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Elevation of serum CXC chemokine ligand-12 levels predicts poor outcome after aneurysmal subarachnoid hemorrhage☆

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ABSTRACT

Objective: CXC chemokine ligand-12 (CXCL12) is involved in the innate immune system. Elevation of its level in the peripheral blood is associated with severity and outcome of ischemic stroke. This study aimed to investigate its relation to severity and prognosis following aneurysmal subarachnoid hemorrhage (aSAH).
Methods: Serum CXCL12 levels were determined in a total of 182 controls and 182 aSAH patients. Hemorrhagic severity was assessed using the World Federation of Neurological Surgeons (WFNS) scale and modified Fisher grading scale. Unfavorable outcome was defined as Glasgow outcome scale score of 1–3. Prognostic predictors of 6-month mortality and unfavorable outcome were identified using multivariate analysis.
Results: The serum CXCL12 levels were significantly higher in patients as compared to controls (14.5 ± 6.7 ng/mL vs. 1.7 ± 0.6 ng/mL, $P < 0.001$) and were independently associated with WFNS scores ($t = 5.927$, $P < 0.001$) and modified Fisher scores ($t = 5.506$, $P < 0.001$). Serum CXCL12 levels predicted 6-month mortality and 6-month unfavorable outcome with the area under curves of 0.815 [95% confidence (CI), 0.751–0.868] and 0.809 (95% CI, 0.745–0.864) respectively and were related independently to 6-month mortality (odds ratio, 4.428; 95% CI, 1.977–12.031; $P = 0.004$) and 6-month unfavorable outcome (odds ratio, 3.821; 95% CI, 1.097–9.251; $P = 0.001$). Moreover, the predictive values of CXCL12 levels were in the range of WFNS scores and modified Fisher scores.
Conclusions: Elevation of serum CXCL12 levels is associated highly with hemorrhagic severity and poor outcome after aSAH, suggesting CXCL12 might have the potential to be a prognostic predictive biomarker of aSAH.

1. Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a major clinical and health-care problem affecting millions of people worldwide [1–3]. It is important to conduct a precise evaluation of the patient's clinical status in the management of treatment modalities and determination of prognosis. The World Federation of Neurological Surgeons (WFNS) scale and Fisher grading scale are commonly used to evaluate the neurologic status and severity of hemorrhage based on the computed tomography (CT) appearance in aSAH patients [4,5]. In addition, interest in the measurement of blood biomarkers as prognostic predictors of aSAH has increased in the last few years [6–8].

The immune system is a complex and tightly regulated system of effectors against external pathogens and endogenous tissue damage. It can be broadly divided into two component parts, the innate immune system and the adaptive immune system [9,10]. The growing body of evidence has shown that the innate immune system affects brain damage after subarachnoid hemorrhage. Chemokines are small chemoattractant cytokines that are produced in response to damage to call inflammatory cells into the area, thus, regulating the innate immune responses after aSAH [10–12]. The CXC chemokine ligand-12 (CXCL12) is a member of the CXC chemokine subfamily that is constitutively expressed in the bone marrow and other tissues including the brain endothelium and is responsible for regulating the trafficking and localization of bone marrow progenitor cells under steady state and stress conditions [13–15]. Following ischemic stroke, CXCL12 mediates the inflammatory response by recruitment of neural progenitor cells, and the mobilization of bone marrow-derived progenitor cells for tissue regeneration and neovascularization [16]. Other animal experiments had also found that CXCL12 played a significantly beneficial role in acute stroke [17,18]. CXCL12 combing CXC motif receptor 4 could inhibit the caspase-3 pathway by up-regulating Bcl-2/Bax ratio, which protects neurons from apoptosis in rats with traumatic brain injury [19]. Interestingly, elevated circulating CXCL12 level at admission is strongly

Abbreviations: aSAH, aneurysmal subarachnoid hemorrhage; AUC, area under curve; CI, confidence interval; CT, computerized tomography; CXCL12, CXC chemokine ligand-12; CXCR 4, CXC receptor 4; GOS, Glasgow outcome scale; OR, odds ratio; ROC, receiver operating characteristic; SDF-1, stromal cell-derived factor 1; WFNS, World Federation of Neurological Surgeons.

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associated with the future stroke [20] and future recurrence of ischemic stroke [21], as well as has close relation to stroke severity and lesion volumes [22]. Still, CXCL12 is identified as an independent diagnostic and prognostic marker in patients with acute ischemic stroke [23]. The present study further investigated the potential prognostic role of CXCL12 in Chinese aSAH patients.

2. Methods

2.1. Ethics statement

This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and has been also approved by the ethical committee at the First Affiliated Hospital, School of Medicine, Zhejiang University. Still, all participants or their representative were informed of the study protocol and their written informed consent was obtained.

