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Cerebrospinal fluid markers of neuroinflammation and coagulation in severe cerebral edema and chronic hydrocephalus after subarachnoid hemorrhage: a prospective study

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Abstract

Background Early severe cerebral edema and chronic hydrocephalus are the primary cause of poor prognosis in patients with subarachnoid hemorrhage (SAH). This study investigated the role of cerebrospinal fluid (CSF) inflammatory cytokines and coagulation factors in the development of severe cerebral edema and chronic hydrocephalus in patients with SAH.

Methods Patients with SAH enrolled in this study were categorized into mild and severe cerebral edema groups based on the Subarachnoid Hemorrhage Early Brain Edema Score at admission. During long-term follow-up, patients were further classified into hydrocephalus and non-hydrocephalus groups. CSF samples were collected within 48 h post-SAH, and levels of inflammatory cytokines and coagulation factors were measured. Univariate and multivariate logistic regression analyses were performed to identify independent factors associated with severe cerebral edema and chronic hydrocephalus. The correlation between inflammatory cytokines and coagulation factors was further investigated and validated in a mouse model of SAH.

Results Seventy-two patients were enrolled in the study. Factors from the extrinsic coagulation pathway and inflammatory cytokines were associated with both severe cerebral edema and chronic hydrocephalus. Coagulation products thrombin-antithrombin complexes (TAT) and fibrin, as well as inflammatory cytokines IL-1β, IL-2, IL-5, IL-7,

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and IL-4, were independently associated with severe cerebral edema. Additionally, Factor VII, fibrin, IL-2, IL-5, IL-12, TNF-α, and CCL-4 were independently associated with chronic hydrocephalus. A positive correlation between extrinsic coagulation factors and inflammatory cytokines was observed. In the SAH mouse model, tissue plasminogen activator was shown to alleviate neuroinflammation and cerebral edema, potentially by restoring glymphatic-meningeal lymphatic function.

Conclusions Elevated levels of inflammatory cytokines and extrinsic coagulation pathway factors in the CSF are associated with the development of early severe cerebral edema and chronic hydrocephalus following SAH. These factors are interrelated and may contribute to post-SAH glymphatic-meningeal lymphatic dysfunction.

Keywords Subarachnoid hemorrhage, Inflammatory cytokines, Coagulation, Glymphatic system, Meningeal lymphatic drainage

Introduction

Spontaneous subarachnoid hemorrhage (SAH) is a severe subtype of stroke, associated with high mortality and morbidity rates [1]. Early brain injury (EBI), occurring within the first 72 h post-SAH, along with the development of long-term hydrocephalus, are recognized as the primary contributors to poor outcomes in SAH patients [2, 3]. Clinical evidence has indicated that cerebral edema in the early phase, as quantified by radiological imaging, is the most prominent manifestation of EBI and is significantly associated with secondary complications and adverse outcomes in SAH patients [4, 5]. Numerous efforts have been made to identify therapeutic targets by elucidating the underlying mechanisms of cerebral edema and chronic hydrocephalus following SAH. Discovering a shared mechanism underlying cerebral edema and hydrocephalus formation could significantly improve the prognosis of SAH patients [1].

Traditionally, cerebral edema formation has been attributed to the dysfunction of ion and water transporters, as well as blood-brain barrier disruption after SAH [6–8]. Meanwhile, hydrocephalus formation has been linked to blood clot obstruction in the ventricular system and arachnoid granulations, along with chronic neuroinflammation and fibrin deposition leading to choroid plexus hypersecretion [9]. Recent preclinical studies have updated the theory of cerebrospinal fluid (CSF) dynamics, emphasizing the importance of CSF circulatory neuroinflammation and coagulation reactions in the development of cerebral edema, hydrocephalus, and subsequent neurological deficits following SAH [6].

Our previous studies revealed that a disordered glymphatic-meningeal lymphatic system contributes to the development of cerebral edema and hydrocephalus after SAH by causing retained CSF in the brain parenchyma and impaired CSF outflow in rodent models [10–12]. Circulating neuroinflammatory cytokines and blood coagulation factors in the CSF play pivotal roles in disrupting the glymphatic-meningeal lymphatic system, further promoting cerebral edema and hydrocephalus formation after SAH [11–13]. Moreover, a significant correlation between circulating inflammatory cytokines and coagulation factors in CSF was observed in our previous preclinical studies [12].

Despite the proposed role of inflammatory responses and blood coagulation products as key contributors to cerebral edema and hydrocephalus in preclinical models, further evidence is needed to confirm the involvement of circulating inflammatory cytokines and coagulation factors in CSF in the formation of cerebral edema and the occurrence of hydrocephalus in SAH patients. In this study, we hypothesized that the levels of inflammatory cytokines and coagulation factors in CSF correlate with the severity of cerebral edema, the occurrence of hydrocephalus, and clinical outcomes in SAH patients. Furthermore, we proposed that these inflammatory factors would correlate with coagulation factors in the CSF.

Methods

Study patients

We included patients diagnosed with spontaneous aneurysmal SAH admitted to the Second Affiliated Hospital of Zhejiang University between August 2022 and August 2023 in an ongoing prospective, observational, single-center cohort study registered on *ClinicalTrials.gov* (NCT06009016).

The inclusion criterion was defined as patients with SAH exhibiting a modified Fisher score (mFS) of 3–4 on computed tomography (CT) within 24 h of ictus. Exclusion criteria included: (1) non-aneurysmal SAH (e.g., trauma, arteriovenous malformation, and angiogram-negative SAH); (2) history of central nervous system (CNS) diseases (e.g., stroke, traumatic brain injury, CNS infection); (3) serious comorbidities prior to SAH onset (e.g., malignant tumors, drug-resistant heart disease, coagulation disorders, or hypertension and other organ dysfunctions within 6 months).

This study was approved by the Institutional Review Board of the Second Affiliated Hospital of Zhejiang University (No. 2023-059). Informed consent was obtained from the patients or their family members, or waived by the Institutional Review Board.

Sample size calculation

Sample size was calculated using G*Power (version 3.1) to determine the proportion of patients with severe cerebral edema and hydrocephalus among those with SAH (one-sample case), with an effect size [g] of 0.2, α error probability of 0.05, study power of 90%, and a constant proportion of 50%. The total sample size required to meet these criteria was determined to be 65 SAH patients for detecting severe cerebral edema and hydrocephalus incidence. This number was increased to 72 patients to enhance the study's statistical power.

Patient management

All patients were treated according to the guidelines proposed by the Neurocritical Care Society and the American Heart Association (AHA), consistent with our previous studies [14–16]. Emergency cerebral digital subtraction angiography (DSA) was performed within 24 h of admission to evaluate the responsible aneurysm. Coiling or clipping was performed by an experienced neurointerventional or neurosurgical team immediately after the initial DSA within 24 h of admission. Lumbar drainage (LD) was routinely performed for patients with an mFS of 3–4, except in cases where external ventricular drainage (EVD) was required for acute hydrocephalus. CT scans of the brain were performed post-surgery, with subsequent scans every 1–3 days based on clinical necessity until discharge. Nimodipine was administered to prevent cerebral vasospasm, and euvolemia was maintained via intravenous hydration for all patients.

Baseline characteristics

Baseline characteristics, including age, gender, body mass index (BMI), past medical history, and social history, were reviewed. Clinical data recorded at admission included the Glasgow Coma Scale (GCS), Hunt and Hess (HH) grade, and World Federation of Neurosurgeons Scale (WFNS). The GCS score at discharge and the length of hospital stay were also documented. Radiological data, evaluated using head CT images, included the mFS, Subarachnoid Hemorrhage Early Brain Edema Score (SEBES) [4], intraventricular hemorrhage (IVH), and the highest Hounsfield Unit (HU) in the hemorrhagic area (higher CT attenuation representing thicker blood clots) [12].

