

Relationship between astrocyte damage and different levels of cerebrospinal fluid markers and prognosis in patients with subarachnoid hemorrhage

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Folia Neuropathol 2024; 62 (2): 147-155

DOI: <https://doi.org/10.5114/fn.2023.132782>

Abstract

Introduction: The aim of the study was to explore the relationship between astrocyte damage and different levels of cerebrospinal fluid markers and prognosis in patients with subarachnoid hemorrhage (SAH).

Material and methods: A total of 168 SAH patients diagnosed and treated in the emergency department of our hospital during the period October 2019 to February 2022 were randomly selected as the study subjects. The severity of these patients' condition was evaluated by Hunt-Hess grading and these subjects were graded as the low-level group (78 patients) and high-level group (90 patients) according to the evaluation results. The Extended Disability Status Scale (EDSS) score was employed to evaluate the astrocyte damage. The content of atypical chemokine receptor 3 (ACKR3), Connexin 43 (Cx43), oxygenated hemoglobin (HbO₂), and endothelin (ET) in cerebrospinal fluid was measured. The relationship between the content of ACKR3, Cx43, HbO₂, and ET in cerebrospinal fluid with EDSS score was analyzed through Pearson correlation analysis. Multivariate logistic regression analysis was adopted to analyze the risk factors.

Results: ACKR3 was mainly expressed in the cytoplasm of cerebrospinal fluid monocytes, and Cx43 was mainly expressed in the cell membrane and cytoplasm. Patients in the high-level group had markedly higher expression rates of ACKR3 and Cx43 positive cells in cerebrospinal fluid than those in the low-level group ($p < 0.05$). Patients in the high-level group had higher content of HbO₂ and ET in cerebrospinal fluid and EDSS score than patients in the low-level group ($p < 0.05$). The content of ACKR3, Cx43, HbO₂, and ET in cerebrospinal fluid of SAH patients was positively correlated with EDSS scores ($p < 0.05$). Systolic blood pressure, Hunt-Hess grade, rebleeding, emotional control, EDSS score, ACKR3, Cx43 positive cell rate, and HbO₂ and ET expression levels were independent risk factors for the prognosis of SAH patients ($p < 0.05$).

Conclusions: Astrocyte damage in SAH patients was positively correlated with the content of ACKR3, Cx43, HbO₂, and ET in cerebrospinal fluid. These indicators increased significantly with the increasing severity of the disease, and had certain value in reflecting the patient's condition. Astrocyte damage combined with cerebrospinal fluid markers had potential value in evaluating the severity and prognosis of patients.

Key words: subarachnoid hemorrhage, cerebrospinal fluid, ACKR3, Cx43, astrocyte damage, relevance.

Introduction

Subarachnoid hemorrhage (SAH) is a clinically common neurological emergency caused mainly by rupture of cerebral aneurysms, and is also the main cause of sudden death in patients [19]. The main manifesta-

tions of SAH are severe headache, nausea, vomiting, and meningeal irritation signs, with or without focal physical signs, among which recurrent hemorrhage, cerebral vasospasm, and hydrocephalus are common complications. According to relevant statistics, SAH can

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Received: 30.03.2023, Accepted: 28.08.2023, Online publication: 13.11.2023

account for about 10% of acute stroke, and about 80% of SAH is caused by rupture of intracranial aneurysm [4]. The pathogenesis of SAH is relatively complex, and the onset is relatively rapid, with high mortality and disability rates, seriously affecting the lives of patients. In recent years, with the rapid development of transportation and increasing social pressure, the incidence rate of SAH has been rising.

According to research findings [12], atypical chemokine receptor 3 (ACKR3, also known as CXC chemokine receptor 7) is overexpressed in many malignant tumors, such as breast cancer, and participates in regulating the proliferation and migration of tumor cells, thus promoting disease progression and metastasis. Research has found that ACKR3 can promote the regeneration of the myelin sheath in the human central nervous system [21]. Connexin 43 (Cx43) is the most abundant gap junction protein in the central nervous system, which can maintain the network stability of astrocytes, thereby affecting the development of oligodendrocytes and participating in the pathological and injury processes of the central nervous system. Previous studies have suggested that [9,17]. Subarachnoid hemorrhage can damage the neurological system, resulting in motor and cognitive dysfunction. With the continuous deepening of disease research, it is gradually being revealed that astrocytes are important for the development and maintenance of the blood brain barrier, brain homeostasis, structural support, cerebral blood flow control, and the secretion of neuroprotective factors. In addition to the nutritional support function, astrocytes can also regulate the information transmission of chemical synapses between neurons and regulate the vasodilation contraction coupling in the brain [22]. According to research results [7], vasoconstriction after intracerebral hemorrhage is related to multiple factors such as the endothelial system, oxygenated hemoglobin (HbO₂), and the protein kinase C (PKC) pathway. Brain vascular endothelial cells produce endothelin (ET), which can cause vasoconstriction, activate PKC, cause vasospasm, and exacerbate ischemia. Currently, the clinical relationship between astrocyte damage and

the expression of ACKR3, Cx43, HbO₂, and ET in cerebrospinal fluid, as well as the prognosis, is unclear. Based on the above related data, this study speculates that the expression of ACKR3, Cx43, HbO₂ and ET in the cerebrospinal fluid of patients with SAH is related to astrocyte damage and prognosis.

