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# Clinical significance of FSTL 1, Bax, Bcl-2 in acute cerebral infarction and its relationship with hemorrhagic transformation

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**Abstract. – OBJECTIVE: Acute cerebral infarction (ACI) is the most common type of acute cerebrovascular disease so far, and its incidence rate has been increasing in recent years. At present, the methods of diagnosing ACI in clinic are extremely complicated, and an effective index that can effectively diagnose ACI is urgently needed in clinic. This study is designed to investigate the clinical significance of Follistatin-like protein 1 (FSTL1), Bax and Bcl-2 in ACI.**

**PATIENTS AND METHODS: A total of 84 cases of ACI patients admitted to our hospital from September 2017 to September 2019 and 90 cases of healthy subjects undergoing physical examination at the same time were selected as the research objects for prospective analysis. The concentrations of FSTL1, Bax and Bcl-2 in the peripheral blood of objects in the two groups were detected to analyze the diagnostic value of FSTL1, Bax and Bcl-2 for ACI, and the correlation of FSTL 1, Bax and Bcl-2 with the infarct size, treatment method and hemorrhagic transformation. Another 20 SD rats were purchased, among which 10 rats were randomly selected for ACI modeling. FSTL1 concentration, Bax and Bcl-2 protein expression in brain tissues of ACI rats and normal rats were detected.**

**RESULTS: FSTL1 and Bax in peripheral blood of ACI patients were higher than those of healthy subjects ($p$<0.050), and Bcl-2 was lower than those of healthy subjects ($p$<0.050). It was detected that FSTL1, Bax and Bcl-2 had good diagnostic value for patients with ACI ($p$<0.001). FSTL1 and Bax decreased while Bcl-2 increased in patients treated with thrombolytic therapy ($p$<0.050). And FSTL1, Bax and Bcl-2 were closely related to infarct size and hemorrhagic transformation ($p$<0.050). Logistic regression analysis showed that NIHSS score, atrial fibrillation, infarct volume, FSTL1 and Bax were independent risk factors affecting hemorrhagic transformation in ACI patients ($p$<0.050), and Bcl-2 was an independent protective factor affecting hemorrhagic transformation in ACI patients ($p$<0.050). The concentration of FSTL1 and the expression of Bax protein in rat brain tissue were also higher than that in normal rats, while Bcl-2 was lower than that in normal rats ($p$ < 0.001).**

**CONCLUSIONS: FSTL1, Bax and Bcl-2 are involved in the occurrence and development of ACI and are closely related to the hemorrhagic transformation of patients. The mechanism by which FSTL1 promotes the occurrence of ACI might be related to promoting the occurrence of inflammatory responses in the brain tissue of patients or accelerating the apoptosis of neurons.**

*Key Words:*
FSTL1, Bax, Bcl-2, ACI, Hemorrhagic transformation.

## Introduction

Acute cerebral infarction (ACI), or ischemic stroke, is the most common type of acute cerebrovascular disease at present[1]. In recent years, continuous studies have indicated that the incidence of ACI is increasing year by year[2]. Moreover, the middle-aged and elderly, the original high-risk population of ACI, also showed a younger trend[3]. The onset of ACI is extremely fast. Once the rescue is not timely, it may cause permanent damage to the patient's neurological function, resulting in disability and paralysis. In severe cases, it directly causes insufficient blood supply to the brain and causes death[4,5]. At present, the diagnosis of ACI is complicated in clinic, which requires not only a series of radiological technologies such as CT, MRI, carotid artery ultrasound, transcranial Doppler, digital subtraction angiography, but also routine bio-

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chemical indexes examination. For the selection of treatment, cerebral perfusion examination and brain function evaluation may even be required[6]. This is not only not conducive to the rapid treatment of ACI patients, but also has great disadvantages in evaluating the rehabilitation of patients. Therefore, an effective index that can effectively reflect ACI is urgently needed in clinic.

Follistatin-like protein 1 (FSTL1) is an extracellular glycoprotein expressed in multiple tissues and organs of most mammals[7]. It has been proved to have extremely important biological functions such as regulating cell proliferation, promoting endothelial cell function and vascular regeneration[8]. In recent years, some studies have shown that FSTL1 is bound up with cardiovascular diseases and inflammatory diseases[9,10], but no study has confirmed its status in ACI. As two common apoptotic protein tissues in clinic, Bax and Bcl-2 have been confirmed to be closely related to neuronal apoptosis in many studies[11]. However, the team of Xu et al[12] proposed that FSTL1 could reduce Bcl-2 and improve Bax, thus promoting chondrocyte apoptosis.

Therefore, we suspected that FSTL1 was also abnormally expressed in ACI, and its pathway of action might be related to Bax and Bcl-2. In order to verify our ideas, this experiment would provide a reliable reference for future clinical diagnosis and treatment of ACI by exploring the clinical significance of FSTL1, Bax and Bcl-2 in ACI.

## Patients and Methods

### General Data

#### Patient Data

A total of 84 cases of ACI patients admitted to our hospital from September 2017 to September 2019 and 90 cases of healthy subjects undergoing physical examination at the same time were selected as the research objects for prospective analysis. ACI patients were enrolled in the study group, including 26 cases of minor infarction (infarction area < 5 $cm^3$), 34 cases of middle infarction (infarction area 5-10 $cm^3$), and 24 cases of major infarction (infarction area > 10 $cm^3$). There were 52 patients with thrombolytic therapy and 32 patients without thrombolytic therapy, and 36 patients with hemorrhagic transformation (CT or MRI reexamination found hemorrhage in the original infarction area or distant part of infarction) and 48 patients without hemorrhagic transformation. The healthy subjects were enrolled in the control group. There was no statistical difference between the two groups in clinical baseline data such as age, gender, body mass index (BMI), etc. ($p > 0.050$). This study was conducted according to Helsinki Declaration of the World Medical Association. Written consent was obtained from all enrolled patients, and the study was approved by the Ethics Com-mittee of our hospital.