Hydrocephalus and quantification of cerebral edema

Hydrocephalus was defined as ventricular enlargement on CT scans (bicaudate index > 0.2 or Evans' index > 0.3) accompanied by hydrocephalus-related symptoms, such as neurological deterioration (aggravated response to painful stimuli in unconscious patients or development of confusion, gait disturbance, or urinary incontinence in conscious patients).

Acute hydrocephalus was characterized by its occurrence within the first 1–2 weeks after SAH, typically due to acute blockage of the ventricular system by blood clots. Chronic hydrocephalus was defined as hydrocephalus persisting for more than 2 weeks following SAH, in the absence of ventricular obstruction, requiring the placement of a ventriculoperitoneal shunt [17].

Cerebral edema was quantified using the SEBES score (ranging from 0 to 4), calculated from CT images as per our previous study (**Additional file 1**) [18]. In cases of discrepancy, the CT was graded by a third reviewer. Patients were categorized into mild cerebral edema (SEBES 0–2) and severe cerebral edema (SEBES 3–4) cohorts based on the first CT at admission.

Clinical complications and outcome

SAH-related complications included seizures and delayed cerebral ischemia (DCI), which was defined as clinical deterioration with focal neurological deficits or a loss of 2 points on the GCS due to cerebral vasospasm or cerebral infarction (new cerebral infarctions appearing on CT or MRI, excluding those occurring within 48 h of surgery or coiling) [19].

Functional outcomes were assessed at 3 months post-discharge using the modified Rankin Score (mRS). A favorable outcome was defined as an mRS score of 0–3, while a poor outcome was defined as a score of 4–6.

Given the reported association of serum inflammatory and coagulation factors with the incidence of DCI and poor functional outcomes in SAH patients [20, 21], patients experiencing DCI and those with poor outcomes at 3 months post-discharge were included as supplementary experimental groups. The relationship between inflammatory cytokines and coagulation factors in CSF with the incidence of DCI and poor outcomes was also examined.

CSF samples collection and processing protocol

All CSF samples from SAH patients were collected before coiling or clipping through LD or EVD within 48 h of SAH onset. The samples were centrifuged at 3000 rpm for 10 min at 4 °C to remove cells (erythrocytes and immune cells) and stored at -80 °C until analysis.

A total of 17 cytokines were measured using a Bio-Plex Pro Human Cytokine 17-plex Assay (M50-00031YV, Bio-Rad, Hercules, CA, USA) following the manufacturer's protocol. The cytokines measured included: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, MCP-1/CCL-2, MIP-1β/CCL-4, and TNF-α.

The concentrations of coagulation factors in human CSF were determined using ELISA kits, which included the following: Human TF ELISA kit (EK0928, Boster, Pleasanton, CA, USA), Human Fibrinogen ELISA kit

(ab108842, Abcam, Cambridge, MA, USA), Human TAT ELISA kit (ab108907, Abcam, Cambridge, MA, USA), Human Factor IX ELISA Kit (NBP2-60518, Novus Biologicals, Centennial, CO, USA), Human Coagulation Factor XI ELISA Kit (NBP2-60626, Novus Biologicals, Centennial, CO, USA), and Human Factor VII ELISA Kit (NBP2-67951, Novus Biologicals, Centennial, CO, USA). All procedures followed the manufacturer's instructions.

Animal SAH model and drug administration

Specific pathogen-free C57BL/6 male mice (6–8 weeks old) were purchased from SLAC Laboratory Animal Co. Ltd. (Shanghai, China). Mice were housed in a controlled animal facility on a 12-hour light/dark cycle and provided with regular rodent chow and sterilized tap water ad libitum. Mice were randomly divided into Sham, SAH + Vehicle, and SAH + recombinant tissue plasminogen activator (rtPA) groups. The experimental groups' identities were blinded to the researchers performing surgeries, outcome assessments, and data analyses. Sample sizes were determined using the resource equation method, as described previously [22].

The prechiasmatic SAH model was employed as previously described [11]. In brief, mice were anesthetized with 1% pentobarbital, and their heads were fixed in a stereotactic apparatus (RWD Life Science, Shenzhen, Guangdong, China). A midline incision exposed the anterior skull, and a burr hole was drilled 4.5 mm anterior to the bregma at a 40° angle using a 0.9 mm drill. Autologous blood (60 μL) from a C57BL/6 donor was injected over a 10-second period using a 26-gauge needle advanced through the burr hole at a 40° angle until reaching the skull base.

rtPA (Actilyse, Boehringer Ingelheim, Ingelheim am Rhein, Germany) was infused at 5 μL/min following blood injection. The rtPA (15 μg) was dissolved in 10 μL artificial CSF (aCSF, Fisher Scientific, Waltham, MA, USA). The needle was left in place for 5 min before retraction to prevent backflow. Mice undergoing the same procedure without blood and drug injections served as the sham group.

Neurological function assessment for mice

Neurological function was assessed using the Modified Garcia scale, Pole test, and Wire Hanging test. The Modified Garcia scale (0–18 points) was employed to evaluate short-term neurological function, including six subtests assessing response capacity, alertness, coordination, motor skills, complex movements, and balance [23]. A blinded investigator conducted the neurological assessments 24 and 72 h post-SAH. A higher score indicated better neurological function.

The Pole test was conducted as previously described [11]. Mice were placed on top of a 50–55 cm vertical pole with an 8–10 mm diameter and trained to turn and descend the pole. The timing began when the mice initiated the turning motion. Two parameters were recorded: the time taken to complete the 180° turn (T_{turn}) and the total time to descend (T_{total}). If a mouse was unable to execute the turn and descended laterally, T_{total} was considered equivalent to T_{turn}. If a mouse completed the turn, descended partway, and fell, timing ceased when it reached the ground. The maximum test time was 60 s.

The Wire Hanging test was conducted as previously described [11]. This test assesses grip strength, balance, and endurance in mice. Mice were trained to hang from a single wire between two posts 50–60 cm above the ground, with their hind limbs taped to prevent the use of all four paws. A pillow was placed below for safety. "Latency to fall" was the main outcome, with a maximum test duration of 60 s.

Training sessions for all tests were conducted three times per day before the initiation of testing. All assessments were performed by a blinded investigator.

CSF tracing and glymphatic-meningeal lymphatic function assessment in mice

To visualize CSF movement and clearance, FluoSpheres carboxylate 0.5 μm-beads 505/515 (F8813, Fisher Scientific) and 0.5% Alexa Fluor 594 hydrazide (A10438, Fisher Scientific) were injected into the cisterna magna (i.c.m.) 24 h post-SAH, as previously described [11, 24].

Mice were anesthetized with 1% pentobarbital, and the neck skin was shaved and disinfected with iodine and ethanol. The mice were secured in a stereotaxic frame, and a midline incision was made through the skin. The muscle layers were retracted to expose the cisterna magna. Using a Hamilton syringe coupled to a 33-gauge needle, 2 μL of beads or 5 μL of Alexa Fluor 594 were injected at a rate of 2 μL/min. To prevent backflow, the needle was inserted through the retracted muscle, and the skin was sutured after injection. Mice were placed on a heating pad to recover.

Mice were euthanized with pentobarbital, and the deep cervical lymph nodes (dCLNs), meningeal whole mounts, and brains were collected 2 h after bead injection, as previously described [11]. The dCLNs were fixed in 4% paraformaldehyde (PFA) for 2 h at 4 °C, followed by the CUBIC clearance protocol [25]. Nodes were incubated in 50% and 100% reagent 1 for 2 days at 37 °C with DAPI, washed twice in PBS for 2 h, incubated overnight with DAPI, and then incubated in 50% and 100% reagent 2 for 2 days at 37 °C with DAPI. Finally, nodes were placed in eight-well chambers (155411, Thermo Fisher) with mineral oil and imaged using confocal microscopy.

