Clinical significance of changes in IL-6, CRP and S100 in serum and NO in cerebrospinal fluid in subarachnoid hemorrhage and prognosis

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Abstract. Clinical significance of changes in interleukin-6 (IL-6), C-reactive protein (CRP) and S100 in serum and NO was investigated in cerebrospinal fluid (CSF) in subarachnoid hemorrhage (SAH) and its prognosis. A total of 43 SAH patients and 23 healthy subjects were selected and divided into cerebral vasospasm (CVS) group and non-CVS group, and favorable prognosis group and unfavorable prognosis group according to Hunt-Hess grade. The levels of IL-6, CRP, S100 and NO in CSF were detected, respectively, followed by statistical analysis of correlation. The higher the Hunt grade was, the higher the factor expression was; the expression levels of IL-6, CRP, S100 and NO in CSF were gradually increased in CVS group and unfavorable prognosis group, and the differences were significant compared with those in the control group. There was a positive correlation between the expression levels of each of the two factors among IL-6, CRP, S100 and NO in CSF, and the differences were statistically significant ($P<0.05$). The expression levels of IL-6, CRP, S100 and NO in CSF in SAH patients are significantly increased, showing positive correlations and participating in the occurrence and development of SAH, which provide new directions for the early clinical diagnosis of SAH.

Introduction

Subarachnoid hemorrhage (SAH) refers to the syndrome where the blood reaches the intracranial or intraspinal subarachnoid space after intracranial vascular rupture caused by many factors (1). It is reported that SAH accounts for approximately 15% of cerebrovascular diseases (2). If there is no effective clinical treatment in the early stages of bleeding, approximately 13% patients will die suddenly (3,4).

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Cerebral vasospasm (CVS) usually occurs after SAH, and it can cause cerebral infarction in case of severe condition (5,6), so the early diagnosis of this disease is particularly important. Increasing number of scholars believe that SAH is closely related to the body's inflammatory response. They think that the occurrence and development of SAH are also associated with the body's cytokine expression (7-10).

In the present study, the changes in interleukin-6 (IL-6), C-reactive protein (CRP) and S100 in serum and NO in cerebrospinal fluid (CSF) in SAH patients were detected to investigate the possible pathogenesis of SAH, so as to provide new directions for the early clinical diagnosis of SAH.

Patients and methods

Patients. A total of 43 patients clinically confirmed as SAH were selected (Fig. 1), including 27 males and 16 females aged 61±5 years. The clinical data were sorted by Hunt-Hess grade and the results were evaluated by the specialist. Exclusion criteria: Patients with the onset time of more than 3 days, used to receive the clinic treatment in other hospitals, with liver, kidney, heart or lung insufficiency or infectious diseases. This study was approved by the Ethics Committee of Binzhou Medical University Hospital (Binzhou, China). Signed written informed consents were obtained from all participants before the study. Control group: 23 healthy subjects were selected, including 13 males and 10 females aged 58±4 years. SAH patients were divided into the CVS and the non-CVS groups according to whether there was CVS, and patients were also divided into the favorable prognosis and the unfavorable prognosis groups according to APACHE II score. All patients and healthy subjects signed the informed consent.

Experimental reagents. Human IL-6, CRP and S100 enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Genetimes ExCell Biotechnology Co., Ltd., Shanghai, China) and NO kit (Wenzhou ERKN Biotechnology Co., Ltd., Wenzhou, China).

Detection of IL-6, CRP and S100 in serum. Human IL-6, CRP and S100 ELISA kits were purchased from Shanghai Genetimes ExCell Biotechnology Co., Ltd. The expression levels of IL-6, CRP and S100 in serum samples were detected according to the instructions.
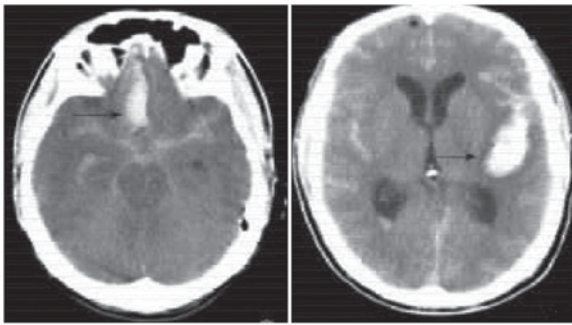
Figure 1. Representative images of SAH with a CT scan. SAH, subarachnoid hemorrhage.

The contents of IL-6, CRP and S100 were detected according to the instructions of IL-6, CR and S100 ELISA kit.