2.2. Participants

This is a prospective study from a cohort of patients with confirmed aSAH admitted to the Department of Neurosurgery, First Affiliated Hospital, School of Medicine, Zhejiang University between January 2011 and May 2014. Patients were enrolled in the study with the first-ever non-traumatic SAH, the clinical history of SAH within the last 24 h before admission, the single intracranial aneurysms confirmed by CT angiography with or without digital subtraction angiography and the treatment through clipping or coiling within the 48 h after admission. Exclusion criteria included surgery, trauma or infectious diseases during the preceding 1 month, autoimmune diseases with or without immunosuppressive therapy, prior neurological diseases such as intracerebral hemorrhage and ischemic stroke, previous use of antiplatelet or anticoagulant medication, rebleeding after admission, suspected pseudoaneurysm, and other prior systemic diseases such as uremia, liver cirrhosis, malignancy, chronic heart disease, chronic lung disease, diabetes mellitus and hypertension. The control group consisted of the healthy age- and sex-matched volunteers.

2.3. Assessment

The neurological status was graded according to the WFNS scale [4] and the radiological severity of SAH was classified based on the modified Fisher grade [5]. The WFNS assessments were performed by at least two clinicians. Radiologists helped to determine the modified Fisher grade. When two raters obtained different scores, a judgment of a third senior clinician was considered. Symptomatic cerebral vasospasm was defined as the development of new focal neurological signs, deterioration in level of consciousness, or the appearance of new infarction on CT when the cause was felt to be ischemia attributable to vasospasm after other possible causes of worsening (e.g. hydrocephalus, seizures, metabolic derangement, infection, or oversedation) had been excluded [24,25].

2.4. Endpoint

Participants were followed up until death or completion of 6 months after aSAH. The endpoints were unfavorable outcome and death after 6 months. The functional outcome was defined by Glasgow outcome scale (GOS) score. GOS scores were dichotomized in favorable and unfavorable outcomes (GOS of 4–5 vs. GOS of 1–3) [26–28]. For follow-up, structured telephone interviews were performed by one doctor who was blinded to clinical information.

2.5. Sampling and laboratory analysis

Blood samples were collected from the patients at admission and from controls at study entry. After centrifugation, aliquots of the samples were immediately stored −80 °C until assay. Serum CXCL12 levels were determined in duplicate samples with a quantitative sandwich enzyme-linked immunosorbent assay kit (Quantikine; R&D Systems, Minneapolis, MN, USA) in accordance with the manufactures' instructions. The investigator was blinded to the clinical outcome and neuroimaging findings.

2.6. Statistical analysis

All the results were statistically analyzed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and MedCalc 9.6.4.0 (MedCalc Software, Mariakerke, Belgium). Statistical significance was defined as a probability value of less than 0.05.

Categorical variables were presented as counts (percentage). Continuous variable were reported as mean ± standard deviation or median (the upper and lower quartiles) as appropriate. The statistical significance of the intergroup observed difference was assessed with the chi-square test, Fisher exact test, Student *t* test or Mann–Whitney U-test as appropriate. All parameters found to be significant in the univariate analysis were further entered in a binary Logistic regression model to identify prognostic predictors.

Linear relationships between CXCL12 levels and other variables were investigated using Spearman's correlation coefficient or Pearson's correlation coefficient as appropriate. We used Bonferroni correction to control for the multiple testing. A multivariate linear regression was carried out further to verify their relationships.

Receiver operating curves (ROCs) were generated to determine cut-off values for optimal prognostic predictive sensitivities and specificities. The area under curves (AUCs) were estimated in accordance with the ROC curves and the additive benefits of CXCL12 levels to the WFNS scores and modified Fisher scores were evaluated in a combined logistic-regression model.

3. Results

3.1. Participants characteristics

During the study period, 239 aSAH patients were initially evaluated. 57 patients were excluded because of the reasons listed in Fig. 1. Eventually, 182 aSAH patients were included in this study. Additionally, 182 gender- and age-matched healthy subjects were enrolled as controls.

This group of aSAH patients was composed of 77 males and 105 females as well as had a mean age of 50.6 ± 11.4 years (range, 34–77 years). The median admission WFNS scores and modified Fisher scores were 3 (2–3) (range, 1–5) and 3 (2–4) (range, 2–5) respectively. Aneurysmal location is as follows: 48 (26.4%) aneurysms were located at posterior communication artery; 31 (17.0%) aneurysms, internal carotid artery; 37 (20.3%) aneurysms, anterior communication artery; 33 (18.1%) aneurysms, middle cerebral artery; 22 (12.1%) aneurysms, anterior cerebral artery; 8 (4.4%) aneurysms, posterior cerebral artery; 3 (1.7%) aneurysms, vertebral artery. 146 (80.2%) patients had cystic aneurysm. The mean aneurysm diameter was 7.9 ± 5.4 mm (range, 2–25 mm). 96 (52.8%) patients underwent clipping and other patients obtained endovascular coiling. 37 (20.3%) patients had acute hydrocephalus; 29 (15.9%) patients, intraventricular hemorrhage; 43 (23.6%) patients, underwent external ventricular drain; 52 (28.6%) patients, symptomatic cerebral vasospasm.

3.2. The change of serum CXCL12 levels in aSAH patients

Patients had the significant elevation of serum CXCL12 levels as compared with normal controls (14.5 ± 6.7 ng/mL vs. 1.7 ±