#### Inclusion and Exclusion Criteria

Inclusion criteria: patients whose ACI was confirmed by imageological examination in our hospital. Patients admitted to hospital within 24 hours of onset. Patients with complete case data. Patients between 30 to 70 years old. Exclusion criteria: patients complicated with tumor, other cardiovascular and cerebrovascular diseases, other autoimmune diseases or infectious diseases, hepatic or renal insufficiency. Patients with drug allergy. Patients who received other antibiotic drugs within 3 months before admission. Patients in gestation period or lactation period. Patients with low treatment compliance or mental disorders. Patients transferred to other hospitals. Patients whose expected survival time was less than 1 month.

#### Animal Data

Twenty male Sprague Dawley (SD) rats, aged 6-8 weeks, weighing 200-250 g, were purchased from the experimental animal center of Chongqing National Biological Industry Base, batch number: SCXK20170125.

### Methods

#### Therapies

After relevant tests, rt-PA intravenous thrombolysis was performed for those who met thrombolytic indications after evaluating the neurologic impairment (NIHSS) score. Twenty-four hours before and after thrombolysis, the vital signs of patients were strictly detected, and the level of consciousness and degree of nerve defect were observed. CT reexamination was performed 24 hours after thrombolysis, and antiplatelet aggregation drugs were given if there was no hemorrhage. Craniocerebral MRI was performed within 3 days after the condition was stable, and symp-

tomatic treatment was given. For those who did not meet thrombolytic indications, antiplatelet aggregation therapy was given in combination with examination results of hemagglutination and blood analysis. Craniocerebral MRI examination was completed in 3 days, and blood glucose adjustment, dehydration and neuroprotective agents were given during treatment.

### Sample Collection

Fasting venous blood was collected from ACI patients at admission (1d), 3 days after admission (3d), and 7 days after admission (7d). Fasting venous blood was collected from the control group in the morning. The blood was placed at room temperature for 30 min, then centrifuged for 10 min (400×g) to obtain the upper serum for subsequent detection.

### Modeling Method

Ten rats were selected for ACI modeling by random number table method, which was considered as the model group. The other 10 rats were raised normally without treatment and served as the normal group. Modeling method: after intraperitoneal injection of chloral hydrate (40 mg/100 g) into anesthetized rats, they were placed at the supine position. The rectal temperature was stabilized at 37.5°C. The skin was incised along the midline of the neck to expose the right common carotid artery. The silicone rubber tube was introduced into the external carotid artery and gently pushed into the middle cerebral artery through the internal carotid artery at about 17-20 nm. When slight resistance was felt, the middle cerebral artery was blocked by sutures. After 2 hours of infarction, the silicone rubber tube was withdrawn, the incision was sutured and sterilized. NIHSS score was performed 24 hours later.

## Detection Methods

### Detection of Human Samples

Enzyme linked immunosorbent assay (ELISA) was used to detect FSTL1, Bax and Bcl-2 in serum, the kit was purchased from Shanghai Yubo Biotechnology Co., Ltd. (KT-1277), Shanghai Hengfei Biotechnology Co., Ltd. (SEB343MU-1), Shanghai Jingkang Bioengineering Co., Ltd. [JK-(a)-5318]. The detection process was strictly carried out in sterile environment according to the kit instructions.

### Detection of Animal Samples

After 48 hours of modeling, all rats were killed by dislocation under anesthesia to obtain their brain tissue. The blood clots and pia mater were removed, and then they were fully ground and divided into two parts. The concentration of FSTL1 of one part in brain tissue was determined by enzyme-linked immunosorbent assay (ELISA). The kit was purchased from Shanghai Fusheng Industrial Co., Ltd., FSE8030. After a portion of 3 mL/g protein lysate was fully lysed in another part, the expression of Bcl-2 and Bax proteins was detected by Western Blotting (WB). After centrifugation for 10 min (1500×g), the supernatant was obtained. Bicinchoninic acid (BCA) was used to detect total protein concentration. Denature was conducted at 100°C for 10 min, then polyacrylamide gel was transferred to polyvinylidene difluoride (PVDF) membrane after polyacrylamide gel electrophoresis. After washing, it was sealed in 5% defatted milk and placed in room temperature for 1 h, added with Bax and Bcl-2 primary antibody to incubate overnight at 4°C. After washing for 3 times, Bcl-2 and Bax secondary antibody were added, placing at 37°C for 1 h. Enhanced chemiluminescence (ECL) was applied to developed after washing, and the gray value of grayscale value of the bands was measured and analyzed by Quantity One software.

### Outcome Measures

Main outcome measures: the concentrations of FSTL1, Bcl-2 and Bax in the peripheral of the study group and the control group, and their predictive values for ACI occurrence. Changes of FSTL1, Bcl-2 and Bax at different time points and their relationship with ACI clinical pathology. The correlation of FSTL1, Bcl-2 and Bax with rebleeding after thrombolysis. Secondary indicators: FSTL1, Bcl-2, Bax expression in rat brain tissue.

### Statistical Analysis

SPSS 22.0 (IBM, Armonk, NY, USA) statistical software was applied to analyze and process the data, and Graphpad software was used to graph the data. Counting data were expressed in the form of (rate), and chi-square test was used for comparison between groups. The measurement data were expressed in the form of (mean±SD). One-way ANOVA and LSD back testing were used for comparison among groups. Repeated measurement analysis of variance and Bonferroni back testing were used for multi-time compar-

ison. The diagnostic predictive value was analyzed by ROC curve. Logistic regression analysis was used for risk factors. $p<0.050$ was considered statistically significant.