In this study, SAH patients were selected as the research objects to explore the relationship between astrocyte damage and the expression of ACKR3, Cx43, HbO₂, and ET in cerebrospinal fluid and prognosis.

Material and methods

General materials

A total of 168 SAH patients diagnosed and treated in the Emergency Department of our hospital during October 2019 to February 2022 were randomly picked as the study subjects. The selection process is shown in Figure 1. Among them, there were 101 males and 67 females, aged from 29 to 75 years, with an average age of 50.37 ± 8.32 years. According to the Hunt-Hess classification, patients were assigned to the low-level group with 78 patients and the high-level group with 90 patients. Inclusion criteria: (1) all patients met the clinical criteria for diagnosis and treatment of SAH [11], confirmed by imaging examination; (2) the onset time did not exceed 3 days; (3) all patients gave informed consent, signed an informed consent form and actively cooperated with the study; (4) the clinical and pathological data of the patient were complete and compatible with the research; (5) all patients were diagnosed for the first time. Exclusion criteria: (1) patients with rheumatoid arthritis, liver cirrhosis, and other diseases; (2) patients with serious impairment of cardiac function; (3) patients with severe mental dysfunction and unable to cooperate with the study; (4) patients with cerebral hemorrhage, cerebral infarction, meningitis, spinal cord disease, central nervous system infection, and peripheral neuropathy; (5) SAH patients due to head trauma. This study was approved by the Ethics Committee of our hospital (Approval number: LWFB20230201). This study followed the ethical guidelines, including respect for autonomy, non-harm, advantage, and justice.

Hunt-Hess classification

The severity of the patient's condition was evaluated by the Hunt-Hess classification [13], and the patients were graded as 0 to V. Among them, no bleeding was considered as level 0; Grade I referred to no obvious symptoms or only slight headache; Grade II referred to moderate to severe headache, meningeal irritation sign, and even cranial nerve palsy; patients with drowsiness, confusion, or mild focal neurological signs were classified as Grade III; patients with coma,

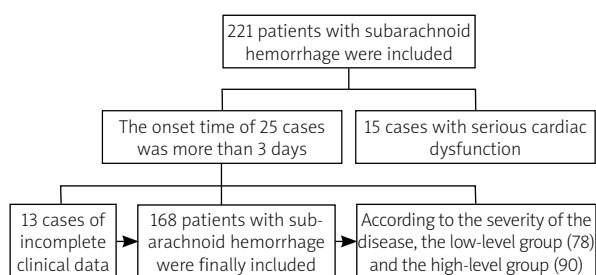


Fig. 1. The selection process of subarachnoid hemorrhage (SAH) patients.

moderate to severe hemiplegia, or early denervation, ankylosis, or autonomic dysfunction were classified as Grade IV; the patients were classified as Grade V when they fell into a deep coma, exhibited decerebrate rigidity, or even were in a terminal situation. Levels 0 to II were classified as low levels, and levels III to V were classified as high levels.

EDSS scores

The Extended Disability Status Scale (EDSS) [3] score was employed to evaluate the astrocyte damage, including 10 items, 10 points in total. Among them, with the patient's normal state and no relevant neurological signs, 0 points were recorded; a score of 1 point was given if the patient had no disability and only had mild abnormalities found during physical examination; if there was slight disability, but only one functional system was involved, 2 points would be recorded; a moderate disability with the ability to walk independently but involving one functional system was scored as 3 points; a score of 4 points was given for a person who was able to walk more than 500 meters independently but had a severe disability that involved one functional system; 5 points for being able to walk independently for more than 200 meters, but having had an impact on normal work; to be able to walk for 100 meters with the help of auxiliary tools, but needing to rest half way, was recorded as 6 points; if the walking distance was less than 5 m with the help of auxiliary tools, but a wheelchair could be used independently, score 7 points; the range of movement was limited to wheelchairs, beds, and other places, with normal upper limb functions, but the patient could only move with help, which was recorded as 8 points; a score of 9 points was given if the patient was able to eat and speak normally, but had no upper limb function, and was bedridden; a patient who died of multiple sclerosis was scored 10 points. The higher the score was, the more severe was the astrocyte damage in the patient.