For brain and meningeal whole-mount collection, mice were perfused transcardially with cold 1× PBS. Skin and muscle were removed from the skull, and the brain

and skullcap were fixed in 2% PFA for 12 h at 4 °C. The meninges were then carefully dissected from the skullcaps for immunofluorescence staining, blocked with 2% donkey serum for 1.5 h at room temperature, and incubated overnight at 4 °C with primary antibodies: rat anti-Lyve1 (1:200, sc-65647, Santa Cruz, Dallas, TX, USA) and goat anti-CD31 (1:100, AF3628, R&D Systems, Minneapolis, MN, USA). After washing, sections were incubated for 2 h with donkey Alexa Fluor 405, 488, or 594 secondary antibodies (1:1000, Thermo Fisher). Tissues were transferred to PBS and mounted with ProLong Gold antifade reagent (P36934, Thermo Fisher) on glass slides with coverslips for further imaging.

Mice were euthanized with pentobarbital, and brains were collected 30 min after Alexa Fluor 594 injection, as previously described [24]. The brains were sliced into coronal Sect. (50-μm thick), and whole-slice montages from the anterior and posterior brain were analyzed to observe Alexa Fluor 594 diffusion, representing glymphatic function.

All slides were imaged using a Leica DMI8 confocal microscope and LAS AF software (Leica Microsystems).

Image quantification

Quantitative analysis of the acquired images was performed using ImageJ software. For lymph nodes, the percent volume of microbead coverage in cleared dCLNs was assessed by creating a 3D reconstruction and calculating the volume covered by beads, divided by the total node volume. The percent volumes of the right and left dCLNs were averaged for each mouse.

For meningeal lymphatic vessels (mLVs), the percent area coverage of Lyve1 was calculated as the percentage of the Lyve1 field divided by the Lyve1−CD31+vessel field. Fluorescence intensity of microbeads on mLVs was also measured. Tracer diffusion in the brain parenchyma was assessed by evaluating fluorescence intensity in the anterior and posterior brain.

Brain water content of mice

Brain edema was quantified using the wet-dry weight method, as previously described [12]. Brain water content (%) was calculated as follows: [(wet weight−dry weight) / wet weight] × 100%.

Brain inflammatory cytokines assessment of mice

Protein samples were extracted from brain tissue, as performed previously [12]. Inflammatory cytokines were assessed using ELISA assays according to the manufacturer's instructions, including IL-1β (MLB00C, R&D Systems), IL-5 (M5000, R&D Systems), IL-17 (M1700, R&D Systems), and GM-CSF (MGM00, R&D Systems).

Statical analysis

Patient characteristics were described using unpaired Student's t-tests or non-parametric Mann–Whitney U tests for continuous variables, and Pearson chi-square or Fisher's exact tests for categorical variables. Normality was assessed using the Shapiro-Wilk test.

Spearman's rank correlation was conducted to analyze associations between coagulation factors and inflammatory cytokines, with Spearman's correlation coefficient (r) and corresponding 95% confidence intervals (CI) reported.

All variables with a p-value < 0.10 in univariate analysis were included in the multivariate logistic regression model. Multivariate logistic regression analysis, using backward selection, was performed to identify independent biomarkers associated with cerebral edema and hydrocephalus. Analysis was conducted using both factors (model 1) and adjusted for clinical and radiological variables (model 2) in Table 1 to obtain adjusted odds ratios (ORs). ORs and 95% CIs from univariate and multivariate analyses were recorded.

One-way analysis of variance (ANOVA) followed by Tukey's or Dunnett's multiple comparisons tests were used to detect differences between animal groups.

Data are presented as mean ± standard deviation (SD), median (interquartile range), or number (percentage). Statistical analysis was performed using GraphPad Prism 8.2.1 (GraphPad Software, San Diego, CA, USA), SPSS 23.0 (IBM, Armonk, NY, USA), and OmicStudio tools (LC Sciences, Hangzhou, Zhejiang, China) at https://www.omicstudio.cn/tool. Adjusted p-values were used for multiple testing. All p-values were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

General characteristics

A total of 72 patients with spontaneous aneurysmal SAH admitted to our hospital were included in the study. The mean age of the subjects was 60.3 ± 11.3 years (range 20–89 years), and 37 (51.4%) were women. Among the patients, 46 underwent aneurysm clipping, while 26 received coil embolization. No patients were lost to follow-up after discharge. Based on the baseline SEBES score, 38 (52.8%) presented with severe cerebral edema, while 34 (47.2%) had mild cerebral edema (Table 1). Chronic hydrocephalus occurred in 13 patients (18.1%) either during hospitalization or the follow-up period (Table 2).

No significant differences were observed in baseline demographic characteristics between the severe and mild cerebral edema groups (all $p > 0.05$). However, patients with severe cerebral edema presented with lower GCS scores ($p = 0.004$) and higher HH grades ($p = 0.004$) at admission compared to those with mild cerebral

Table 1 Demographic and clinical data of SAH patients between mild cerebral edema and severe cerebral edema

	Mild cerebral edema (*n* = 34)	Severe cerebral edema (*n* = 38)	*p* value†
Baseline characteristics			
Age (y)	63.0 ± 10.5	57.9 ± 11.6	0.052
Gender, male	18(52.9)	17(44.7)	0.487
BMI	22.5 ± 3.0	23.8 ± 3.2	0.062
Smoking	12(35.3)	9(23.7)	0.279
Drinking	11(32.4)	9(23.7)	0.412
Diabetes	2(5.9)	2(5.3)	0.909
Hypertension	19(55.9)	16(42.1)	0.243
Clinical data at admission			
GCS grade	15(13–15)	12.5(5–15)	0.004
HH grade	2(2–3)	3(2–4)	0.004
Radiological data at admission			
IVH	9(26.5)	26(68.4)	< 0.001
Highest HU	64.8 ± 6.0	73.2 ± 6.9	< 0.001
Treatment			
Coiling	12(35.3)	14(36.9)	0.891
Clipping	22(64.7)	24(63.1)	0.891
EVD	5(14.7)	13(34.2)	0.056
LD	31(91.2)	27(71.1)	0.031
Craniectomy	0(0)	8(21.1)	0.006
Complications			
DCI	12(35.3)	28(73.7)	< 0.001
Seizure	1(2.9)	2(5.3)	0.999
Acute hydrocephalus	7(20.6)	15(39.5)	0.082
Chronic hydrocephalus	5(14.7)	8(21.1)	0.485
Outcome			
Length of hospital stay	16.1 ± 9.7	25.3 ± 42.6	0.223
GCS at discharge	15(9–15)	11.5(4–15)	0.021
3-month mRS (4–6)	12(35.3)	23(60.5)	0.032

Variables are presented as mean ± SD, median (interquartile range), or number (%)

† p values were calculated by Pearson chi-square test or Fisher's exact test, or Student's t test or Mann–Whitney U test as appropriate

SAH subarachnoid hemorrhage; BMI body mass index; CGS Coma Glasgow Scale; DCI delayed cerebral ischemia; HH Hunt-Hess; HU hounsfield unit; IVH intraventricular hemorrhage; EVD external ventricular drainage; LD lumbar drainage; mRS modified Rankin Scale

Table 2 Demographic and clinical data of SAH patients between non-hydrocephalus and hydrocephalus