## Results

### Comparison of FSTL1, Bax and Bcl-2 Between Study Group and Control Group

The concentration of FSTL1 and Bax in the peripheral blood of the study group was higher than those of the control group ($p<0.050$), with the lowest concentration at 1d, increasing trend at 3d and the highest concentration at 7d ($p<0.050$). However, Bcl-2 was lower than that of the control group ($p<0.050$), with the highest level at 1d, decreasing trend at 3d and the lowest level at 7 d ($p<0.050$), as shown in Figure 1.

### Diagnostic Value of FSTL1, Bax and Bcl-2 in ACI

ROC analysis was carried out on the detection results of FSTL1, Bax and Bcl-2 of patients in the study group on the 1st day after admission. The results revealed that the diagnostic sensitivity of FSTL1 concentration in peripheral blood for the diagnosis of ACI was 79.76%, and the specificity was 61.11% ($p < 0.001$). However, the diagnostic sensitivity and specificity of Bax for ACI were 75.00% and 80.00%, respectively ($p < 0.001$). The diagnostic sensitivity and specificity of Bcl-2 for ACI in patients were 75.00% and 70.00%, respectively ($p < 0.001$), as shown in Table I and Figure 2.

### Comparison of FSTL1, Bax and Bcl-2 Concentrations in Patients With Different Infarct Volumes

Comparing the concentrations of FSTL1, Bax and Bcl-2 in patients with different infarct volumes, it could be seen that FSTL1 and Bax were the highest in patients with major infarction, followed by patients with middle infarction and the lowest in patients with minor infarction ($p<0.050$). However, Bcl-2 was lowest in patients with major infarction, and it was higher in patients with minor infarction than in patients with middle infarction ($p<0.050$). Both FSTL1 and Bax in infarcted patients were the lowest at 1d, and the highest at 7d ($p<0.050$), while Bcl-2 had the highest at 1d and the lowest at 7d ($p<0.050$), as shown in Figure 3.

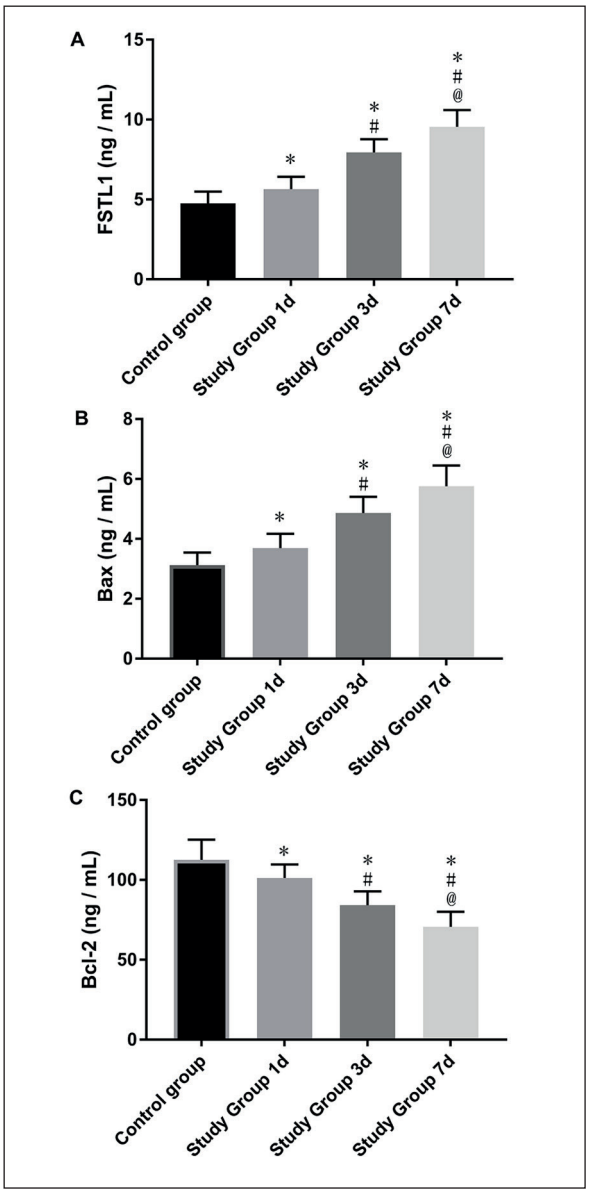


**Figure 1.** Comparison of FSTL1, Bax and Bcl-2 between study group and control group. **A**, Comparison of FSTL1 between study group and control group. **B**, Comparison of Bax between study group and control group. **C**, Comparison of Bcl-2 between study group and control group. *Means compared with the control group, *$p<0.050$. #Means compared with the study group at 1d, #$p<0.050$. @Means compared with the study group at 3d, @$p<0.050$.