Outcome measures

The patient was placed in a lateral position and subjected to local anesthesia. We performed lumbar puncture on the patient and entered the subarachnoid space within the spinal canal to extract 3 ml of cerebrospinal fluid. The cerebrospinal fluid was centrifuged at a speed of 4500 r/min to remove the supernatant.

Detection of expression of ACKR3 and Cx43: The immunohistochemical (IHC) method [10] was used to detect the expression of ACKR3 and Cx43 in patients' cerebrospinal fluid monocytes. 1 ml of cerebrospinal fluid was centrifuged to conduct IHC staining. The slide with cells was fixed in neutral formaldehyde (purchased

from Shanghai Yuanye Biotechnology Co., Ltd., Songjiang District, Shanghai, China) for 10 min, then washed with distilled water, soaked in 0.1 M phosphate-buffered saline (PBS) for 5 min, and incubated in 3% H₂O₂ deionized water for 10 min to eliminate endogenous peroxidase activity. The slides were sealed with goat serum working solution (purchased from Beijing Bio Labbo Technology Co., Ltd., Haidian District, Beijing, China), incubated at room temperature for 10-15 minutes, and then poured out. One slide was incubated with appropriately diluted monoclonal mouse anti human ACKR3 antibody (purchased from Abcam Company, United States), and the other slide was dripped with Cx43 antibody (purchased from Abcam, United States). PBS buffer (purchased from Shanghai Guangrui Biotechnology Co., Ltd., Fengxian District, Shanghai, China) was used as a negative control instead of the primary antibody. The slides were incubated at 37°C for 3 hours and washed with PBS three times for 3 minutes each time. The slides were incubated with biotinylated secondary antibody working solution (purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Daxing District, Beijing, China) at room temperature for 10-15 minutes, and then washed with PBS three times. The slides were incubated with horseradish enzyme labeled chain enzyme ovalbumin working solution at room temperature or 37°C for 10-15 minutes, rinsed with PBS three times, washed with DAB (purchased from Wuhan Etillet Biotechnology Co., Ltd., East Lake New Technology Development Zone, Wuhan, China) for 5 minutes, and washed with flowing water for 10 minutes. The slides were stained with hematoxylin for 10 minutes, and then the floating color was washed with tap water. The cerebrospinal fluid images were observed under an optical microscope (purchased from Jinan Like Medical Equipment Co., Ltd., Tianqiao District, Jinan City, Shandong Province, China) and the results were judged on a text analyzer. A monocyte with a brown cytoplasm was positive, while a monocyte with a colorless nucleus was negative.

Detection of HbO₂ and ET content: Colorimetric assay and radioimmunoassay were used to detect the content of oxygenated hemoglobin (HbO₂) and endothelin (ET), respectively, in the cerebrospinal fluid of patients. The kit was purchased from Shanghai Xinyu Biotechnology Co., Ltd. (Songjiang District, Shanghai, China).

Correlation analysis: Pearson's correlation test was adopted to analyze the relationship between the levels of ACKR3, Cx43, HbO₂, and ET in cerebrospinal fluid and EDSS scores in SAH patients.

Prognosis influencing factors: Follow-up was 6 months, and all 168 patients completed 6 months follow-up. 168 patients were assigned to the good prog-

nosis group (4 points \leq GOS score \leq 5 points, $n = 112$ cases) or poor prognosis group (1 point \leq GOS score \leq 3 points, $n = 56$ cases) according to the Glasgow Outcome Score (GOS). The age, gender, education level (junior high school, senior high school/junior college, university and above), smoking history, drinking history, diabetes history, hypertension history, onset to hospital time, consciousness level, blood pressure, whether there was an epileptic seizure, Hunt-Hess classification, rebleeding, cerebral vasospasm, emotional control, and EDSS score were collected.

Statistical analysis

According to different observation indicators and data, the SPSS 21.0 statistics software package was used for calculation. The enumeration data were expressed as cases (%), and compared using the χ^2 test. The measurement data were all tested for normal distribution, and they all conformed to normal distribution. The measurement data were expressed in the form of $\bar{x} \pm s$ and a t -test was used between two groups. Pearson correlation analysis was used for correlation testing; logistic regression analysis of multiple factors affecting the prognosis of SAH patients was performed; the difference was statistically significant at $p < 0.05$.

Results

Expression of ACKR3 and Cx43 in cerebrospinal fluid of patients in different conditions

As shown in Figure 2, ACKR3 was mainly expressed in the cytoplasm of cerebrospinal fluid monocytes, and Cx43 was mainly expressed in the cell membrane and cytoplasm. As shown in Table I, the expression rate of ACKR3-positive cells in cerebrospinal fluid ($84.85 \pm 5.73\%$) in the high-grade group was significantly higher than that in the low-grade group ($47.18 \pm 3.25\%$) ($p < 0.05$); the expression rate of Cx43-positive cells in the high-grade group ($89.14 \pm 20.72\%$) was significantly higher than that in the low-grade group ($47.18 \pm 3.25\%$) ($p < 0.05$).

