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Biomarkers for stroke recovery

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Biomarkers for stroke recovery

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Editorial: Biomarkers for stroke recovery

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Editorial on the Research Topic
Biomarkers for stroke recovery

Stroke is a significant contributor to disability and mortality worldwide, posing a substantial burden on individuals, families, and healthcare systems (1, 2). Despite advancements in acute interventions like thrombolysis and mechanical thrombectomy, stroke patients' post-stroke recovery process remains intricate and multi-dimensional, often falling short of optimal patient outcomes (3–5). Several factors influence the complex nature of stroke recovery, such as stroke severity, lesion location, comorbidities, and patient characteristics (6–8). To improve stroke care, it is crucial to comprehend the biological mechanisms underlying stroke recovery and identify reliable biomarkers that can predict recovery outcomes (9–11).

Blood biomarkers are advantageous as a source of biomarkers due to their easy accessibility, non-invasiveness, and the ability to detect systemic changes associated with stroke (12, 13). Several promising blood biomarkers, such as inflammatory markers like C-reactive protein (CRP) and interleukin-6 (IL-6), as well as neurotrophic factors like brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), have been associated with stroke recovery (14–17). While these biomarkers show potential as prognostic tools, their reliability is limited due to patient variability resulting from co-morbidities, demographics, and the type of stroke (18, 19).

The Research Topic titled "*Biomarkers for stroke recovery*" in Frontiers in Neurology was aimed at providing new insights into the application of biomarkers in predicting stroke outcomes and identifying potential treatment targets. It comprises 19 articles that investigate different biomarkers for stroke recovery. Zhou et al. found that neurofilament light chain and S100B serum levels were associated with disease severity and outcome in patients with aneurysmal subarachnoid hemorrhage (aSAH). This study highlights the potential of these biomarkers in predicting outcomes and monitoring disease progression in aSAH patients. He et al. investigated the relationship between hyper-homocysteinemia and poor postoperative angiogenesis in adult patients with Moyamoya disease. The study found that hyperhomocysteinemia was a predictor for poor postoperative angiogenesis with this particular cerebrovascular disease. Using a discovery-based SWATH-MS proteomic approach, Misra et al. identified a panel of blood-based protein biomarkers for the diagnosis of acute stroke. The study highlights the potential for these biomarkers to enable earlier treatment and improve patient outcomes. Ye et al. developed and validated a nomogram that utilizes serum amyloid A as a biomarker to predict the risk of cognitive impairment after lacunar infarction. The authors suggest that this blood-based biomarker has potential for identifying at-risk patients and facilitating early interventions to improve patient outcomes

treatment strategies. Wang et al. conducted a matched cohort study to determine the relationship between the mean of 24-h venous blood glucose and in-hospital mortality among patients with subarachnoid hemorrhage. The study found that high mean 24-h venous blood glucose levels were associated with increased in-hospital mortality, which could provide an early warning for additional care for these patients.

Mo et al. explored the dual function of microglial polarization and its treatment targets in ischemic stroke. The study found that regulating microglial polarization could be a potential treatment strategy for ischemic stroke. Zhong C. et al. analyzed the association between high serum anion gap and all-cause mortality in non-traumatic subarachnoid hemorrhage. The study found that a high serum anion gap was correlated with increased all-cause mortality in patients with non-traumatic subarachnoid hemorrhage. Peng et al. investigated the relationship between red blood cell distribution width and post-stroke fatigue in the acute phase of acute ischemic stroke. High red blood cell distribution width levels were associated with increased post-stroke fatigue that affects many stroke patients.

Chen et al. evaluated the predictive value of the neutrophil-to-lymphocyte ratio on stroke outcome after intravenous thrombolysis and mechanical thrombectomy. High neutrophil-to-lymphocyte ratio on day 1 was a predictor of poor stroke outcome, which could be used for early identification of patients requiring additional care. Zhong J. et al. investigated the predictive value of reduced plasma levels of RGM-A on stroke-associated pneumonia in patients with acute ischemic stroke, in which stroke patient have a 30% infection rate leading to poorer clinical outcomes (20). The study found that reduced plasma levels of RGM-A could predict stroke-associated pneumonia in patients with acute ischemic stroke. Zhang et al. evaluated the association of the systemic immune-inflammation index (SII) with carotid plaque vulnerability in patients.

