Original Article
The role of serum and cerebrospinal fluid levels of C reactive protein in evaluating neurological functional recovery of acute cerebral infarction patients

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Abstract: As one non-specific acute reactive factor, C reactive protein (CRP) is closely related with the occurrence of cerebral infarction and can work as one evaluating index reflecting nervous system injury. This study recruited acute cerebral infarction (ACI) patients, whose neurological functions and CRP levels in serum and cerebrospinal fluid (CSF) were tested to analyze its correlation with functional recovery of ACI patients. ACI patients in our hospital were recruited to analyze the CRP level in serum and CSF by enzyme-linked immunosorbent assay (ELISA). With concurrent neurological function evaluation, the correlation between CRP level and functional recovery was analyzed. ACI patients had higher serum and CSF CRP levels compared to recovered patients or healthy ones. On day 7, both CRP levels reached peak values, with gradually decrease till day 14. Those patients with severe neurological functional deficits had higher CRP contents (both serum and CSF) compared to those having mild or moderate ACI. A positive correlation existed between serum and CSF levels of CRP (P<0.05). CRP level was found to be positively correlated with the severity of neurological deficits. The combined assay of serum and CSF levels of CRP may work as effective index reflecting the functional recovery after ACI.

Keywords: Serum, cerebrospinal fluid, C reactive protein, acute cerebral infarction

Introduction
With the aging of total population in China, the incidence of acute cerebral infarction (ACI) has been increasing gradually. With high morbidity and mortality rate, ACI severely affects the lifespan and life quality of elder people [1]. Inflammatory response has been found to play an important role in the occurrence of atherosclerosis and its complications, as it can facilitate the formation of arterial thrombosis for progression of ACI [2]. C reactive protein (CRP) is one non-specific acute reactive protein produced by the liver under the stimulus from interleukin-6 (IL-6). As one critical inflammatory mediator, CRP participates in multiple diseases including hypertension and diabetes, and also can reflect the prognosis of ischemia stroke [3]. Studies have suggested the specificity of serum CRP level in predicting the severity of ACI [4]. The combined assay of serum and cerebrospinal fluid (CSF) levels of CRP may help to analyze the functional recovery of ACI patients. One critical factor of ACI treatment to decrease the incidence of multiple organ failure and hence force mortality rate, is the suppression of body CRP level for alleviating inflammatory response [5]. This study recruited ACI patients with various severities, to evaluate their neurological functions, along with both serum and CSF levels of CRP, in order to analyze the correlation between CRP level and functional recovery of ACI patients.

Materials and methods

General information of patients and sample collection
A total of 40 ACI patients from January 2014 to January 2015 in Yan’an University Affiliated Hospital were recruited as the experimental
group. There were 20 males and 20 females in this group, aging between 42 and 80 years old (average age = 51.2 years). ACI was diagnosed by brain CT or MRI within 2 to 4 days of primary disease onset. A total of 26 patients were at acute stage, while the other 14 patients were at recovery stage for routine follow-ups. All patients were divided into 15 mild, 16 moderate and 9 severe cases based on the neurological deficits score of ACI. In acute stage patients, there were 12 mild, 9 moderate and 5 severe cases. For recovery patients, the figures were 4, 6 and 4, respectively. General information of patients were listed in Table 1.

The study protocol was approved by the Research Ethics Committee of Yan'an University Affiliated Hospital, and all patients gave their informed consent before study commencement.

**Inclusive criteria:** Confirmed ACI diagnosis by head CT or MRI; No major disease of liver or kidney; no thrombolysis treatment previously; No mental illness history.

**Exclusive criteria:** Having infectious disease within recent two weeks; Accompanied with malignant tumors; Complicated with immune disease or hematological disease; with severe heart, liver or kidney disease or diabetes.

Another cohort of 40 healthy individuals were admitted as the control group. There were 22 males and 18 females, aging between 45 and 80 years old (average age = 54.2 years). No significant different existed between disease and control groups regarding sex or age distribution (P>0.05).

**Sample collection**

Fasted blood samples (4 mL) were collected at day 3, day 7 and day 14 after primary disease onset, and were kept in EDTA-containing tubes. After incubation at room temperature for 1 hour, the blood was centrifuged at 1,500 g for 10 min to collect the supernatant, which was kept at -20°C.

At day 3, day 7 and day 14, 2 mL CSF sample was collected by lumbar puncture, during which intracerebral pressure (ICP) was measured. CSF was kept -20°C.

**Enzyme linked immunosorbent assay (ELISA)**

Using CRP ELISA kit (Kaiji Biotech, China), CRP levels were determined in both serum and CSF samples. In brief, samples were added into 96-well plate along with standard samples, followed by mixing with incubation buffer (containing 5% anti-CRP biotin-conjugated antibody). The plate was vibrated under room temperature for 1 hour, followed by washing 6 times. TMB-HRP substrates were then added for 30-min incubation, and were quenched by stopping buffer. A microplate reader was used to quantify optical density (OD) values of each well at 405 nm. A standard curve was firstly plotted based on standard samples. The concentration of samples was then determined by the standard curve and linear regression.

**Neurological score**

Based on NIHSS system, mild, moderate and severe neurological dysfunction was determined as <4, 4~15, and >15 scores.