Levels of HbO₂ and ET in cerebrospinal fluid of patients with different conditions

The content of HbO₂ in cerebrospinal fluid in the high-level group (1.58 ± 0.28 mmol/l) was obviously higher than that in the low-level group (1.00 ± 0.24 mmol/l), and the content of ET in cerebrospinal fluid in the high-level group (217.10 ± 30.31 ng/l) was also obviously higher than that in the low-level group (89.14 ± 20.72 ng/l) ($p < 0.05$, Table II and Fig. 3).

Changes in EDSS scores in patients with different conditions

Patients in the high-level group (6.64 ± 1.23) had a much higher EDSS score than patients in the low-level group (2.46 ± 0.58) ($p < 0.05$, Table III).

Relationship between levels of ACKR3, Cx43, HbO₂, and ET in cerebrospinal fluid and EDSS scores in patients with SAH

Pearson correlation analysis showed that the content of ACKR3, Cx43, HbO₂, ET in cerebrospinal fluid of SAH patients was positively correlated with EDSS scores ($r = 0.635, 0.723, 0.691$ and 0.837 , $p < 0.05$; Table IV and Fig. 4).

Univariate analysis of prognostic factors in SAH patients

The clinical data of patients with good prognosis and those with poor prognosis showed statistically significant differences in age, educational level, smoking history, hypertension history, systolic blood pressure, epileptic seizures, Hunt-Hess grade, rebleeding, cerebral vasospasm, emotional control, and EDSS scores ($p < 0.05$, Table V).

Multivariate logistic regression analysis of prognosis in SAH patients

The statistically significant indicators in Table V were included in the multivariate logistic regression analysis. The results shown in Table VI indicate that systolic blood pressure, Hunt-Hess classification, rebleeding, emotional control, EDSS score, ACKR3, Cx43 positive cell rate, and HbO₂ and ET expression levels were independent risk factors for the prognosis of SAH patients ($p < 0.05$).

Discussion

Subarachnoid hemorrhage is a medical emergency. The onset of SAH is relatively rapid, with over 30% of patients dying within 48 hours of onset [5]. Astrocytes are important supports for neuronal cells and play an important role in the regulation of cerebral microcirculation and blood flow. The dysfunction of astrocytes can cause apoptosis of nerve cells, leading to the occurrence of neural dysfunction. A large number of studies have shown that [1,2] brain injury begins with the rupture of an aneurysm and develops over time. After intracerebral hemorrhage, a pseudoaneurysm can form around the rupture. Rupture of intracranial aneurysms is the most common cause of SAH, with a high mortality and