Chen et al. investigated the longitudinal changes in the hypothalamic-pituitary-adrenal axis and sympathetic nervous system in relation to stroke prognosis. The authors demonstrated that changes in these systems are linked to worse patient outcomes, highlighting the need for targeted interventions to improve patient outcomes. Yao et al. examined the effect of mean heart rate on 30-day mortality in ischemic stroke patients with atrial fibrillation using data from the MIMIC-IV database. Their findings suggest that higher mean heart rate is associated with an increased risk of mortality, underscoring the need for targeted interventions to reduce heart rate and improve patient outcomes. A systematic review and meta-analysis by Huang et al. investigated the association of stress hyperglycemia ratio (SHR) with clinical outcomes in patients with stroke. The study found that elevated SHR was significantly associated with poor outcomes, including increased mortality, disability, and length of hospital stay. This suggests that monitoring SHR may be a useful biomarker for predicting stroke recovery. Another study by Jiang et al. explored the use of the monocyte-to-lymphocyte ratio (MLR) as a biomarker for acute kidney injury (AKI) after acute hemorrhagic stroke. The results indicated that MLR was a significant predictor of AKI, and higher MLR values were associated with a greater risk of AKI. This finding highlights the potential of MLR as a prognostic biomarker for stroke recovery. In addition, Guo et al. investigated the use of optic nerve sheath diameter (ONSD) and ONSD/eyeball transverse diameter (ETD) ratio as biomarkers for predicting malignant progression in ischemic stroke. The study found that elevated ONSD and ONSD/ETD ratio were significantly associated with malignant progression, indicating their potential as biomarkers for stroke recovery.

Another promising avenue of biomarker research in stroke recovery is the use of imaging biomarkers. Neuroimaging techniques, such as magnetic resonance imaging (MRI) and positron emission tomography (PET), can provide insights into structural and functional changes in the brain following stroke. Several imaging biomarkers have been proposed as potential predictors of recovery outcomes, including measures of white matter integrity, cortical thickness, and functional connectivity. Imaging biomarkers offer unique advantages, such as the ability to detect changes in specific brain regions, their utility is limited by the high cost and technical expertise required for acquisition and analysis. Wei et al. investigated the potential of combining cerebral small vessel disease with cerebral collaterals to predict the prognosis of patients with acute large artery atherosclerotic stroke. Their study suggests that this approach could be useful for predicting patient outcomes and developing personalized treatment strategies. Tahmi et al. evaluated neuroimaging biomarkers of cognitive recovery after ischemic stroke. The study found that different neuroimaging techniques could be used to predict cognitive recovery after ischemic stroke. In addition, there is a need for larger, multicentric studies that can validate and replicate findings across different populations and settings.

In conclusion, the "*Biomarkers for stroke recovery*" Research Topic provides a comprehensive overview of the latest advances in this field. The 19 studies included in this topic demonstrate the potential of biomarkers to predict stroke recovery and improve treatment outcomes. The research presented in this topic underscores the importance of further investigations to validate these biomarkers and their role in clinical practice. We hope that this Research Topic will stimulate further research in this field and pave the way for improved stroke recovery outcomes.

Author contributions

PK conceptualized the idea of this research topic and drafted the editorial. KP contributed to writing the editorial to its final version. Both authors approved the submitted version for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, et al. Global and regional burden of stroke during 1990–2010: findings from the Global Burden of Disease Study 2010. *Lancet.* (2014) 383:245–54. doi: 10.1016/s0140-6736(13)61953-4

2. GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* (2019) 18:459–80. doi: 10.1016/S1474-4422(18)30499-X

3. Parr E, Ferdinand P, Roffe C. Management of acute stroke in the older person. *Geriatrics.* (2017) 2:27. doi: 10.3390/geriatrics2030027

4. Tawil SE, Muir KW. Thrombolysis and thrombectomy for acute ischaemic stroke. *Clin Med.* (2017) 17:161. doi: 10.7861/clinmedicine.17-2-161

5. Reverté-Villarroya S, Dávalos A, Font-Mayolas S, Berenguer-Poblet M, Sauras-Colón E, López-Pablo C, et al. Coping strategies, quality of life, and neurological outcome in patients treated with mechanical thrombectomy after an acute ischemic stroke. *Int J Environ Res Public Health.* (2020) 17:6014. doi: 10.3390/ijerph17176014

6. Boehme AK, Esenwa C, Elkind MSV. Stroke risk factors, genetics, and prevention. *Circ Res.* (2017) 120:472–95. doi: 10.1161/CIRCRESAHA.116.308398

7. Nath M, Swarnkar P, Sharma R, Kumar A, Misra S, Kumar P. Association of modifiable risk factors with ischaemic stroke subtypes in Asian versus Caucasian populations: a systematic review and meta-analysis. *Eur J Clin Invest.* (2022) 52:e13849. doi: 10.1111/eci.13849