### Comparison of FSTL1, Bax and Bcl-2 Concentrations Between Patients Treated With Thrombolytic Therapy and Non-Thrombolytic Therapy

Comparing the FSTL1, Bax and Bcl-2 concentrations of patients treated with thrombolytic therapy and non-thrombolytic theraphy, it

**Table I.** Diagnostic value of FSTL 1, Bax and Bcl-2 for ACI.

| | FSTL1 | Bax | Bcl-2 |
|---|---|---|---|
| Cut-off | > 5.005 ng/mL | > 3.445 ng/mL | < 106.300 ng/mL |
| AUC | 0.747 | 0.854 | 0.785 |
| Std. Error | 0.037 | 0.028 | 0.034 |
| 95% CI | 0.674-0.819 | 0.799-0.908 | 0.718-0.852 |
| Sensitivity (%) | 79.76 | 75.00 | 75.00 |
| Specificity (%) | 61.11 | 80.00 | 70.00 |
| Youden index (%) | 40.87 | 55.00 | 45.00 |
| *p* | < 0.001 | < 0.001 | < 0.001 |

could be seen that there was no difference in FSTL1, Bax and Bcl-2 between thrombolytic and non-thrombolytic patients at 1d ($p > 0.050$), while FSTL1 and Bax of thrombolytic patients were higher than non-thrombolytic patients ($p<0.050$) and Bcl-2 was lower than non-thrombolytic patients ($p<0.050$) at 3d and 7d. FSTL1 and Bax of patients treated with both methods were the lowest at 1d, increasing at 3d and the highest at 7d ($p<0.050$). However, Bcl was the highest at 1d, decreasing at 3d, and the lowest at 7d ($p<0.050$), as shown in Figure 4.

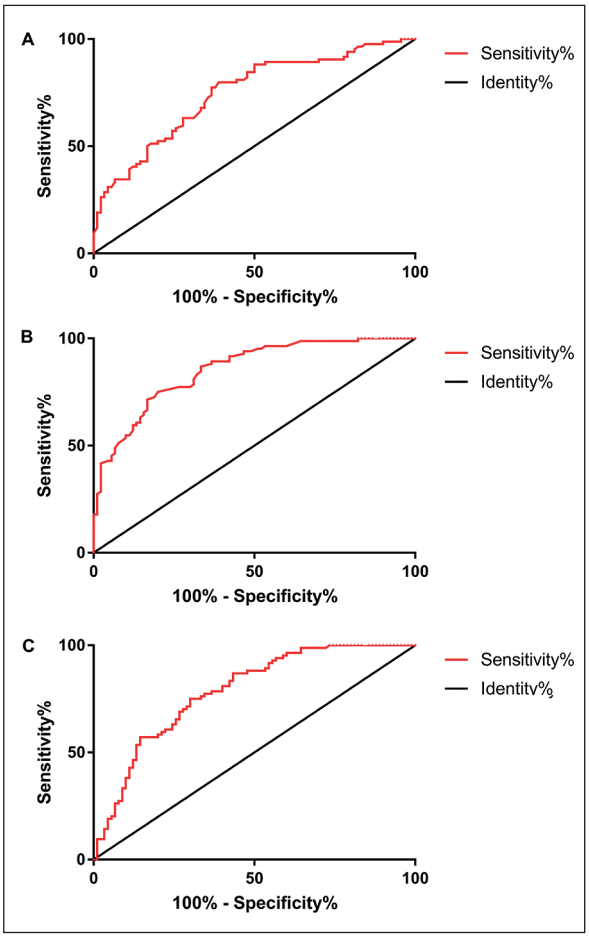


**Figure 2.** Diagnostic value of FSTL1, Bax and Bcl-2 for ACI. **A**, ROC curve of FSTL1 concentration in peripheral blood to ACI of patients. **B**, ROC curve of Bax concentration in peripheral blood to ACI of patients. **C**, ROC curve of Bcl-2 concentration in peripheral blood to ACI of patients.

### *Comparison of FSTL1, Bax and Bcl-2 Concentrations in Patients With Hemorrhagic Transformation and Patients Without Hemorrhagic Transformation*

FSTL1 and Bax in patients with hemorrhagic transformation were higher than those without hemorrhagic transformation, while Bcl-2 was lower than those without hemorrhagic transformation ($p<0.050$). Both FSTL1 and Bax in patients with hemorrhagic transformation and those without hemorrhagic transformation were the lowest at 1d, increasing at 3d and the highest at 7d ($p<0.050$). However, Bcl was highest at 1d, decreasing at 3d, and lowest at 7d ($p<0.050$), as shown in Figure 5.

### *Risk Factors Affecting Hemorrhagic Transformation in ACI Patients*

Univariate analysis showed that NIHSS score, homocysteine (Hcy), atrial fibrillation and infarct volume were the single factors affecting hemorrhagic transformation in ACI patients ($p<0.050$). Combined with the above results, the indicators with differences were assigned values, and then LR: forward was selected for Logistic regression analysis. The results revealed that Hcy was not a multi-factor influencing hemorrhagic transformation of ACI patients ($p > 0.050$). While NIHSS score, atrial fibrillation, infarct volume, FSTL1 and Bax were independent risk factors affecting hemorrhagic transformation in ACI patients ($p<0.050$), and Bcl-2 was am independent protective factor affecting hemorrhagic transformation in ACI patients ($p<0.050$), as shown in Tables II, III, and IV.