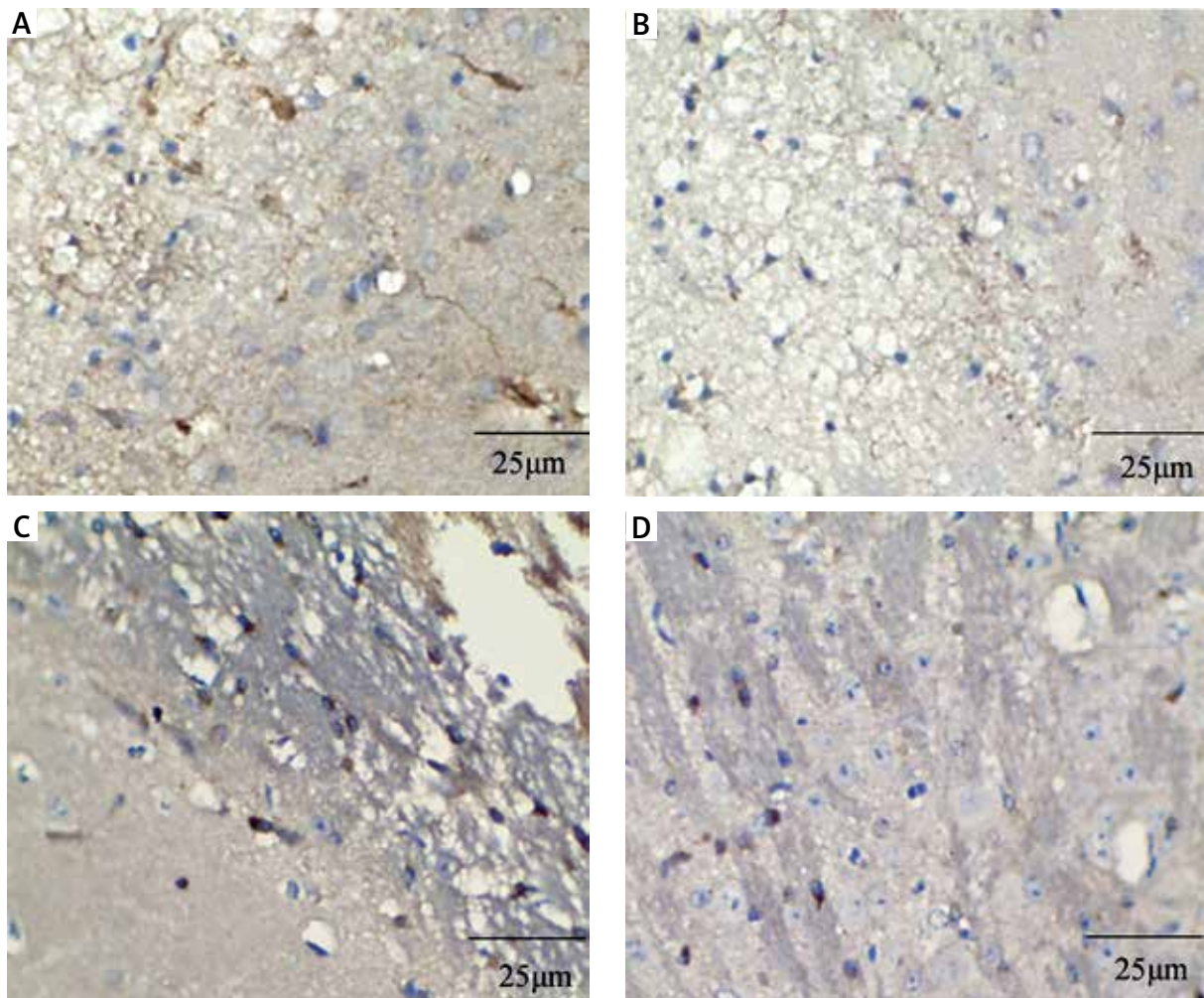


Fig. 2. Expression of ACKR3 and Cx43 in cerebrospinal fluid of patients in different condition (400×). **A)** Expression of ACKR3 in the high-level group; **B)** Expression of ACKR3 in low-level group; **C)** Expression of Cx43 in high-level group; **D)** Expression of Cx43 in low-level group.

disability rate. Some scholars have found [20] that the nature of early brain damage after SAH appears to be ischemic and closely related to survival. Early prediction of disease progression and effective individualized treatment strategies are important for preventing early death and the development of delayed ischemic injury.

ACKR3 is an atypical chemokine receptor with a seven transmembrane domain, belonging to class A G protein coupled receptors. Research has found that [8] ACKR3 can combine with CXCL12 and CXCL11, thereby participating in multiple biological processes. In addition, studies have found that [6] the expression of ACKR3 is upregulated in various pathological conditions related to inflammation, infection, or ischemia, such as inflammatory bowel disease, encephalitis, and rheumatoid arthritis. With the continuous deepening of research on ACKR3, people have gradually discovered

that altered ACKR3 expression patterns have also been detected in various cancer types [12,18]. In research, some scholars have demonstrated that CXCR7 is the main mediator of CXCL12 signal transduction in cultured astrocytes [14]. In addition, astrocytes control the integrity of synaptic plasticity, cerebral blood flow, brain energy metabolism, and neurotransmitter homeostasis in the brain. Similarly, in the diseased central nervous system, astrocytes play multiple roles, including controlling immune processes, neuronal survival and regeneration, and the permeability of the blood-brain barrier. The gap junction channel composed of gap junction proteins is the most important pathway for information transmission between adjacent cells. In the central nervous system, Cx43 is mainly expressed in astrocytes and blood vessel walls. Changes in the activity of Cx43 and the half channel and gap junction on astrocytes

Table I. Positive cell rates of ACKR3 and Cx43 in cerebrospinal fluid of patients with different conditions (%)

Groups	Cases	ACKR3	Cx43
Low-level group	78	47.18 ±3.25	32.40 ±6.02
High-level group	90	84.85 ±5.73	62.07 ±3.29
<i>t</i>		51.333	40.332
<i>p</i>		< 0.001	< 0.001

Table II. Levels of HbO₂ and ET in cerebrospinal fluid of patients with different conditions ($\bar{x} \pm s$)

Groups	Cases	HbO ₂ (mmol/l)	ET (ng/l)
Low-level group	78	1.00 ±0.24	89.14 ±20.72
High-level group	90	1.58 ±0.28	217.10 ±30.31
<i>t</i>		14.299	31.451
<i>p</i>		< 0.001	< 0.001