Detection of NO level in CSF. The chemical property of NO is more active, which can be quickly metabolized and converted into NO_2^- and NO_3^-, and NO_2^- will be further converted into NO_3^-. In this study, the nitrate reductase method was used to specifically reduce NO_3^- into NO_2^-, the latter of which can react with the developer and produce colored substances. Finally, the absorbance value was detected.

The level of NO in CSF was detected according to the instructions of NO kit.

Statistical analysis. In this study, SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) software was used for analysis. Data were presented as mean ± standard deviation. Comparison between groups was done using One-way ANOVA test followed by post hoc test (Least Significant Difference). Pearson's analysis was used to test the correlation between the two factors. $\alpha=0.05$ was set as the inspection standard.

Results

Contents of serum IL-6, CRP and S100 in SAH patients. The expression levels of IL-6, CRP and S100 in serum of SAH patients were significantly higher than those in the normal control group, and the differences of each Hunt grade were statistically significant compared with the normal control group ($P<0.05$). The expression levels of IL-6, CRP and S100 in serum of SAH patients were gradually increased with the increase of Hunt grade (Fig. 2).

Content of NO in CSF. The expression level of NO in CSF of SAH patients was significantly higher than that in the normal control group, and the difference of each Hunt grade was statistically significant compared with the normal control group ($P<0.05$). The expression level of NO in CSF of SAH patients was gradually increased with the increase of Hunt grade (Fig. 3).

Contents of serum IL-6, CRP and S100 and NO in CSF in the CVS and non-CVS groups. The levels of IL-6, CRP, S100 and NO in CSF in CVS group were higher than those in the normal control group at 1, 4, 7 and 10 days, and the differences were significant. The contents of serum IL-6,
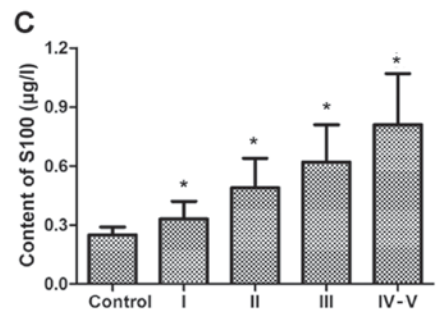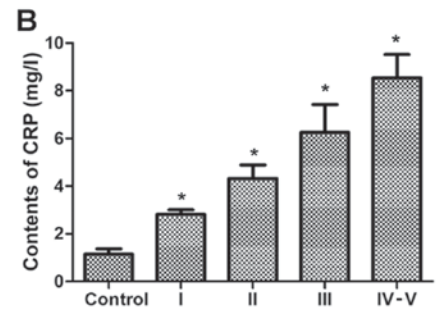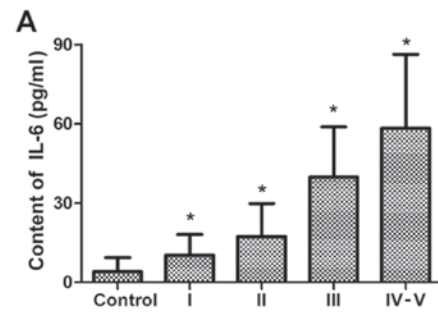

Figure 2. Contents of serum IL-6, CRP and S100 in SAH patients. The expression levels of IL-6, CRP and S100 in serum of SAH patients were significantly higher than those in the normal control group. *$P<0.05$, compared with the normal control group. IL-6, interleukin-6; CRP, C-reactive protein; SAH, subarachnoid hemorrhage.
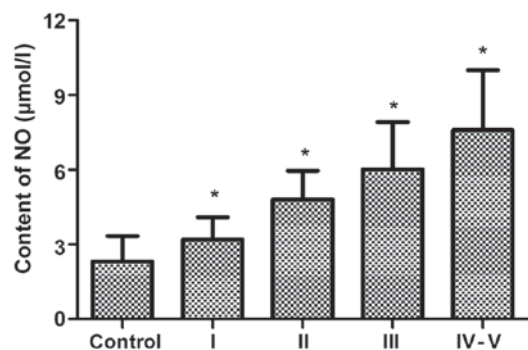

Figure 3. Content of NO in CSF. The expression level of NO in CSF of SAH patients was significantly higher than that in the normal control group. *$P<0.05$, compared with the normal control group. CSF, cerebrospinal fluid; SAH, subarachnoid hemorrhage.

CRP and S100 and NO in CSF in the CVS group were significantly higher than those in the non-CVS group, and the differences were statistically significant ($P<0.05$). The

Table I. Contents of serum IL-6, CRP, S100 and NO in CSF in the CVS and the non-CVS groups (mean ± standard deviation).