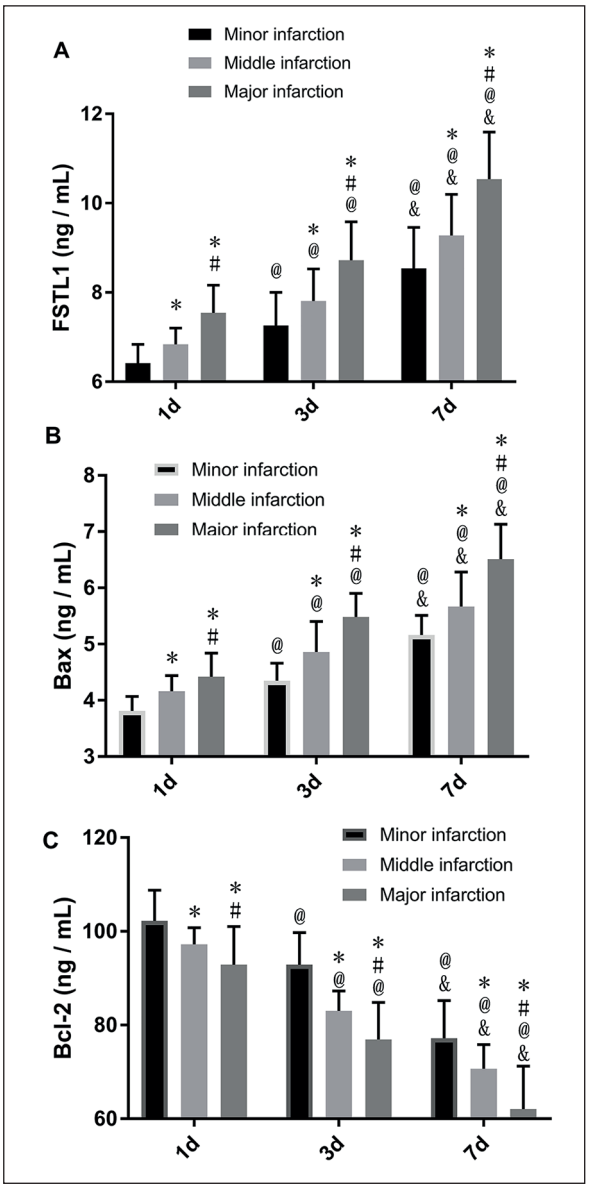


**Figure 3.** Comparison of FSTL1, Bax and Bcl-2 concentrations in different infarct volumes. **A**, Comparison of FSTL1 concentration in different infarct volumes. **B**, Comparison of Bax concentration in different infarct volumes. **C**, Comparison of Bcl-2 concentration in different infarct volumes. * represents comparison with the minor infarction during the same time, *$p$<0.050. #Represents comparison with patients with middle infarction at the same time, #$p$<0.050. @ Represents comparison patients with the same infarct size patients at 1d, @$p$<0.050. &Represents comparison with patients with the same infarct size at 3d, &$p$<0.050.

### Comparison of FSTL1, Bax and Bcl-2 in Rat Brain

Among the 10 rats that performed ACI modeling, 9 were successfully modeled, with a success rate of 90.0%. After the rats were killed, FSTL1, Bax and Bcl-2 in the brain tissue of the rats in the model group were detected, and it was found that concentrations of FSTL1 and Bax were significantly higher than those in the normal group,

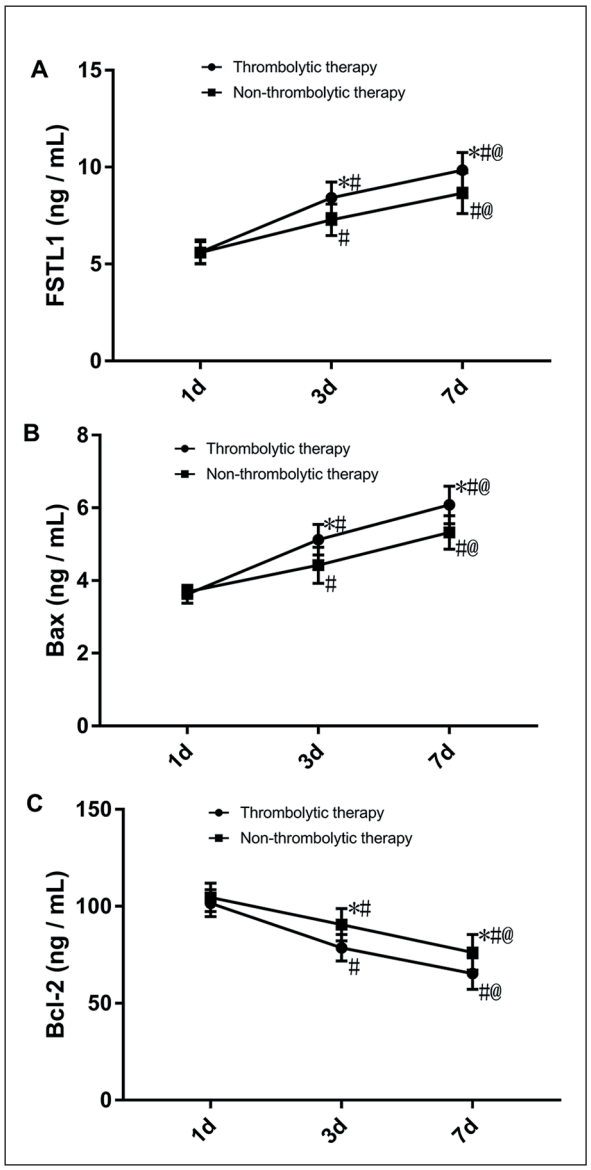


**Figure 4.** Comparison of FSTL1, Bax and Bcl-2 concentrations between patients treated with thrombolytic therapy and non-thrombolytic therapy. **A**, Comparison of FSTL1 concentration between patients receiving treatment of thrombolytic and non-thrombolytic. **B**, Comparison of Bax concentration between patients receiving treatment of thrombolytic and non-thrombolytic. **C**, Comparison of Bcl-2 concentration between patients receiving treatment of thrombolytic and non-thrombolytic patients. *Represents comparison with patients treated with non-thrombolytic therapy patients at the same time, *$p$<0.050. #Represents comparison with patients treated in the same way at 1 d, #$p$<0.050. @Represents comparison with patients treated in the same way in 3 d, @$p$<0.050.