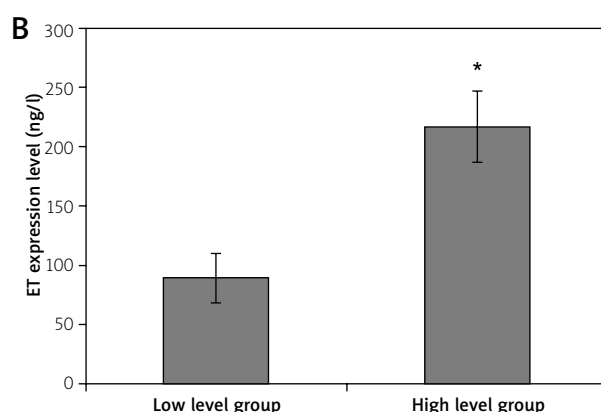
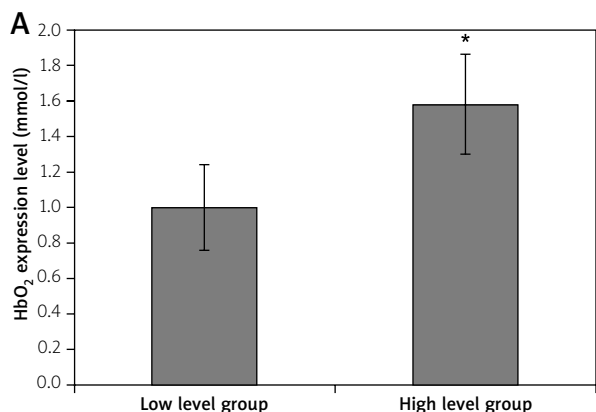


Fig. 3. Levels of HbO₂ and ET in cerebrospinal fluid of patients with different conditions. **A)** HbO₂ expression; **B)** ET expression. **P* < 0.05 compared with the low-level group.

Table III. Changes in EDSS scores in patients with different conditions ($\bar{x} \pm s$)

Groups	Cases	EDSS score
Low-level group	78	2.46 ±0.58
High-level group	90	6.64 ±1.23
<i>t</i>		27.475
<i>p</i>		< 0.001

Table IV. Relationship between levels of ACKR3, Cx43, HbO₂, and ET in cerebrospinal fluid and EDSS scores in patients with subarachnoid hemorrhage (SAH)

Indicators	EDSS score	
	<i>r</i>	<i>p</i>
ACKR3	0.635	< 0.001
Cx43	0.723	< 0.001
HbO ₂	0.691	< 0.001
ET	0.837	< 0.001

can affect the activity of neurons, which can participate in neuronal damage caused by neuroimmune responses, exacerbating cerebral ischemic damage. Hippo signaling pathway kinase LATS1/2 and transcriptional activator YAP/TAZ may be involved in this process [16]. Some studies have confirmed [22] that Cx43 might be involved in the pathological process of cerebral vasospasm after SAH. In this study, by analyzing the expression of ACKR3 and Cx43 in cerebrospinal fluid of patients with different severity of SAH, it was found that the expression of ACKR3 and Cx43 in cerebrospinal fluid strongly increased with the progression of SAH. In this study, the degree of astrocyte damage was evaluated by the EDSS score. The results showed that there was a significant correlation between the expression of ACKR3 and Cx43 in cerebrospinal fluid and the degree

of astrocyte damage, and both of them could be used as relevant indicators to judge the condition of patients.

Cerebral vasospasm is an important complication of SAH, and the two important candidates for causing cerebral arterial spasm are the red blood cell product HbO₂ and the vasoconstrictor ET. Some scholars have found [15] that the combination of HbO₂ and ET activates diffusion neurons in the cerebral cortex *in vivo*, and suggests the nitric oxide scavenging function of HbO₂. When the availability of nitric oxide decreases, the threshold concentration of ET induced cerebral ischemia significantly decreases through the complex interaction between the neuronal astrocyte network