	Non-hydrocephalus (*n* = 59)	Hydrocephalus (*n* = 13)	*p* value†
Baseline characteristics			
Age (y)	59.8 ± 10.3	62.7 ± 15.1	0.403
Gender, male	27(45.8)	8(61.5)	0.303
BMI	23.1 ± 3.0	23.7 ± 3.7	0.961
Smoking	17(28.8)	4(30.8)	0.888
Drinking	16(27.1)	4(30.8)	0.790
Diabetes	4(6.8)	0(0)	0.999
Hypertension	28(47.5)	7(53.8)	0.677
Clinical data at admission			
GCS grade	15(8–15)	15(8–15)	0.454
HH grade	2(2–4)	2(2–4)	0.468
Radiological data at admission			
SEBES	3(1–4)	3(1–4)	0.579
IVH	25(42.4)	10(76.9)	0.024
Highest HU	68.6 ± 7.8	72.2 ± 6.7	0.121
Treatment			
Coiling	21(35.6)	5(38.5)	0.845
Clipping	38(64.4)	8(61.5)	0.845
EVD	11(18.6)	7(53.8)	0.008
LD	48(81.4)	10(76.9)	0.715
Craniectomy	6(0)	2(15.4)	0.629
Complications			
DCI	28(47.5)	12(92.3)	0.002
Seizure	2(3.4)	1(7.7)	0.455
Outcome			
Length of hospital stay	14.3 ± 8.3	51.2 ± 66.7	< 0.001
GCS at discharge	15(8–15)	14(7–15)	0.010
3-month mRS (4–6)	22(37.3)	13(100)	< 0.001

Variables are presented as mean ± SD, median (interquartile range), or number (%)

† p values were calculated by Pearson chi-square test or Fisher's exact test, or Student's t test or Mann–Whitney U test as appropriate

SAH subarachnoid hemorrhage; BMI body mass index; CGS Coma Glasgow Scale; DCI delayed cerebral ischemia; HH Hunt-Hess; HU hounsfield unit; IVH intraventricular hemorrhage; EVD external ventricular drainage; LD lumbar drainage; mRS modified Rankin Scale

edema. Additionally, patients in the severe edema group had a higher incidence of IVH ($p<0.001$) and thicker blood clots ($p<0.001$) on initial CT imaging, as well as increased rates of LD ($p=0.031$) and craniectomy ($p=0.006$) compared to those with mild cerebral edema. Furthermore, patients with severe cerebral edema experienced higher rates of DCI ($p<0.001$), had poorer GCS scores at discharge ($p=0.021$), and worse mRS scores at the 3-month follow-up ($p=0.032$).

No significant differences in demographic and clinical data were noted between patients with and without hydrocephalus, except for a longer hospital stay ($p<0.001$), poorer outcomes at discharge ($p=0.010$), and worse outcomes at the 3-month follow-up ($p<0.001$). Higher rates of IVH ($p=0.024$), EVD ($p=0.008$), and DCI ($p=0.002$) were also observed in the hydrocephalus group compared to the non-hydrocephalus group.

Coagulation factors and inflammatory cytokines in CSF are associated with severe cerebral edema after SAH

Our previous study demonstrated that both intrinsic (Factors IX, XI), extrinsic (Factors III, VII), and common coagulation pathway factors (Fibrin, TAT) were significantly upregulated in the CSF of SAH patients [12].

As depicted in Fig. 1, the levels of extrinsic coagulation factors, Factor III ($p=0.008$) and Factor VII ($p=0.011$), as well as common coagulation products, Fibrin ($p=0.007$) and TAT ($p=0.032$), were significantly higher in patients with severe cerebral edema compared to those with mild

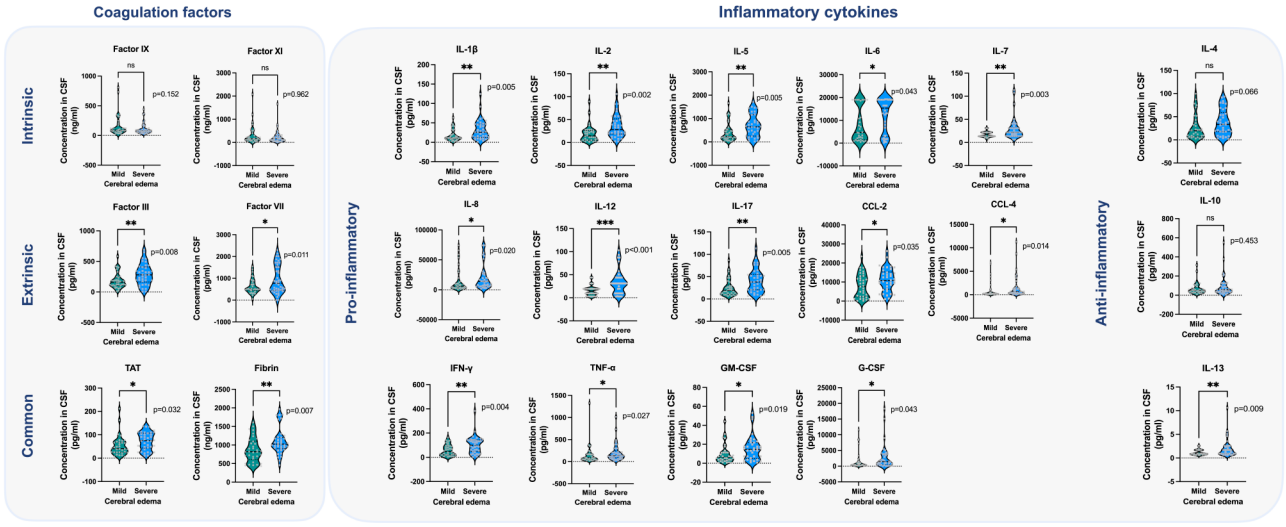


Fig. 1 Levels of coagulation factors and inflammatory cytokines in patients with subarachnoid hemorrhage (SAH) presented with mild and severe cerebral edema. Data are presented as median with interquartile range. P values were calculated by Student's t test or Mann–Whitney U test. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, ns represents no statistical difference

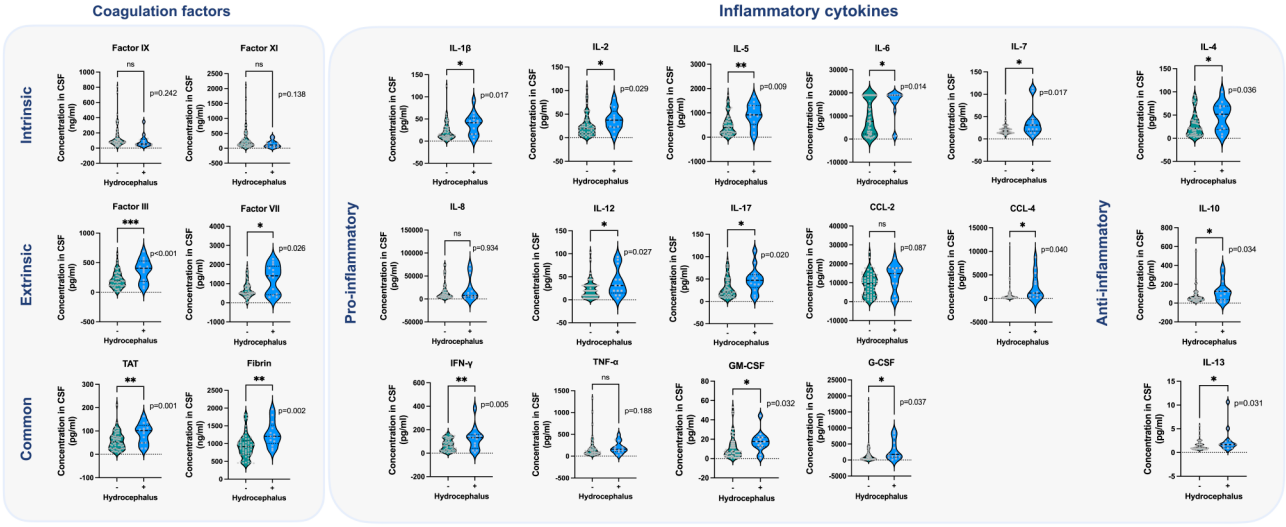


Fig. 2 Levels of coagulation factors and inflammatory cytokines in patients with subarachnoid hemorrhage (SAH) presented with and without hydrocephalus. Data are presented as median with interquartile range. P values were calculated by Student's t test or Mann–Whitney U test. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, ns represents no statistical difference

cerebral edema. However, no statistical differences were observed in the levels of intrinsic coagulation factors Factor IX ($p=0.152$) and Factor XI ($p=0.962$) between the two groups.