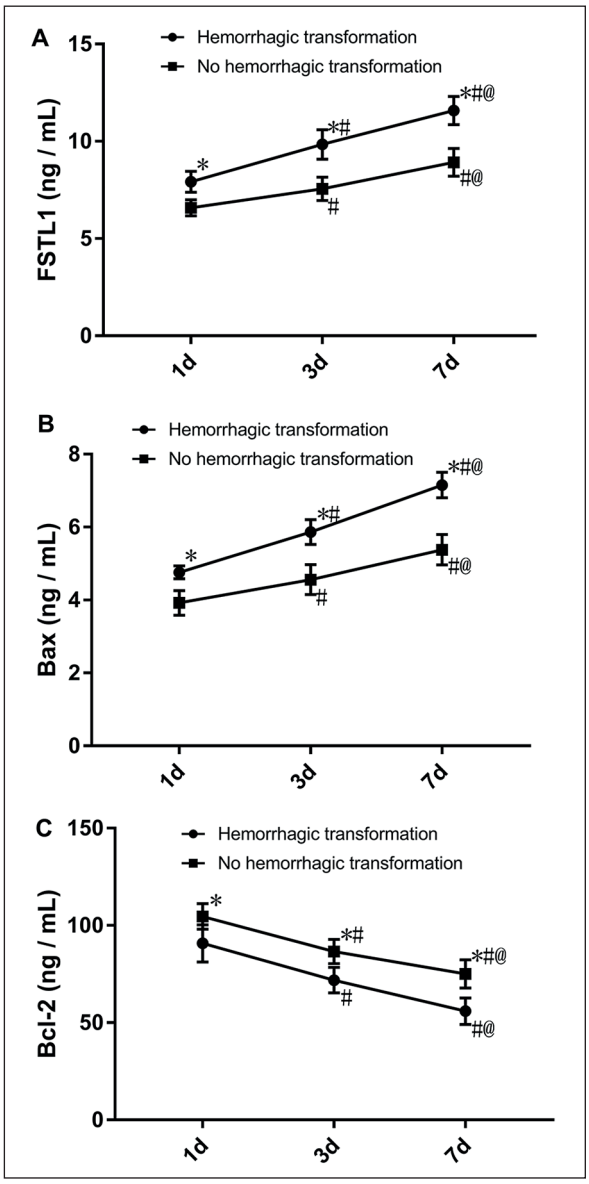


***Figure 5.*** Comparison of FSTL1, Bax and Bcl-2 concentrations in patients with hemorrhagic transformation and patients without hemorrhagic transformation. **A**, Comparison of FSTL1 concentration between patients with hemorrhagic transformation and patients without hemorrhagic transformation. **B**, Comparison of Bax concentration between patients with hemorrhagic transformation and patients without hemorrhagic transformation. **C**, Comparison of Bcl-2 concentration between patients with hemorrhagic transformation and patients without hemorrhagic transformation. *Represents comparison with patients without hemorrhagic transformation at the same time, *$p$<0.050. #Represents comparison with patients in the same group at 1 d, #$p$<0.050. @Represents comparison with patients in the same group in 3 d, @$p$<0.050.

while Bcl-2 concentration was significantly lower than that in the normal group ($p < 0.001$), as shown in Figure 6.

## Discussion

ACI, as an extremely common cerebrovascular disease in clinic, is also one of the most common diseases causing disability in the elderly[13]. ACI refers to the rapid loss of brain function due to the interruption of blood supply, and the formation of a complex infarcted area by means of excitotoxicity, oxidative stress, apoptosis, and inflammatory response[14,15]. At present, the diagnostic requirements of ACI are extremely high, and the selection of treatment is also highly limited. Therefore, a full understanding of the pathogenesis and molecular mechanism of ACI is of great significance for the future clinical diagnosis and treatment of ACI.

The results of this study showed that FSTL1 and Bax in peripheral blood of ACI patients were higher than those in healthy control group, while Bcl-2 was lower than that in control group, suggesting that FSTL1, Bax and Bcl-2 may participate in the occurrence and development of ACI. However, through referring to previous studies, we found that FSTL1, Bax and Bcl-2 are also abnormally expressed in rats with breast cancer, leukemia and vascular dementia[16-18], which can also support our experimental results. FSTL1, as an inflammatory factor discovered in recent years, has both anti-inflammatory and pro-inflammatory effects, and has been proved to be the occurrence of heart failure and stroke[19,20]. In the study of Deng et al[21], FSTL1 can inhibit the proliferation and migration of airway smooth muscle cells induced by platelet-derived growth factor BB (PDGF-BB). The onset of ACI is closely related to atherosclerosis. Previous studies have confirmed that FSTL1 is involved in the process of atherosclerosis[22]. Therefore, we speculated that the mechanism of FSTL1 in ACI might be to promote the occurrence of atherosclerosis by activating the inflammatory reaction of macrophages, thus causing ACI. However, the abnormal expression of Bax and Bcl-2 confirmed that neuronal apoptosis was more obvious in ACI patients. We found that it has been reported that FSTL1 inhibits the apoptosis of liver cancer cells through Protein kinase B(AKT) / Glycogen synthase kinase 3β (GAS-3β)[23], so it is speculated that another function of FSTL1 in ACI may be related to promoting neuronal apoptosis, but this still needs further experimental proof. Through ROC curve, it was detected that FSTL1, Bax, Bcl-2 concentration in patients' peripheral blood has a good predictive value for the occurrence of ACI,