**Statistical analysis**

SPSS 17.0 software was used to process all collected data, of which numeration data was compared by chi-square test, while measurement data were compared by analysis of variance and were presented as mean ± standard deviation (SD). Logistic regression analysis was used to reveal the correlation. A statistical significance was defined when P<0.05.

### Table 1. General information of disease group

<table>
<thead>
<tr>
<th></th>
<th>Recovery stage</th>
<th>Acute stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female (N)</td>
<td>8/6</td>
<td>12/14</td>
</tr>
<tr>
<td>Age (year)</td>
<td>54.2±5.1</td>
<td>51.2±4.6</td>
</tr>
<tr>
<td>Disease period (hr)</td>
<td>7.8±3.3</td>
<td>2.1±0.7</td>
</tr>
<tr>
<td>Neurological deficit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Severe</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1±2.1</td>
<td>22.9±2.6</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>125.2±19.4</td>
<td>131.5±18.9</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>75.2±8.1</td>
<td>76.1±8.9</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.1±8.0</td>
<td>5.3±8.7</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.1±1.5</td>
<td>2.7±1.9</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>10.23±1.1</td>
<td>10.98±1.9</td>
</tr>
</tbody>
</table>

Note: BMI, body-mass index; SBP, systolic blood pressure; DBP, diabolic blood pressure; TC, total cholesterol; TG, total glycerol; FPG, fast plasma glucose.
CRP in cerebral infarction

Results

CRP level across different time points

CRP levels in both serum and CSF revealed higher levels in acute phase patients compared to those in recovery phase patients, which also had higher CRP levels than control ones (P<0.05, Table 2). A longitudinal observation found the peak level of CRP at day 7 after primary disease onset, followed by gradually decrease till day 14 (P<0.05, Table 2). Such pattern also occurred in recovery patients and control group.

CRP levels and neurological dysfunctions

We found that those patients with severe neurological dysfunctions had higher CRP levels at each time point compared to moderate or mild group (P<0.05, Table 3). Similar time-dependent patterns occurred in all sub-groups, as CRP levels reached the peak at day 7 and gradually decreased till day 14.

Correlation between serum and CSF levels of CRP

The correlation analysis revealed a significantly positive correlation between serum CRP and CSF CRP level (r = 0.920, P<0.05, Figure 1).

CRP levels and neurological functions

Further analysis revealed positive correlations between neurological deficits and serum CRP level (r = 0.856, P<0.05, Figure 2) or CSF CRP level (r = 0.893, P<0.05, Figure 3).

Discussion

As one common disease in elder population, ACI has high incidence and mortality rate, thus severely affecting patients’ life span and quality [6]. The occurrence of ACI is mainly attributed to the interruption of focal cerebral blood flow for causing ischemia, hypoxia and tissue necrosis [7]. As early as 1930s, CRP was identified as one non-antibody protein factor produced by hepatocytes under the stimulus of cytokines. As one responsive protein during acute stage, CRP is abundantly expressed during tissue damage, thus reflecting body injury and inflammation/tissue repair [8]. Previous studies have found the positive correlation between CRP level and the severity of cardiovascular disease. It can deposit on the arterial wall for binding lipoprotein and producing inflammatory mediators, causing injury of vascular endothelial tissues, dispatching of plaques, making it one risk factor of cardiovascular disease [9, 10]. Those with CRP contents higher than 10 mg/L had significantly elevated risk of vascular disease than those with lower CRP levels [11].

In this study, we selected ACI patients, whose CRP level in serum and CSF was measured, in parallel with neurological functional evaluation. We found significantly elevated CRP contents in both serum and CSF at day 3, day 7 and day 14.
CRP in cerebral infarction

Correlation analysis between serum and CSF levels of CRP found positive correlation between these two factors, both of which were also positively correlated with neurological dysfunctions. Previous study has demonstrated the predictive role of body CRP level in reflecting the condition of ACI-related inflammation. The potent inflammatory response may accelerate the deterioration of ischemia lesion, further affecting the future recovery of neurological function [16, 17]. CRP can participate in body inflammatory response via activating complements, to mediating vascular constriction and permeability, for further regulation on nutrition of neurons, thus protecting neural tissues from several injuries [18, 19]. The differential level of CSF and serum CRP levels may be due to the different distances of lesions toward the arachnoid cavity. The tolerability of neurons at different sites are variable, making the endocytosis of CRP by macrophage [20]. Previous study has postulated the correlation between blood CRP contents and ACI patients’ severity. However, the single blood assay may be biased by multiple factors. The combined
assay of serum and CSF levels of CRP, however, may help to better analyze its effects on functional recovery of ACI patients. Therefore, CRP may be one independent risk factor of ACI, whose functional recovery can be evaluated by combined assay of serum and CSF CRP levels.

In summary, the correlation between CRP level and ACI occurrence suggests the value of combined assay of serum and CSF levels of CRP in both evaluating the severity of ACI and describing the functional recovery condition. It is thus of clinical values to further explore the possibility of using CRP inhibitor to increase the treatment efficacy of ACI and to improve the prognosis.

Disclosure of conflict of interest

None.

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