In terms of pro-inflammatory cytokines, IL-1β ($p=0.005$), IL-2 ($p=0.002$), IL-5 ($p=0.005$), IL-6 ($p=0.043$), IL-7 ($p=0.003$), IL-8 ($p=0.020$), IL-12 ($p<0.001$), IL-17 ($p=0.005$), G-CSF ($p=0.019$), GM-CSF ($p=0.043$), IFN-γ ($p=0.004$), CCL-2 ($p=0.035$), CCL-4 ($p=0.014$), and TNF-α ($p=0.027$) were significantly elevated in patients with severe cerebral edema compared to those with mild cerebral edema. Although no significant differences were noted for the anti-inflammatory cytokines IL-4 ($p=0.066$) and IL-10 ($p=0.453$), IL-13 ($p=0.009$) was markedly higher in patients with severe cerebral edema compared to those with mild cerebral edema.

Coagulation factors and inflammatory cytokines in CSF are associated with chronic hydrocephalus after SAH

As shown in **Fig.** 2, the levels of extrinsic coagulation factors, Factor III ($p<0.001$) and Factor VII ($p=0.026$), as well as common coagulation products, Fibrin ($p=0.002$) and TAT ($p=0.001$), were significantly elevated in patients who developed chronic hydrocephalus compared to those without the condition. No significant differences

were observed in the levels of intrinsic coagulation factors Factor IX (p=0.242) and Factor XI (p=0.138) between the two groups.

The levels of pro-inflammatory cytokines, including IL-1β (p=0.017), IL-2 (p=0.029), IL-5 (p=0.009), IL-6 (p=0.014), IL-7 (p=0.017), IL-12 (p=0.027), IL-17 (p=0.020), G-CSF (p=0.037), GM-CSF (p=0.032), IFN-γ (p=0.005), and CCL-4 (p=0.040), were significantly higher in patients who developed chronic hydrocephalus compared to those without. The levels of TNF-α (p=0.188) and CCL-2 (p=0.087) in patients with chronic hydrocephalus tended to be higher than in those without. Additionally, the levels of anti-inflammatory cytokines IL-4 (p=0.036), IL-10 (p=0.034), and IL-13 (p=0.031) were markedly higher in patients who developed chronic hydrocephalus compared to those without the condition.

Coagulation factors and inflammatory cytokines in CSF associated with DCI and 3-months poor outcome after SAH

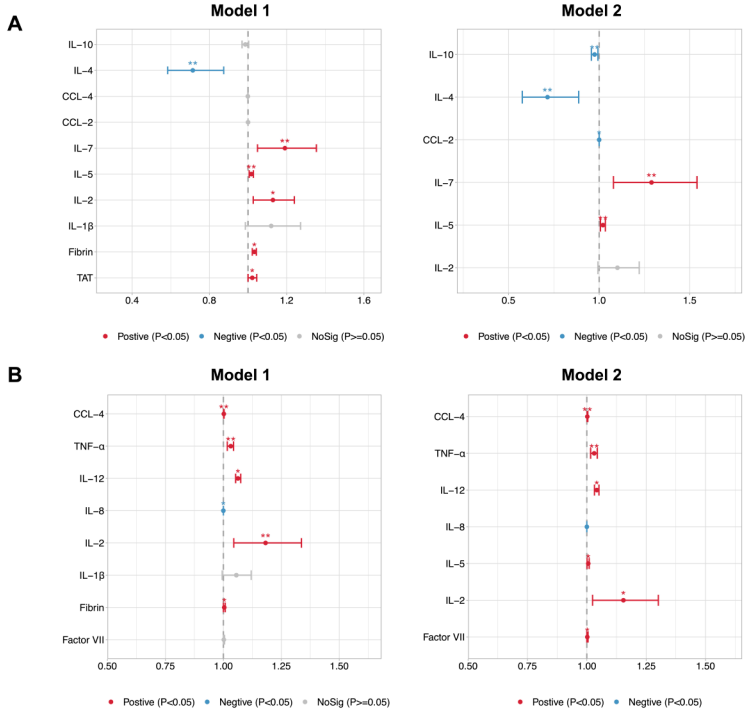
Given that DCI is a major complication affecting the outcomes of SAH patients, univariate analysis was conducted to explore the potential associations between coagulation and inflammatory factors, DCI, and poor outcomes at 3 months.

As shown in Supplementary Table 1 in **Additional File 2**, levels of coagulation factors Factor III (p=0.005), TAT (p=0.001), and Fibrin (p=0.004) were higher in patients with DCI compared to those without. However, no significant differences were observed in the levels of inflammatory factors between patients with and without DCI (all p>0.05).

In contrast, levels of coagulation factors Factor III (p<0.001), Factor VII (p=0.024), TAT (p=0.002), and Fibrin (p=0.002) were higher in patients with poor outcomes at 3 months compared to those with favorable outcomes. Pro-inflammatory cytokines, including IL-1β (p=0.021), IL-2 (p=0.044), IL-5 (p=0.013), IL-6 (p=0.015), IL-7 (p=0.019), IL-12 (p=0.006), IL-17 (p=0.019), G-CSF (p=0.011), GM-CSF (p=0.012), and IFN-γ (p=0.045), as well as the anti-inflammatory cytokine IL-13 (p=0.010), were also elevated in patients with poor outcomes at 3 months compared to those with favorable outcomes (Supplementary Table 2 in **Additional file 2**).

Independent biomarkers in CSF for severe cerebral edema and chronic hydrocephalus after SAH

As depicted in Fig. 3, multivariate logistic regression analyses were performed stepwise to identify independent coagulation and inflammatory biomarkers associated with severe cerebral edema and chronic hydrocephalus. All levels of factors in CSF were included



Independent variables contribute to severe cerebral edema

	Model 1			Model 2		
Factors	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
TAT	1.022	1.000-1.045	0.048			
Fibrin	1.033	1.022-1.043	0.047			
IL-β	1.120	0.986-1.272	0.081			
IL-2	1.129	1.027-1.240	0.012	1.101	0.993-1.221	0.067
IL-5	1.017	1.006-1.028	0.002	1.021	1.007-1.034	0.002
IL-7	1.191	1.049-1.354	0.007	1.289	1.079-1.540	0.005
IL-4	0.714	0.584-0.874	0.001	0.715	0.576-0.887	0.033
IL-10	0.986	0.969-1.003	0.098	0.975	0.957-0.993	0.007
CCL-2	1.000	1.000-1.001	0.052	1.000	1.000-1.001	0.033
CCL-4	0.099	0.999-1.000	0.094			

Independent variables contribute to chronic hydrocephalus

	Model 1			Model 2		
Factors	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Factor VII	1.002	1.000-1.005	0.079	1.002	1.000-1.005	0.035
Fibrin	1.004	1.000-1.008	0.034			
IL-β	1.056	0.995-1.120	0.075			
IL-2	1.182	1.045-1.337	0.008			
IL-5				1.006	1.001-1.010	0.011
IL-8	1.000	1.000-1.001	0.003	1.000	1.000-1.001	0.060
IL-12	1.063	1.053-1.075	0.017	1.042	1.032-1.051	0.045
TNF-α	1.032	1.017-1.044	0.004	1.031	1.016-1.044	0.008
CCL-4	1.002	1.001-1.003	0.003	1.002	1.001-1.004	0.008

Fig. 3 Independent variables contributed to severe cerebral edema and hydrocephalus. **A**. Odd ratios (ORs) of variables contributed to severe cerebral edema. **B**. ORs of variables contributed to hydrocephalus. ORs and p values were calculated by multivariate logistic regression analysis. ORs are presented with OR and 95% confidence interval (CI). Analysis was performed with both factors (model 1) and adjusted for clinical and radiological variables (model 2)

in model 1, while clinical and radiographic variables (age, sex, BMI, smoking, alcohol use, hypertension, diabetes, GCS score, and IVH) were included in model 2. Due to high collinearity between the HH score and GCS score, as well as between IVH and the highest HU on CT scan, GCS score and IVH were used as variables in model 2.