8. Chauhan G, Debette S. Genetic risk factors for ischemic and hemorrhagic stroke. *Curr Cardiol Rep.* (2016) 18:124. doi: 10.1007/s11886-016-0804-z

9. Grefkes C, Fink GR. Recovery from stroke: current concepts and future perspectives. *Neurol Res Pract.* (2020) 2:17. doi: 10.1186/s42466-020-00060-6

10. Kane E, Ward NS. Neurobiology of stroke recovery. In: Platz T, editor. *Clinical Pathways in Stroke Rehabilitation: Evidence-based Clinical Practice Recommendations*. Cham: Springer. (2021). Available online at: http://www.ncbi.nlm.nih.gov/books/NBK585590/ (accessed February 20, 2023).

11. Boyd LA, Hayward KS, Ward NS, Stinear CM, Rosso C, Fisher RJ, et al. Biomarkers of stroke recovery: consensus-based core recommendations from the stroke recovery and rehabilitation roundtable. *Neurorehabil Neural Repair.* (2017) 31:864–76. doi: 10.1177/1545968317732680

12. Dunckley T, Coon KD, Stephan DA. Discovery and development of biomarkers of neurological disease. *Drug Discov Today.* (2005) 10:326–34. doi: 10.1016/S1359-6446(04)03353-7

13. Mayeux R. Biomarkers: potential uses and limitations. *NeuroRx J Am Soc Exp Neurother.* (2004) 1:182–8. doi: 10.1602/neurorx.1.2.182

14. Lasek-Bal A, Jedrzejowska-Szypulka H, Student S, Warsz-Wianecka A, Zareba K, Puz P, et al. The importance of selected markers of inflammation and blood-brain barrier damage for short-term ischemic stroke prognosis. *J Physiol Pharmacol.* (2019) 70:209–17. doi: 10.26402/jpp.2019.2.04

15. Wiseman S, Marlborough F, Doubal F, Webb DJ, Wardlaw J. Blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-lacunar stroke and non-stroke: systematic review and meta-analysis. *Cerebrovasc Dis.* (2014) 37:64–75. doi: 10.1159/000356789

16. Ahmad O, Wardlaw J, Whiteley WN. Correlation of levels of neuronal and glial markers with radiological measures of infarct volume in ischaemic stroke: a systematic review. *Cerebrovasc Dis.* (2012) 33:47–54. doi: 10.1159/000332810

17. Escudero C, Acurio J, López E, Rodríguez A, Benavente A, Lara E, et al. Vascular endothelial growth factor and poor prognosis after ischaemic stroke. *Eur J Neurol.* (2021) 28:1759–64. doi: 10.1111/ene.14641

18. Kamtchum-Tatuene J, Jickling GC. Blood biomarkers for stroke diagnosis and management. *Neuromolecular Med.* (2019) 21:344–68. doi: 10.1007/s12017-019-08530-0

19. Simpkins AN, Janowski M, Oz HS, Roberts J, Bix G, Doré S, et al. Biomarker application for precision medicine in stroke. *Transl Stroke Res.* (2020) 11:615–27. doi: 10.1007/s12975-019-00762-3

20. Westendorp WF, Nederkoorn PJ, Vermeij J-D, Dijkgraaf MG, van de Beek D. Post-stroke infection: a systematic review and meta-analysis. *BMC Neurol.* (2011) 11:110. doi: 10.1186/1471-2377-11-110

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Hyperhomocysteinemia Is a Predictor for Poor Postoperative Angiogenesis in Adult Patients With Moyamoya Disease

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Background and Purposes: The risk factors of poor postoperative angiogenesis in moyamoya disease (MMD) patients remain unknown. We aimed to investigate the association between hyperhomocysteinemia (HHcy) and postoperative angiogenesis of adult patients with MMD.

Methods: A total of 138 adult patients with MMD were prospectively recruited from July 1 to December 31, 2019. After excluding 10 patients accepting conservative therapy and 77 individuals without postoperative digital subtraction angiography (DSA), all 51 MMD patients were enrolled, and 28 patients received bilateral operations separately. Patients were grouped according to postoperative angiogenesis and HHcy presentation, respectively. Clinical data and laboratory examinations were compared. Potential risk factors were evaluated by univariate and multivariate logistic regression analysis. Nomogram was further performed. The biological functions of homocysteine (Hcy) were explored *in vitro*.