**Table II.** Single factors affecting hemorrhagic transformation in ACI patients [n (%)].

| | Hemorrhagic transformation (n = 36) | Without hemorrhagic transformation (n = 48) | *t* or $\chi^2$ | *p* |
|---|---|---|---|---|
| Age (years) | | | 0.266 | 0.606 |
| ≤ 60 | 23 (63.89) | 28 (58.33) | | |
| > 60 | 13 (36.11) | 20 (41.67) | | |
| BMI (kg/cm$^2$) | | | 0.041 | 0.840 |
| < 21 | 12 (33.33) | 15 (31.25) | | |
| ≥ 21 | 24 (66.67) | 33 (68.75) | | |
| NIHSS score | | | 5.124 | < 0.001 |
| | 15.26 ± 5.63 | 9.52 ± 4.63 | | |
| Hcy (mmol/L) | | | 4.132 | < 0.001 |
| | 15.62 ± 6.24 | 22.63 ± 8.62 | | |
| Family medical history | | | 0.145 | 0.703 |
| With | 15 (41.67) | 22 (45.83) | | |
| Without | 21 (58.33) | 26 (54.17) | | |
| Living environment | | | 0.108 | 0.742 |
| Town | 29 (80.56) | 40 (83.33) | | |
| Countryside | 7 (19.44) | 8 (16.67) | | |
| Atrial fibrillation | | | 8.811 | 0.003 |
| Yes | 26 (72.22) | 19 (39.58) | | |
| No | 10 (27.78) | 29 (60.42) | | |
| Infarct volume (cm$^3$) | | | 12.190 | < 0.001 |
| < 5 | 8 (22.22) | 18 (37.50) | | |
| 5-10 | 10 (27.78) | 24 (50.00) | | |
| > 10 | 18 (50.00) | 6 (12.50) | | |
| Smoking | | | 0.259 | 0.611 |
| Yes | 28 (77.78) | 35 (72.92) | | |
| No | 8 (22.22) | 13 (27.08) | | |
| Thrombolytic therapy | | | 0.017 | 0.897 |
| Yes | 22 (61.11) | 30 (62.50) | | |
| No | 14 (38.89) | 18 (37.50) | | |

**Table III.** Assignment table.

| Factors | Assignment |
|---|---|
| NIHSS score | The data conform to continuous variables are analyzed using original data. |
| Hcy (mmol/L) | The data conform to continuous variables are analyzed using original data. |
| Atrial fibrillation | No = 0, Yes = 1 |
| Infarct volume (cm$^3$) | < 5 = 0, 5-10 = 1, > 10 = 2 |
| FSTL1 (ng/mL) | The data conform to continuous variables are analyzed using original data. |
| Bax (ng/mL) | The data conform to continuous variables are analyzed using original data. |
| Bcl-2 (ng/mL) | The data conform to continuous variables are analyzed using original data. |

**Table IV.** Multiple factors affecting hemorrhagic transformation in ACI patients.

| Factors | B | SE | Wald $\chi^2$ | *p* | OR | 95% CI |
|---|---|---|---|---|---|---|
| NIHSS score | 2.423 | 1.182 | 10.623 | < 0.001 | 10.523 | 2.623-54.631 |
| Atrial fibrillation | 2.423 | 0.892 | 4.826 | 0.012 | 8.623 | 4.623-12.623 |
| Infarct volume | 2.165 | 0.923 | 5.623 | 0.007 | 9.623 | 3.624-18.623 |
| FSTL1 | 2.874 | 0.942 | 9.842 | < 0.001 | 9.842 | 2.642-26.634 |
| Bax | 1.524 | 0.715 | 4.842 | 0.013 | 4.124 | 1.624-19.524 |
| Bcl-2 | -0.642 | 0.154 | 21.642 | < 0.001 | 0.214 | 0.214-0.864 |