In multivariate model 1 for severe cerebral edema (Fig. 3A), higher levels of TAT (OR=1.022, p=0.048), Fibrin (OR=1.033, p=0.047), IL-2 (OR=1.129, p=0.012), IL-5 (OR=1.017, p=0.002), IL-7 (OR=1.191, p=0.007), and lower IL-4 (OR=0.714, p=0.001) were independently associated with severe cerebral edema. Additionally, higher levels of IL-1β (OR=1.120, p=0.081), CCL-2 (OR=1.000, p=0.052), and lower levels of IL-10 (OR=0.986, p=0.098) and CCL-4 (OR=0.099, p=0.094) tended to be associated with severe cerebral edema. In multivariate model 2, higher levels of IL-5 (OR=1.021, p=0.002), IL-7 (OR=1.289, p=0.005), and lower levels of IL-4 (OR=0.715, p=0.033), IL-10 (OR=0.975, p=0.007), and CCL-2 (OR=1.000, p=0.033) remained associated with severe cerebral edema. Higher levels of IL-2 (OR=1.101, p=0.067) also tended to be associated with severe cerebral edema.

In multivariate model 1 for chronic hydrocephalus (**Fig.** 3B), higher levels of Fibrin (OR=1.004, p=0.034), IL-2 (OR=1.182, p=0.008), IL-8 (OR=1.000, p=0.003), IL-12 (OR=1.063, p=0.017), TNF-α (OR=1.032, p=0.004), and CCL-4 (OR=1.002, p=0.003) were independently associated with chronic hydrocephalus. Factor VII (OR=1.002, p=0.079) and IL-1β (OR=1.056, p=0.075) also tended to be associated with chronic hydrocephalus. In multivariate model 2, higher levels of Factor VII (OR=1.002, p=0.035), IL-5 (OR=1.006, p=0.011), IL-12 (OR=1.042, p=0.045), TNF-α (OR=1.031, p=0.008), and CCL-4 (OR=1.002, p=0.008) remained associated with chronic hydrocephalus, while IL-8 (OR=1.000, p=0.060) tended to be associated with chronic hydrocephalus.

Extrinsic coagulation factors correlated with inflammatory cytokines in CSF after SAH

Given the well-established link between the coagulation and inflammatory systems in the cardiovascular system [26], we further analyzed the correlation between coagulation factors and inflammatory cytokines in the CSF. As shown in Fig. 4A, the levels of the coagulation products Fibrin and TAT in the CSF were strongly correlated with the extrinsic coagulation factors Factor III and Factor VII (TAT and Factor III: OR=0.61, p<0.001; TAT and Factor VII: OR=0.38, p=0.001; Fibrin and Factor III: OR=0.67, p<0.001; Fibrin and Factor VII: OR=0.55, p<0.001).

Moreover, both extrinsic coagulation factors (Factor III, Factor VII) and coagulation products (Fibrin, TAT) were highly correlated with all measured inflammatory cytokines (p<0.05). As depicted in Fig. 4B, the top four cytokines exhibiting the strongest correlation with Fibrin were IL-17 (OR=0.69), IL-5 (OR=0.68), GM-CSF (OR=0.65), and IL-1β (OR=0.61). The top four cytokines most strongly correlated with TAT were IL-2 (OR=0.44), IL-10 (OR=0.41), IL-12 (OR=0.41), and IL-6 (OR=0.39). Similarly, the top four cytokines exhibiting the strongest correlation with Factor III were IL-17 (OR=0.60), IL-5 (OR=0.58), IL-12 (OR=0.57), and IL-6 (OR=0.55), while for Factor VII, the strongest correlations were with IL-5 (OR=0.56), IL-17 (OR=0.55), IL-12 (OR=0.55), and IL-7 (OR=0.52).

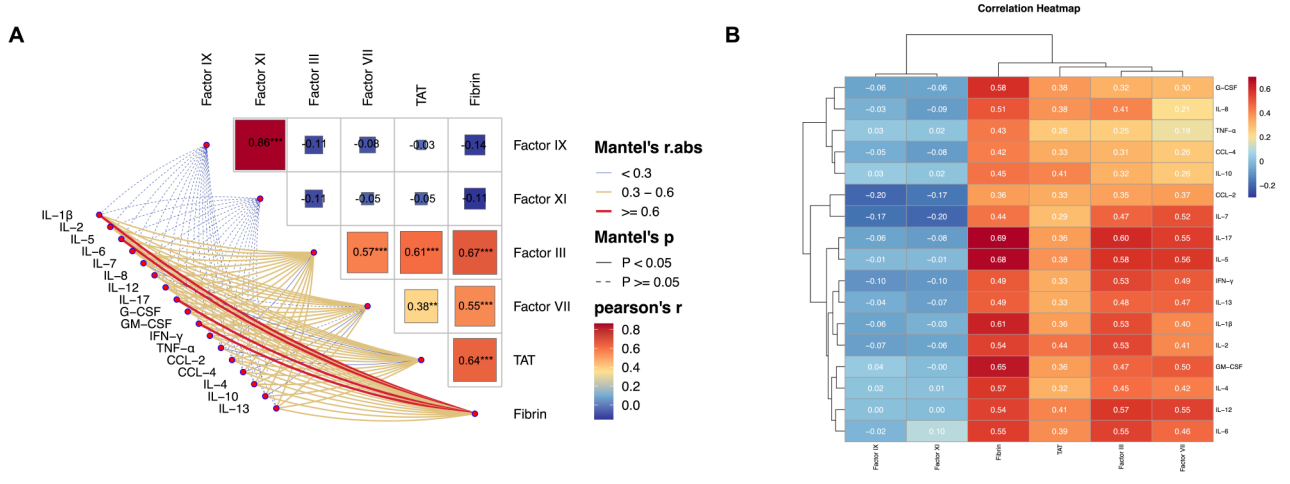


Fig. 4 Correlation heatmaps between coagulation factors and inflammatory cytokines. **A**. Pairwise comparisons of coagulation factors, with a color gradient denoting the Spearman's correlation coefficients. Mantel tests for correlation between inflammatory cytokines and coagulation factors. **B**. Correlation heatmap between coagulation factors and inflammatory cytokines. Correlation coefficient r and p value were calculated by Spearman's rank test. *p<0.05, **p<0.01, *** p<0.001

Administration of rtPA mitigates neuroinflammation and restores glymphatic-meningeal lymphatic function after SAH in mice

Given the strong correlation between coagulation products and inflammatory cytokines, we administered rtPA (a fibrinolytic agent) to mitigate the coagulation response and neuroinflammation in CSF following SAH in mice. A total of 42 mice were used in this part of the study (14 mice per group).