Group	n	Days	IL-6 (pg/ml)	CRP (mg/l)	S100 (µg/l)	NO (µmol/l)
Control	23		4.01±5.36	1.15±0.22	0.25±0.04	2.31±1.02
CVS	25	1	10.65±4.91[a,b]	5.99±1.03[a,b]	0.53±0.02[a,b]	4.14±0.69[a,b]
		4	29.26±9.49[a,b]	13.83±0.95[a,b]	0.81±0.14[a,b]	5.97±1.16[a,b]
		7	37.93±14.07[a,b]	15.17±2.36[a,b]	1.03±0.22[a,b]	7.82±1.63[a,b]
		10	52.39±20.81[a,b]	25.82±4.41[a,b]	1.71±0.14[a,b]	8.85±1.07[a,b]
Non-CVS	18	1	7.11±4.25[a]	3.05±0.27[a]	0.39±0.03[a]	3.77±0.65[a]
		4	25.94±6.35[a]	11.48±1.33[a]	0.68±0.08[a]	5.31±1.04[a]
		7	22.19±10.31[a]	9.29±0.37[a]	0.55±0.11[a]	4.85±0.97[a]
		10	15.88±8.37[a]	6.73±1.29[a]	0.51±0.04[a]	4.42±0.73[a]

[a]$P<0.05$, compared with the control group; [b]$P<0.05$, compared with non-CVS group. IL-6, interleukin-6; CRP, C-reactive protein; CSF, cerebrospinal fluid; CVS, cerebral vasospasm.

Table II. Contents of IL-6, CRP, S100 and NO in CSF in the favorable prognosis and the unfavorable prognosis groups (mean ± standard deviation).

Group	n	Days	IL-6 (pg/ml)	CRP (mg/l)	S100 (µg/l)	NO (µmol/l)
Control	23		4.01±5.36	1.15±0.22	0.25±0.04	2.31±1.02
Favorable prognosis	32	1	6.48±3.13[a]	2.94±0.18[a]	0.28±0.03[a]	3.18±0.47[a]
		4	23.74±5.96[a]	10.85±1.48[a]	0.55±0.08[a]	5.22±0.97[a]
		7	19.86±7.75[a]	8.37±0.29[a]	0.47±0.05[a]	4.77±0.58[a]
		10	13.03±9.16[a]	6.15±0.77[a]	0.39±0.04[a]	3.35±0.26[a]
Unfavorable prognosis	11	1	9.03±3.37[a,b]	4.52±0.99[a,b]	0.44±0.03[a,b]	3.27±0.55[a,b]
		4	28.47±8.59[a,b]	13.03±1.25[a,b]	0.67±0.16[a,b]	4.85±0.94[a,b]
		7	35.14±12.55[a,b]	18.74±1.99[a,b]	1.33±0.19[a,b]	6.69±1.36[a,b]
		10	48.02±17.83[a,b]	27.85±2.76[a,b]	1.68±0.25[a,b]	7.57±1.11[a,b]

[a]$P<0.05$, compared with control group; [b]$P<0.05$, compared with favorable prognosis group. IL-6, interleukin-6; CRP, C-reactive protein; CSF, cerebrospinal fluid.

contents of IL-6, CRP, S100 and NO in CSF in the CVS group were increased gradually with the extension of time, while the contents of IL-6, CRP, S100 and NO in CSF in the non-CVS group reached the peak at 4 days and then gradually declined (Table I).

Factor contents in favorable prognosis group and unfavorable prognosis group. Compared with those in the normal control group, the expression levels of IL-6, CRP, S100 and NO in CSF in unfavorable prognosis group were higher at 1, 4, 7 and 10 days, and the differences were statistically significant (P<0.05). Compared with those in favorable prognosis group, the levels in the unfavorable prognosis group were significantly higher at 1, 4, 7 and 10 days (P<0.05). The levels of IL-6, CRP, S100 and NO in CSF in the unfavorable prognosis group were gradually increased, while the levels in favorable prognosis group reached the peak at 4 days and then declined gradually (Table II).

Correlation among IL-6, CRP, S100 and NO in CSF. The correlations among IL-6, CRP, S100 and NO in CSF were detected via Pearson's analysis. It was found that there was a positive correlation between IL-6 and NO, between CRP and NO and between S100 and NO (r_1=0.417, P<0.05; r_2=0.552, P<0.05; r_3=0.505, P<0.05), and the differences were statistically significant (Fig.4). The expression of IL-6 was elevated when the inflammatory response occurred, and the contents of CRP, S100 and NO in CSF were also increased.

