart disease, and atrial fibrillation. Multivariate logistic regression analysis showed that variables independently related to 1-year mortality were age (OR 1.142, 95% CI 1.021-1.403, $P = .012$), plasma HMGB1 concentration (OR 1.418, 95% CI 1.101-3.284, $P = .003$) and baseline NIHSS score (OR 1.224, 95% CI 1.109-2.417, $P < .001$).