The levels of the top four cytokines exhibiting the strongest correlation with Fibrin, as shown in Fig. 4B, were evaluated. As depicted in Fig. 5A, the levels of IL-1β, IL-5, IL-17, and GM-CSF in the brain at 24 h post-SAH were markedly increased (compared to Sham, $p<0.05$) and significantly reduced following rtPA administration (compared to SAH+Vehicle, $p<0.05$). Similarly, brain water content at 24 h post-SAH was elevated (compared to Sham, $p<0.05$) but decreased after rtPA treatment (compared to SAH+Vehicle, $p<0.05$, **Fig**. 5B). Neurological deficits induced by SAH were also attenuated by rtPA administration (compared to SAH+Vehicle, $p<0.05$, **Fig**. 5B).

Our previous work demonstrated that blood coagulation and neuroinflammatory responses in CSF directly impaired glymphatic-meningeal lymphatic function, leading to cerebral edema and hydrocephalus after SAH in rodents [12]. Here, we examined the effect of rtPA on glymphatic-meningeal lymphatic function at 24 h post-SAH. As shown in Fig. 5C, the rate of Alexa Fluor 594 penetration into the brain was significantly reduced after SAH (compared to Sham, $p<0.05$) but was reversed by rtPA administration (compared to SAH+Vehicle, $p<0.05$). Additionally, the rate of CSF tracer fluorescent bead drainage into mLVs and dCLNs was significantly reduced after SAH (compared to Sham, $p<0.05$) and was restored by rtPA administration (compared to SAH+Vehicle, $p<0.05$, **Fig**. 5D, E). SAH-induced impairment of mLVs was also reversed by rtPA treatment (compared to SAH+Vehicle, $p<0.05$).

Discussion

In this study, we investigated the acute immune response and coagulation activity in the CSF of patients with SAH in relation to the development of severe cerebral edema and chronic hydrocephalus. Our findings indicated that elevated levels of inflammatory cytokines and coagulation factors in the CSF were closely associated with the severity of cerebral edema and the occurrence of chronic

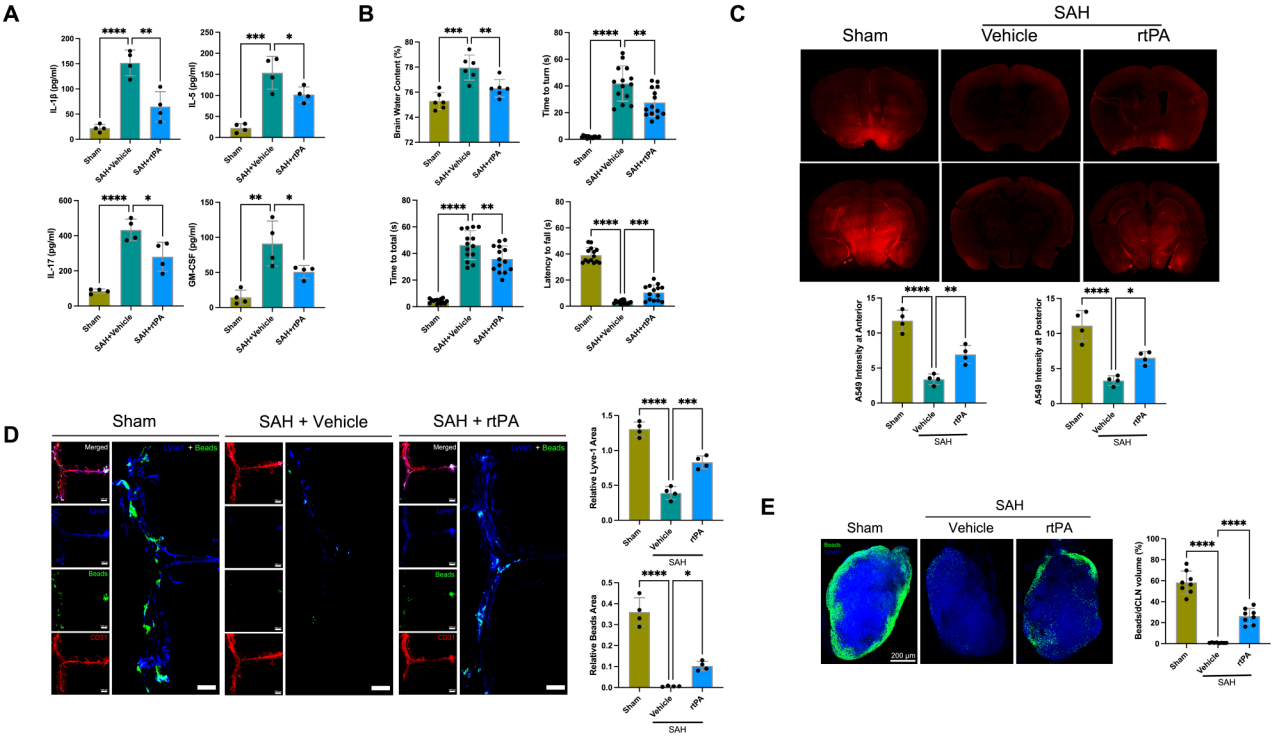


Fig. 5 rtPA administration reversed SAH induced neuroinflammation, cerebral edema and neurological deficits potentially by restoring glymphatic-lymphatic meningeal function in mice. **A**. Levels of neuroinflammation cytokines, $n=4$ per group. **B**. Brain water content ($n=6$ per group) and neurofunction scores ($n=14$ per group, tested by each animal). C. Representative image and quantification of the intensity of A594 penetration into the brain parenchyma, $n=4$ per group. D. Representative image and quantification of the mLVs area and beads area, $n=4$ per group. (E) Representative image and quantification of the beads volume at dCLN, $n=4$ per group. All data was represented as mean ± SD. * $P<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. One-way ANOVA, Tukey's post hoc test

hydrocephalus following SAH. Specifically, the key findings are as follows: (1) Levels of extrinsic coagulation pathway factors and inflammatory cytokines in CSF were associated with severe cerebral edema, chronic hydrocephalus, and poor 3-month outcomes after SAH. Additionally, extrinsic coagulation pathway factors were linked to the occurrence of DCI post-SAH; (2) Coagulation products, TAT and Fibrin, along with inflammatory cytokines IL-1β, IL-2, IL-5, IL-7, and IL-4 in CSF, independently contributed to severe cerebral edema after SAH; (3) Levels of Factor VII, Fibrin, and inflammatory cytokines IL-2, IL-5, IL-12, TNF-α, and CCL-4 in CSF independently contributed to chronic hydrocephalus post-SAH; (4) A positive correlation was observed between extrinsic coagulation pathway factors and inflammatory cytokines following SAH; (5) Administration of rtPA mitigated neuroinflammation and cerebral edema, potentially by restoring glymphatic-meningeal lymphatic function in mice after SAH.

The role of neuroinflammation in the development of cerebral edema and hydrocephalus following SAH has been extensively discussed [27, 28]. In experimental studies, levels of TNF-α and IL-1β were observed to increase in both serum and brain after SAH. Inhibiting IL-1β converting enzyme resulted in a reduction of cerebral edema and EBI [29]. Clinical studies have also shown elevated levels of cytokines, including IL-1β, IL-4, IL-6, IL-8, IL-10, IL-15, IL-17, IL-18, TNF-α, CCL-1, and IFN-γ, in both serum and CSF during the first 7 days post-SAH [30, 31]. Moreover, significantly higher levels of IL-1β, IL-5, IL-6, IL-8, IL-10, IFN-γ, and TNF-α were noted in the high WFNS group and poor prognosis group compared to the low WFNS group and favorable prognosis group [31]. Similarly, our study revealed that elevated levels of inflammatory cytokines in CSF were associated with the severity of cerebral edema during the first 3 days post-SAH and with outcomes at the 3-month follow-up. Specifically, high levels of IL-1β, IL-2, IL-5, and IL-7 independently contributed to severe cerebral edema in SAH patients, while high levels of IL-4 independently contributed to mild cerebral edema. Research on the roles of IL-2, IL-4, IL-5, and IL-7 in SAH is limited; however, prior studies have demonstrated that an IL-2 antagonist mitigates cerebral edema and neurological deficits after traumatic brain injury in mice [32], and IL-4 administration reduces cerebral edema and neurological deficits following ICH in mice [33].