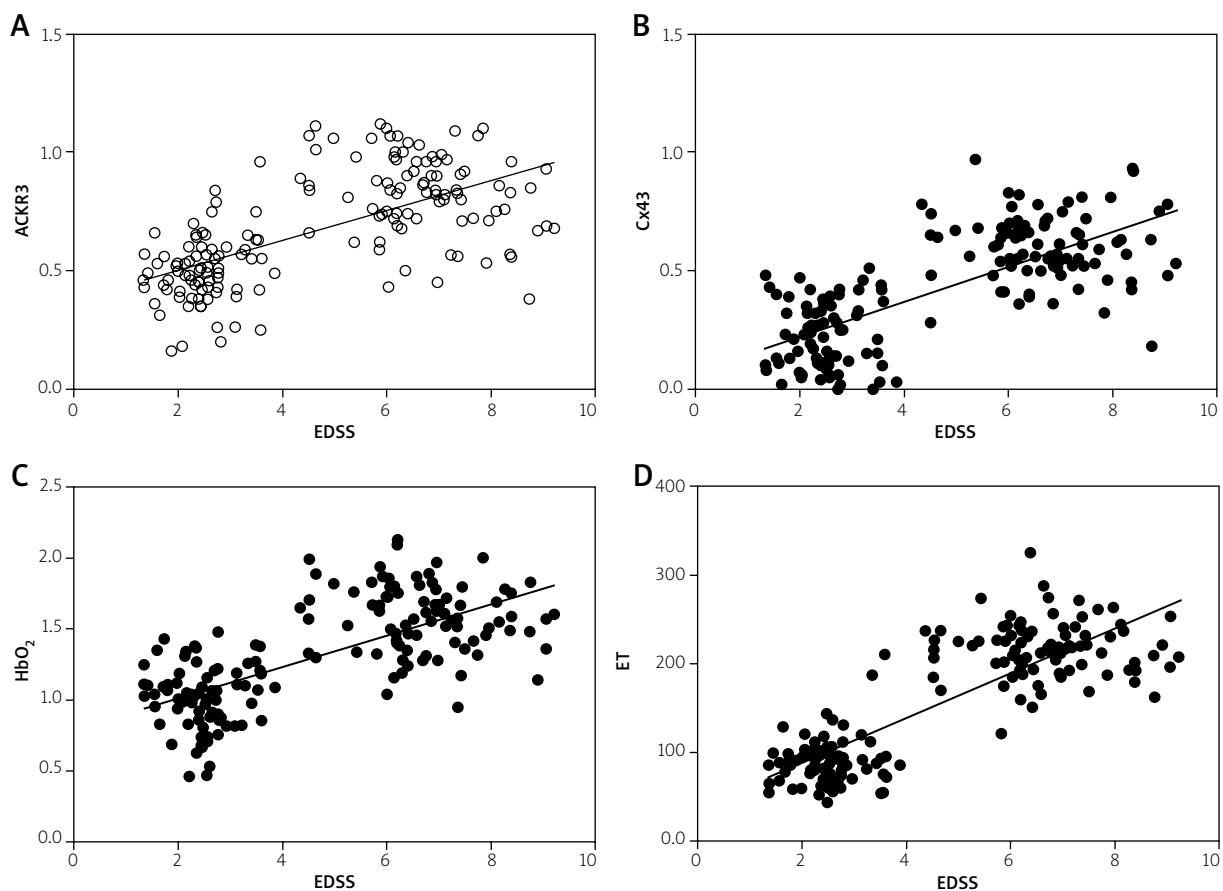


Fig. 4. Correlation analysis chart. **A)** Correlation between ACKR3 and EDSS; **B)** Correlation between Cx43 and EDSS; **C)** Correlation between HbO₂ and EDSS; **D)** Correlation between ET and EDSS.

and the cortical microcirculation. In this study, by analyzing the expression of HbO₂ and ET in cerebrospinal fluid of SAH patients with different severity of the condition, it was found that the expression of HbO₂ and ET in cerebrospinal fluid strongly increased with the progress of SAH. In addition, by analyzing the relationship between HbO₂ and ET levels and EDSS scores, the results showed that the expression of HbO₂ and ET in cerebrospinal fluid was closely related to EDSS scores, which was significant for judging astrocyte damage. In addition, the results of this study showed that systolic blood pressure, Hunt-Hess grade, rebleeding, emotional control, EDSS score, positive cell rates of ACKR3, Cx43, and HbO₂ and ET expression levels were independent risk factors for the prognosis of SAH patients. It can be seen that astrocyte damage combined with cerebrospinal fluid markers can be used as an indicator for evaluating the severity and prognosis of SAH. By comprehensively understanding the degree of brain tissue damage after SAH and providing information for assessing prognosis and guiding clinical treatment, it can make up for the shortcomings of individual

clinical characteristic indicators in this regard, enable patients to benefit from early treatment, reduce mortality in patients with aneurysmal SAH, and improve prognosis.

In general, astrocyte damage in SAH patients was positively correlated with the content of ACKR3, Cx43, HbO₂, and ET in cerebrospinal fluid. These indicators were significantly increased with the severity of the disease, and had certain value in reflecting the patient's condition. Astrocyte damage combined with cerebrospinal fluid markers had potential value in evaluating the severity and prognosis of patients.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This research received no external funding.

