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Detection of high serum concentration of CXC chemokine ligand-12 in acute intracerebral hemorrhage

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ABSTRACT

Background: CXC chemokine ligand-12 (CXCL12), a member of the CXC chemokine subfamily, is involved in both focal angiogenesis and inflammatory reactions. We examined serum CXCL12 concentration in intracerebral hemorrhage (ICH) patients and its correlation to stroke severity and outcome.

Methods: The study was carried out on 105 ICH patients on 105 healthy controls. Serum samples were at admission obtained to measure CXCL12 concentrations. The National Institutes of Health Stroke Scale (NIHSS) and hematoma volume were recorded to assess stroke severity.

Results: As compared to the controls, CXCL12 concentrations were significantly increased in the patients. Also, non-survivors within 6 months and patients with an unfavorable outcome (modified Rankin Scale score > 2) at 6 months had higher CXCL12 concentrations than other remaining ones. CXCL12 concentrations had positive correlation with NIHSS scores and hematoma volume. Serum CXCL12 significantly discriminated patients at risk of 6-month mortality and 6-month unfavorable outcome under receiver operating characteristic curve. Moreover, serum CXCL12 was independently associated with the mortality, overall survival and unfavorable outcome.

Conclusions: Serum CXCL12 concentrations are enhanced after ICH and CXCL12 in serum has the potential to reflect severity and prognosis following hemorrhagic stroke.

1. Introduction

Intracerebral hemorrhage (ICH) is a type of common cerebrovascular diseases and is associated with considerable resource consumption, disability, and mortality [1–4]. The National Institutes of Health Stroke Scale (NIHSS) and hematoma volume have been accepted as the important clinical and radiological parameters to assess the severity and outcome of patients with ICH [5,6]. In recent decades, biomarkers have gained importance in the determination of brain damage related to ICH [7–12]. The research of such biomarkers not only provides an opportunity for discovering the pathophysiological mechanisms underlying hemorrhagic brain injury, but also is beneficial for an early risk assessment with estimate of the ICH severity and prognosis.

Chemokines are a family of small cytokines secreted by a great variety of cells in several different conditions and are implicated in cell maturation, differentiation, infection, autoimmunity, inflammation and cancer [13–15]. After ICH, hematoma components initiate inflammatory signaling via activation of microglia, subsequently releasing chemokines to attract peripheral inflammatory infiltration, which is involved in secondary brain injury [16–18]. Thus, chemokines contribute to neuroinflammation and can reflect the extent of hemorrhagic brain injury. CXC chemokine ligand-12 (CXCL12), a member of the CXC chemokine subfamily, is a regulatory signaling protein of central nervous system and participates in migration, survival and proliferation of neurons [19–21]. In addition, CXCL12 plays a crucial role in both focal angiogenesis and inflammatory reactions [22,23]. Of note, there are data suggesting that increased admission plasma CXCL12 concentrations have a strong association with the future recurrence of ischemic stroke [24]. Meanwhile, serum CXCL12 is found to be an independent diagnostic and prognostic marker for acute ischemic stroke [25,26]. Moreover, its close association with severity and prognosis of traumatic brain injury and aneurysmal subarachnoid

Abbreviations: ICH, intracerebral hemorrhage; AUC, area under curve; CI, confidence interval; CT, computerized tomography; CXCL12, CXC chemokine ligand-12; NIHSS, National Institutes of Health Stroke Scale; HR, hazard ratio; OR, odds ratio; ROC, receiver operating characteristic

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hemorrhage has been confirmed [27,28]. Nevertheless, the change of CXCL12 concentrations up to date remains unclear in peripheral blood of ICH patients.

2. Materials and methods

2.1. Design and subjects

This prospective, observational study was carried out between April 2013 and April 2016 on patients with a diagnosis of first-ever acute spontaneous basal ganglia hemorrhage confirmed by brain computed tomography (CT) scan, who presented to the Emergency Service of our Hospital within 24 h after the onset of symptoms. Excluded were patients with an infection within recent a month, a secondary cause (e.g., brain tumor, vascular malformation and trauma), a history of other neurological disease (e.g., severe head trauma, ischemic stroke and brain tumor), use of antiplatelet or anticoagulant medication, a surgical procedure or other concurrent comorbidities including autoimmune diseases, uremia, liver cirrhosis, malignancy, and chronic heart or lung disease.

A control group was formed from healthy individuals who were age- and sex-matched to ICH patients. Exclusion criteria were infection within recent a month, other neurological diseases (e.g., severe head trauma, ischemic stroke and brain tumor), use of antiplatelet or anticoagulant medication and other concurrent comorbidities including autoimmune diseases, uremia, liver cirrhosis, malignancy, and chronic heart or lung disease. The study was performed with approval of the ethics committee at our hospital. We obtained written informed consent from all participants or their legal guardians.