Although research linking SAH-induced early inflammatory cytokine cascades to chronic hydrocephalus is limited, several studies have demonstrated correlations between elevated levels of inflammatory cytokines in CSF and brain tissue and clinical severity after IVH [34]. Additionally, higher levels of IL-6, IL-8, and IL-10 in CSF were observed in children with non-obstructive hydrocephalus compared to those with obstructive hydrocephalus [35]. Our study revealed that levels of inflammatory cytokines, including IL-6 and IL-10, in CSF were associated with the development of chronic hydrocephalus post-SAH. Furthermore, high levels of IL-2, IL-5, and IL-7 in CSF independently contributed to the formation of chronic hydrocephalus. In contrast, IL-4 and IL-10 appeared to have protective roles against chronic hydrocephalus following SAH. Research on the role of IL-2, IL-5, IL-7, and IL-4 in the development of chronic hydrocephalus after SAH remains scarce. However, IL-10 has been widely recognized as a protective cytokine in hydrocephalus, suppressing the production of other pro-inflammatory cytokines such as IL-1β and TNF-α [36].

Following aneurysm rupture, the influx of a substantial volume of blood into the CSF results in a significant elevation of coagulation factors and products within the CSF [12]. Our previous study revealed that intrinsic (Factors IX and XI), extrinsic (Factors III and VII), common pathway factors (Factor XIII), and coagulation products TAT and Fibrin all increased in CSF following SAH [12]. Levels of TAT and prothrombin fragment F1+2 in CSF were significantly elevated in SAH patients compared to both blood samples and CSF from control patients, with even higher levels observed in SAH patients with vasospasm. Furthermore, Factor III levels in the CSF of SAH patients were significantly increased compared to their blood samples [37]. Elevated thrombin concentration and activity in CSF have been associated with poor functional outcomes at 6 weeks and 6 months in patients with intracerebral hemorrhage [38]. Intracisternal fibrinolysis has been shown to significantly reduce poor neurological outcomes, the incidence of DCI, chronic hydrocephalus, and mortality [39].

In this study, we demonstrated that high levels of extrinsic and common coagulation factors were associated with severe cerebral edema, chronic hydrocephalus, DCI, and poor 3-month outcomes following SAH. Specifically, increased levels of Fibrin and TAT independently contributed to severe cerebral edema, while elevated levels of Fibrin and Factor VII independently contributed to chronic hydrocephalus. These findings align with preclinical studies, where inhibition of thrombin or Factor III improved glymphatic function, alleviating cerebral edema and neurological deficits related to hydrocephalus in rodent models [12, 40, 41].

It is well recognized that extensive cross-talk occurs between the coagulation and inflammatory systems in the cardiovascular system [42]. Coagulation activation is mediated by inflammatory activities, such as IL-1β, IL-6, and TNF-α. The main mechanisms of coagulation disruption during systemic inflammation involve Factor III (tissue factor)-mediated thrombin generation and dysfunction of normal anticoagulant processes, with

impaired fibrin removal due to suppression of the fibrinolytic system [42]. Coagulation activation and fibrin deposition in response to inflammation further promote localized inflammatory activity at the site of injury or infection. Thrombin and Fibrin can directly stimulate mononuclear cells and endothelial cells to produce cytokines and chemokines, including TNF-α, IL-1β, IL-6, IL-8, and CCL-2 [43, 44]. While thrombin is considered a cause of peri-hematoma inflammation in intracerebral hemorrhage, the correlation between coagulation and inflammation in the brain, particularly in CSF, after SAH remains unclear.

In this study, we found that the coagulation response in CSF after SAH was predominantly dependent on the extrinsic coagulation pathway and was highly correlated with inflammatory cytokines. Furthermore, the administration of intracisternal fibrinolytics (rtPA) significantly reduced inflammatory cytokine levels and cerebral edema at 24 h post-SAH in mice. The potential mechanism may involve the restoration of glymphatic flow and meningeal lymphatic drainage, both of which are obstructed by blood and impaired by inflammatory cytokines. These preclinical findings support the potential of intraventricular rtPA administration in patients with SAH, offering a promising approach for improving outcomes and reducing the incidence of DCI and hydrocephalus [39]. However, previous randomized controlled trials have shown that rtPA administration may induce a transient local inflammatory response, the severity of which is closely associated with the degree of fibrinolysis, suggesting that it may be triggered by the release of hematoma breakdown products rather than the drug itself [45]. In our study, rtPA was administered immediately following blood injection, which may have resulted in lower pro-inflammatory effects and more prolonged anti-inflammatory effects by accelerating CSF circulation.

Several limitations exist in this study. First, it is a single-center observational study with a relatively small sample size, which may introduce potential confounders and bias. Nonetheless, investigating the dynamic pattern of serially measured cytokines in relation to changes in cerebral edema and the development of chronic hydrocephalus could provide valuable insights into the underlying mechanisms. Future studies should aim to collect more data to further establish the association between cytokine dynamics and cerebral edema as well as hydrocephalus. Second, patients underwent either aneurysm clipping or coiling treatments, which could potentially impact clinical outcomes. However, a detailed analysis of demographics, clinical data, and outcomes for SAH patients in both groups was performed (Supplementary Table 3 in **Additional file 2**). Although the clipping group was younger, no significant differences were found in demographic or clinical data (particularly SEBES scores, DCI rates, hydrocephalus rates, and 3-month outcomes). Moreover, SEBES scores were based on admission CT scans, and CSF samples were collected prior to surgery, reducing the likelihood of surgical choice influencing these measures. Third, while this study and our previous preclinical research provide partial explanations, the exact mechanisms involving inflammation and coagulation-mediated cerebral edema and hydrocephalus formation remain elusive. There is evidence that these processes may be related to alterations in the glymphatic-meningeal lymphatic system after SAH [11, 12]. Lastly, although we identified several cytokines (IL-2, IL-5, IL-7, and IL-4) associated with severe cerebral edema and chronic hydrocephalus, the detailed cellular and molecular mechanisms remain unclear and warrant further preclinical studies.

Conclusions

The present study demonstrates that elevated levels of inflammatory cytokines and extrinsic coagulation pathway factors in the CSF are associated with the development of severe cerebral edema and chronic hydrocephalus following SAH. Extrinsic coagulation pathway factors were positively correlated with inflammatory cytokines in CSF after SAH. Inhibition of coagulation by rtPA in CSF may mitigate the neuroinflammatory response and cerebral edema by restoring post-SAH glymphatic-meningeal lymphatic function.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

Y.F, S.C, X.W, JH.Z and J.Y contributed to the design of study. Y.F, X.W and J.W performed the clinical sample collection and analysis, medical data recording and statistical analysis. S.C, J.Z and Y.F performed radiological image analysis and statistical analysis. Y.L, H.Z, A.Z and J.Z carried out the animal study and statistical analysis. Y.F, L.C, and S.C wrote the manuscript. S.T revised the manuscript. All authors reviewed the manuscript and approved the final version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Institutional Review Board of the Second Affiliated Hospital of Zhejiang University School of Medicine (No. 2023-059). Informed consent was obtained from the patients or their family members, or was waived by the Institutional Review Board. All animal procedures were approved by the Animal Use and Care Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine, Guangzhou, China.

Consent for publication
All authors have approved the manuscript for publication.

Competing interests
The authors declare no competing interests.

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