Table V. Univariate analysis of prognostic factors in subarachnoid hemorrhage (SAH) patients [cases (%)] ($\bar{x} \pm s$)

Related factors		Good prognosis group (n = 112)	Poor prognosis group (n = 56)	χ^2/t	P
Age	< 60	93 (83.04)	39 (69.64)	3.977	0.046
	\geq 60	19 (16.96)	17 (30.36)		
Gender	Male	63 (56.25)	38 (67.86)	2.098	0.148
	Female	49 (43.75)	18 (32.14)		
Educational level	Junior high school	57 (50.89)	40 (71.43)	6.455	0.040
	Senior high school/ junior college	27 (24.11)	8 (14.29)		
	University and above	28 (25.00)	8 (14.29)		
Smoking history	Yes	68 (60.71)	44 (78.57)	5.357	0.021
	No	44 (39.29)	12 (21.43)		
Drinking history	yes	105 (93.75)	56 (100.00)	3.652	0.056
	No	7 (6.25)	0 (0.00)		
Diabetes history	Yes	46 (41.07)	27 (48.21)	0.775	0.379
	No	66 (58.93)	29 (51.79)		
Hypertension history	Yes	57 (50.89)	40 (71.43)	6.452	0.011
	No	55 (49.11)	16 (28.57)		
Onset to hospital time	< 12 h	99 (88.39)	45 (80.36)	1.969	0.161
	\geq 12 h	13 (11.61)	11 (19.64)		
Consciousness level	Unconscious disorder	76 (67.86)	33 (58.93)	1.306	0.253
	Conscious disorder	36 (32.14)	23 (41.07)		
Blood pressure (mmHg)	Diastolic pressure	85.42 \pm 18.26	88.21 \pm 19.45	0.913	0.362
	Systolic pressure	142.59 \pm 21.22	155.26 \pm 28.73	3.230	0.002
Epileptic seizure	Yes	4 (3.57)	9 (16.07)	8.171	0.004
	No	108 (96.43)	47 (83.93)		
Meningeal irritation sign	Yes	110 (98.21)	53 (94.64)	1.649	0.199
	No	2 (1.79)	3 (5.36)		
Hunt-Hess classification	Grade 0-II	108 (96.43)	15 (26.79)	92.332	< 0.001
	Grade III-V	4 (3.57)	41 (73.21)		
Rebleeding	Yes	10 (8.93)	15 (26.79)	9.400	0.002
	No	102 (91.07)	41 (73.21)		
Cerebral vasospasm	Yes	13 (11.61)	16 (28.57)	7.523	0.006
	No	99 (88.39)	40 (71.43)		
Emotional control	Good	109 (97.32)	17 (30.36)	89.286	< 0.001
	Poor	3 (2.68)	39 (69.64)		
EDSS score		2.69 \pm 0.78	6.33 \pm 1.85	17.918	< 0.001
ACKR3 (%)		42.33 \pm 5.48	78.95 \pm 10.49	29.757	< 0.001
Cx43 (%)		30.15 \pm 6.48	65.88 \pm 9.52	28.640	< 0.001
HbO ₂ (mmol/l)		1.00 \pm 0.15	1.62 \pm 0.45	13.218	< 0.001
ET (ng/l)		75.24 \pm 21.33	205.26 \pm 29.49	32.641	< 0.001

Table VI. Multivariate logistic regression analysis of prognosis in subarachnoid hemorrhage (SAH) patients

Factors	B	SE	Wald	P	OR	95% CI
Age	0.542	0.298	3.574	0.058	1.687	0.952~2.949
Educational level	0.271	0.233	1.342	0.248	1.276	0.815~2.012
Smoking history	0.357	0.086	1.551	0.558	2.613	1.225~3.269
Hypertension history	0.545	0.914	2.042	0.085	1.614	0.897~1.996
Systolic blood pressure	0.625	0.231	7.156	0.000	1.462	1.015~5.261
Epileptiform seizures	1.112	0.872	2.497	0.069	2.548	1.158~2.785
Hunt-Hess classification	1.512	0.283	9.082	0.000	4.521	1.721~15.369
Rebleeding	1.281	0.186	6.692	0.000	3.871	2.135~6.748
Cerebral vasospasm	0.122	0.875	1.698	0.078	1.524	0.974~2.331
Emotional control	2.212	0.415	9.055	0.000	9.057	2.415~11.621
EDSS score	0.789	0.385	4.828	0.000	4.281	1.132~12.358
ACKR3 (%)	0.033	0.005	6.079	< 0.001	1.032	1.025~2.352
Cx43 (%)	0.998	0.161	8.485	< 0.001	2.710	1.110~5.421
HbO ₂ (mmol/l)	0.667	0.249	7.156	0.007	1.949	1.126~2.48
ET (ng/l)	0.015	0.006	6.372	0.011	1.015	1.001~1.698

Disclosures

This study was approved by The Ethics Committee of Xi'an Gaoxin Hospital (Approval number: LWFB-20230201).

The authors report no conflict of interest.

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