2.2. Assessment

We used NIHSS score to assess severity of neurological deficit of each patient. When any patient had an increase of ≥ 4 points in the NIHSS score or death at 24 h from symptoms onset, early neurologic deterioration was considered [29]. Hematoma volume was estimated according to ABC/2 method [30]. All patients underwent at least twice CT scans, namely an initial CT scan at admission and a second CT scan at 24 h. Hematoma growth was defined as hematoma enlargement $>$ 33% at 24 h [31].

ICH patients were followed up until death or completion of 6 months after stroke. Functional outcome was evaluated using the modified Rankin Scale. A modified Rankin Scale score $>$ 2 at 6 months was considered as an unfavorable outcome. The clinical end points contained death within 6 months as well as unfavorable outcome within 6 months after stroke.

2.3. Assays

Serum samples were separated from blood obtained from patients and controls by centrifuging at 3000 $\times g$ for 10 min. Then, serum was stored -80 °C until assayed. CXCL12 concentrations were measured in duplicate samples with a quantitative sandwich enzyme-linked immunosorbent assay kit (Quantikine; R & D Systems) in accordance with the manufactures' instructions. All determinations were performed by the same laboratory technician blinded to all clinical data.

2.4. Statistical analysis

All analyses were conducted using SPSS 19.0 and MedCalc 9.6.4.0. Continuous variables are expressed in the form of medians (interquartile ranges), and categorical variables are reported as frequencies (percentages) unless other indicated. We used a Mann-Whitney *U* test to compare continuous variables and a χ^2 test or Fisher exact test to compare categorical variables. The dependence between two variables was assessed by Spearman's correlation coefficient. In this study, serum CXCL12 concentrations, NHISS scores, hematoma volume and other continuous variables were dichotomized in accordance with their median values.

We constructed a receiver operator characteristic (ROC) curve with death within 6 months as a classification variable and using CXCL12 concentration, NIHSS score or hematoma volume as a prognostic variable. The area under curve (AUC) and 95% CI were reported based on the ROC curve. In a combined logistic-regression model, the additive benefit of CXCL12 concentrations to NIHSS scores and hematoma volume was estimated.

We used a binary logistic regression analysis to determine the independent association between serum concentrations of CXCL12 and outcome at 6 months after adjustment for other confounding factors which were significant in univariate analysis. Odds ratio (OR) and its 95% CI values were calculated to determine the impact of each variable.

Overall survival was estimated using the Kaplan–Meier method and intergroup comparisons of survival time were performed using the log-rank test. All parameters which were verified to be significant in the univariate analysis were further incorporated into a multivariate Cox's proportional hazard model to identify predictors of 6-month overall survival. Hazard ratio (HR) and the corresponding 95% CI were reported. Statistical significance was defined as a $p < 0.05$.

3. Results

3.1. Study population characteristics

Initially, a total of 141 acute ICH patients, who met the inclusion criteria, presented to the emergency center. According to the exclusion criteria, 34 patients were excluded because of an infection within recent a month (2 cases), bleeding due to brain tumor (2 cases), vascular malformation (3 cases) and trauma (2 cases), a history of severe head trauma (2 cases), ischemic stroke (5 cases) and brain tumor (2 cases), use of antiplatelet or anticoagulant medication (6 cases), a surgical procedure (4 cases) as well as other concurrent comorbidities (6 cases). Also, 2 patients were lost to follow-up and eventually, 105 patients were assessed. Meanwhile, 105 sex- and age- matched healthy individuals were chosen as controls.

This group of patients, of whom 63 were males, had a median age of 67 (61–74) y (range, 45–79 y). Hypertension accounted for 88.6% (93/105) of all patients. Diabetes mellitus occurred in 27 cases (25.7%). A total of 31 patients (29.5%) suffered from intraventricular extension of hematoma. In addition, the admission median NIHSS score was 9 (7–13) (range, 2–19) and the median hematoma volume was 31 (25–37) ml (range, 5–60 ml). The patients presented to emergency center at our hospital from 0.4 to 20.2 h after stroke onset (median, 5.2 h; interquartile range, 1.2–9.8 h). We collected blood samples of patients at the median time of 7.2 (4.2–12.3 h) (range, 1.8–23.2 h) after stroke onset. A total of 14 cases (13.3%) showed hematoma growth. Early neurological deterioration was found in 18 cases (17.1%). Moreover, the median systolic arterial pressure and diastolic arterial pressure were 173 (158–190) mmHg (range, 124–213 mmHg) and 97 (93–101) mmHg (range, 74–117 mmHg) respectively. When completion of 6-month follow-up, we found that 28 patients (26.7%) were dead and 52 patients (49.5%) experienced an unfavorable outcome. Alternatively, mean overall survival time was 143.5 days (95% CI: 131.0–156.1) during 6-month follow up.

3.2. Assessment of serum CXCL12 concentrations

Fig. 1 showed that the ICH patients had significantly higher serum CXCL12 concentrations than the controls; moreover, serum CXCL12 concentrations were markedly increased in non-survivors when compared with survivors within 6 months after ICH. In addition, there was a significant difference between patients with unfavorable outcome and