Discussion

SAH is a clinical syndrome caused by many factors with a high mortality rate, which refers to the blood reaching the intracranial or intraspinal subarachnoid space after intracranial vascular rupture (11,12). IL-6, as a kind of glycoprotein, can participate in the collective inflammatory response and anti-infective defense mechanisms (13,14). At present, it is recognized that IL-6 is a
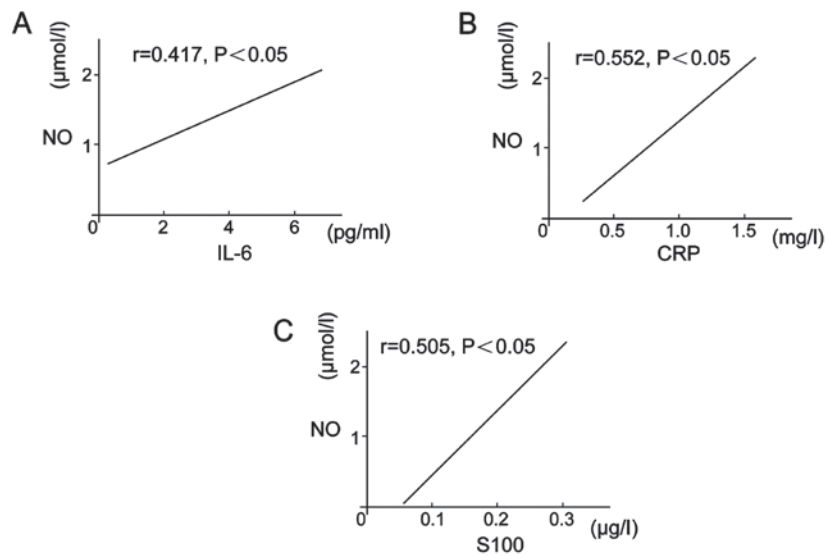
Figure 4. Correlation analysis among IL-6, CRP, S100 and NO in CSF were detected via Pearson's analysis. (A) Between IL-6 and NO; (B) between CRP and NO; and (C) between S100 and NO.

kind of multi-functional glycoprotein cell inflammatory factor involved in various inflammatory reactions of central nervous system, which can cause immune response to the brain tissue damage and inflammation (15). CRP can be synthesized after IL-6 induction, thus activating the body's complement system and improving the immunity (16,17). Some scholars have pointed out that CRP can be associated with prognosis as a continuous variable (18). When SAH occurs, neuroglia cells will be damaged, thus increasing the S100 protein level. Therefore, S100 is often used as one of the markers of SAH (19,20).

In this study, 43 patients diagnosed as SAH were selected and the levels of IL-6, CRP, S100 and NO in CSF were detected. The results showed that the expression of IL-6, CRP, S100 and NO in CSF in SAH patients were higher than those in the healthy normal control group. And the difference of each Hunt grade was significant compared with the normal control group. The contents of IL-6, CRP, S100 and NO in CSF in SAH patients were gradually increased with the increase of Hunt grade. The levels of IL-6, CRP, S100 and NO in CSF in CVS group at 1, 4, 7 and 10 days were higher than those in the normal control group. Compared with those in the non-CVS group, the levels of the four factors in the CVS group were significantly higher. The levels of IL-6, CRP, S100 and NO in CSF in the CVS group were increased gradually with the extension of time, while the levels of IL-6, CRP, S100 and NO in CSF in the non-CVS group reached the peak at 4 days, and then gradually declined. Compared with those in the normal control group, the expression levels of IL-6, CRP, S100 and NO in CSF in the unfavorable prognosis group were higher at 1, 4, 7 and 10 days. Compared with those in the favorable prognosis group, the levels in the unfavorable prognosis group at 1, 4, 7 and 10 days were higher. The levels of IL-6, CRP, S100 and NO in CSF in the unfavorable prognosis group were gradually increased, while the levels in the favorable prognosis group reached the peak at 4 days, and then declined gradually. The expression levels of IL-6, CRP, S100 and NO in CSF in SAH patients were generally higher than those in the healthy normal control group. And the difference of each Hunt grade was significant compared with the normal

control group. The contents of IL-6, CRP, S100 and NO in CSF in SAH patients were gradually increased with the increase of Hunt grade. There were positive correlations among the levels of IL-6, CRP, S100 and NO in CSF in SAH patients, confirming that IL-6, CRP, S100 and NO in CSF in SAH patients can reflect the severity and development process of inflammatory response, and may be involved in the occurrence and development of disease, late CVS and prognosis.

In conclusion, the levels of IL-6, CRP, S100 and NO in CSF of SAH patients were detected in this study, and it was found that there is a positive correlation between each of the two factors, which can reflect the development process of SAH and has a certain guiding significance for the clinical diagnosis of SAH.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

WZ designed the study, LS and LM collected the data, ZL analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Binzhou Medical University Hospital (Binzhou, China). Signed

informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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