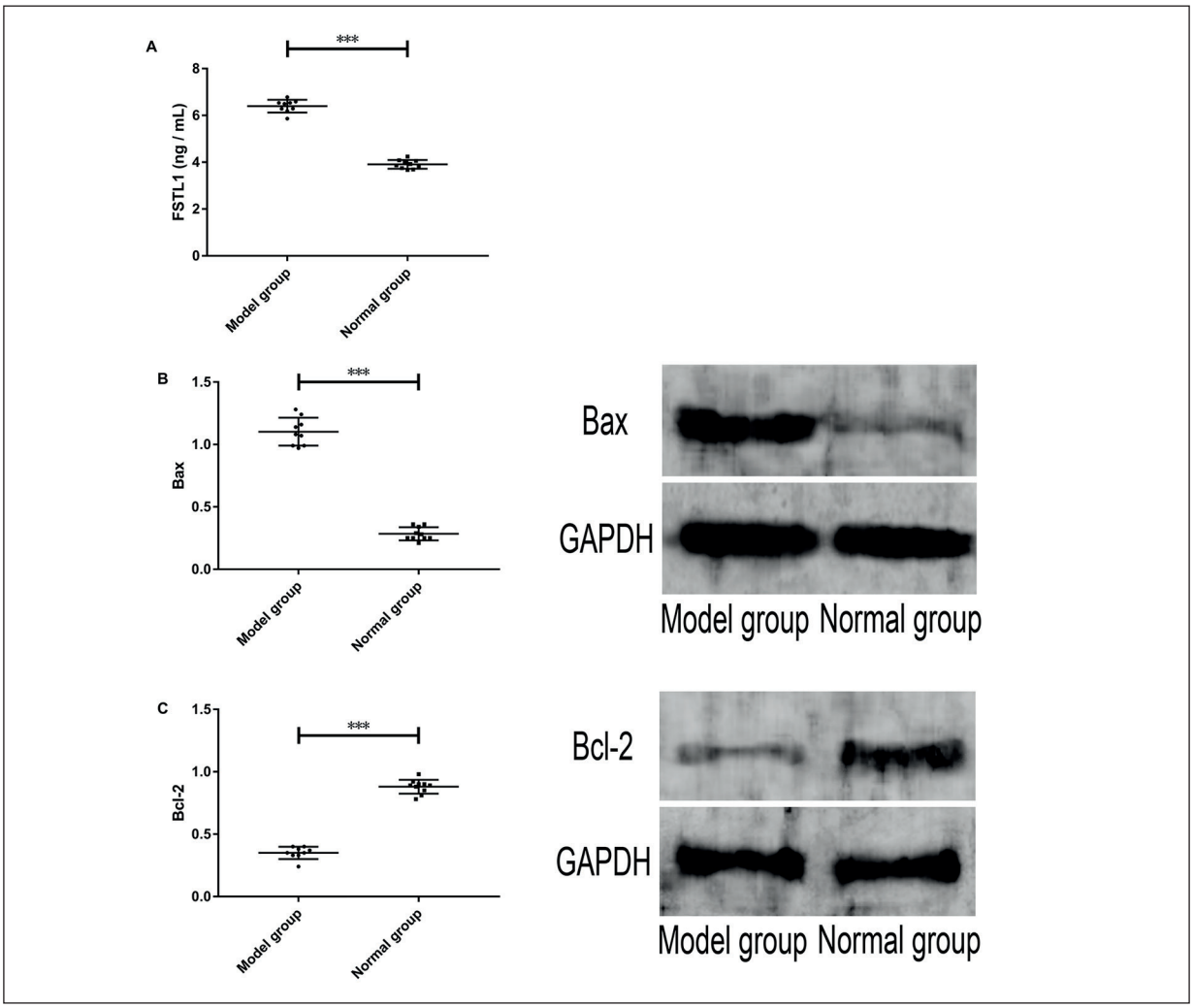


***Figure 6.*** Comparison of FSTL1, Bax and Bcl-2 in rat brain tissue. **A**, Comparison of FSTL1 in rat brain tissue. **B**, Comparison of Bax in rat brain tissue. **C**, Comparison of Bcl-2 in rat brain tissue. FSTL1 and Bax in model group were significantly higher than those in normal group, and Bcl-2 was significantly lower than that in normal group, ***$p < 0.001$.

suggesting that it can be used as a blood marker of ACI for auxiliary diagnosis in the future. Compared with the current clinical diagnosis of ACI which requires complicated radiological technologies, the application of blood markers can greatly shorten the treatment time of patients and improve the early detection rate of ACI. In addition, the blood sample is more convenient to obtain, and the preservation period is longer, which is more favorable for clinical review or further examination at any time. In addition, the objective detection results of blood markers reduce the subjective factors of human judgment in imaging and the possibility of misdiagnosis, which are of great significance for the diagnosis and treatment of ACI in the future.

To further understand the clinical significance of FSTL1, Bax and Bcl-2 to ACI, we detected FSTL1, Bax and Bcl-2 in peripheral blood of patients with different infarct sizes, and found that the three are closely related to the infarct size of patients, which further confirmed that FSTL1, Bax and Bcl-2 are involved in the development of ACI. And we detected FSTL1, Bax, Bcl-2 in patients receiving treatment of thrombolytic therapy and non-thrombolytic therapy, and it was found that the improvement of the three in patients receiving treatment of thrombolytic therapy was more significant, indicating that thrombolytic therapy was more effective for ACI. Thrombolytic therapy is the key to the opening of occluded vessels, which

should be implemented as soon as possible for patients meeting thrombolytic indications. However, some studies suggest that the use of thrombolytic therapy may result in the risk of hemorrhagic transformation[24], which may be related to extracellular proteolysis disorder in neurovascular matrix. By comparing FSTL1, Bax and Bcl-2 in patients with hemorrhagic transformation and those without hemorrhagic transformation, it could be seen that FSTL1 and Bax in patients with hemorrhagic transformation increased while Bcl-2 decreased, suggesting that FSTL1, Bax and Bcl-2 also participate in the occurrence of ACI hemorrhagic transformation. Through multivariate analysis, we found that NIHSS score, atrial fibrillation, infarct volume, FSTL1 and Bax were independent risk factors affecting hemorrhagic transformation in ACI patients, and Bcl-2 was an independent protective factor affecting hemorrhagic transformation in ACI patients, which further confirmed our above conjecture. NIHSS score is a commonly used index for evaluating neurological function. It has been confirmed by many studies on the relationship between NIHSS score and hemorrhagic transformation of ACI. The infarct volume reflects the severity of ACI, and the relationship between NIHSS score and hemorrhagic transformation is self-evident, so it will not be repeated here. Atrial fibrillation is proved to be an inflammatory reaction, and its occurrence is always accompanied by inflammatory reaction[25]. The differential results also confirmed that inflammatory response plays an important role in ACI and confirmed that the pro-inflammatory effect of FSTL1 produces a marked effect on ACI. Finally, by establishing ACI rat model, we detected the concentration of FSTL1 and the expression of Bax and Bcl-2 proteins in the brain tissue, and the results were consistent with our above detection, confirming that FSTL1 participates in the ACI process, which may be achieved through promoting the occurrence of inflammatory reaction in the brain tissue of patients or (and) accelerating neuronal apoptosis.

However, due to limited conditions, there are still some shortcomings in this experiment. For example, only FSTL1, Bax and Bcl-2 were detected in this experiment at 1d, 3d and 7d after the patient was admitted to hospital, and the time points were too few to evaluate the dynamic changes of the three in the patient's disease cycle. It is still necessary to expand the research cycle to obtain more comprehensive monitoring results. As for the exact mechanism of FSTL1's involvement in ACI, we need to conduct more in-depth experiments to determine it and carry out experimental verification for the targeted treatment of ACI for FSTL1, so as to provide more detailed clinical references.

## Conclusions

This study showed that FSTL1, Bax and Bcl-2 are involved in the occurrence and development of ACI and are closely related to hemorrhagic transformation of patients. The mechanism by which FSTL1 promotes the occurrence of ACI might be related to promoting the occurrence of inflammatory responses in the brain tissue of patients or accelerating the apoptosis of neurons.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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