Results: Comparing to the normal, patients with poor postoperative angiogenesis were higher in serum Hcy ($p = 0.004$), HHcy ratio ($p = 0.011$), creatinine (Cr) ($p < 0.001$), uric acid (UA) ($p = 0.036$), Triglyceride ($p = 0.001$), high-density lipoprotein cholesterol (HDL-C) ($p = 0.001$), low-density lipoprotein cholesterol (LDL-C) ($p = 0.009$), ApoA ($p = 0.022$), apolipoprotein B (ApoB) ($p = 0.013$). Furthermore, HHcy was more common in men ($p = 0.003$) than women. Logistic analysis results showed that Hcy ($OR = 0.817$, 95% $CI = 0.707$–0.944, $p = 0.006$) was an independent risk factor. HHcy and Cr were significantly associated with poor postoperative angiogenesis in MMD patients. Further, Hcy could inhibit the proliferation, migration, and tube formation of human brain microvascular endothelial cells (HBMECs), which can be reversed by vascular endothelial growth factor (VEGF).

Conclusion: The HHcy was significantly correlated with poor postoperative angiogenesis in adult patients with MMD. Hcy significantly inhibits HBMECs proliferation, migration, and tube formation. Furthermore, VEGF could reverse the inhibition effect induced by Hcy. Lowering the level of Hcy may be beneficial for postoperative MMD patients. Focusing on the pathophysiology and mechanism of HHcy might help to guide postoperative clinical management.

Keywords: moyamoya disease, angiogenesis, risk factor, homocysteine, hyperhomocysteinemia, prognosis

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INTRODUCTION

Moyamoya disease (MMD) is a rare cerebrovascular disease characterized by progressive stenosis of the intracranial internal carotid arteries (ICA) whose major branches with the emergence of co-existing compensatory abnormal net-like vessels (1–4). MMD is a major cause of stroke in children and young adults and has been observed in different ethnic backgrounds throughout the world, which is reported to be most common in Asian countries such as China, Japan, and Korea (5, 6). In surgical practice, indirect, direct, or combined revascularization is frequently applied, but the risk factors affecting poor postoperative angiogenesis need further research.

Homocysteine (Hcy) is reported to be a sulfur-containing amino acid, and an important intermediate in folate, vitamin B12, and one-carbon metabolism (7). It was reported that the genetic factors such as the mutation in 5,10-methylenetetrahydrofolate reductase (MTHFR) can change the plasma Hcy level. For normal and healthy individuals, the Hcy level in serum is between 5 and 15 μM, and an increase exceeding 16 μM is called hyperhomocysteinemia (HHcy) which may be harmful to vessels (8–10). Earlier studies have reported that the HHcy as an independent risk factor for poor health, such as cancer, coronary, Parkinson's disease, and Alzheimer's disease (8, 10–12). Recently, Ge et al. showed that the Hcy was associated with higher ischemic complications rates in MMD patients (3). However, the postoperative follow-up was limited, and the role of Hcy in postoperative angiogenesis remains unclear.

In our current study, the characteristics of adult MMD patients who underwent surgical options were collected to explore the relationship between Hcy and postoperative angiogenesis and performed experiments to explore the potential role of Hcy on brain vessels.

METHODS

Study Participants

A total of 138 adult patients with MMD were prospectively recruited from July 1 to December 31, 2019 at the Department of Neurosurgery, Beijing Tiantan Hospital. A total of 10 individuals did not receive surgical treatment and 77 individuals without postoperative digital subtraction angiography (DSA) from the previous cohort were excluded. Finally, 51 MMD patients were prospectively enrolled in total, and 28 patients received bilateral operations separately. Guidelines of the Research Committee on Spontaneous Occlusion of the Circle of Willis in 2012 were used to diagnose MMD byDSA (13). All participants were signed the informed consent. The study was approved by the Ethics Committee of Beijing Tiantan Hospital, Capital Medical University (No. KY2016-048-01). The patients were grouped according to postoperative angiogenesis and HHcy presentation, respectively.

Data Collection

The possible risk factors associated with poor postoperative angiogenesis were obtained, such as demographic data, clinical features, laboratory examinations, image examination, and surgical options. Age and sex are included in demographic information. Blood pressure, heart rate, and body mass index (BMI) were considered in clinical features. Laboratory examinations were levels of Hcy, blood glucose (Glu), albumin (ALB), total cholesterol (TC), triglyceride (TG), apolipoprotein A (ApoA), apolipoprotein B (ApoB), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), uric acid (UA), creatinine (Cr), white blood cells (WBC), and platelets (PLT). Suzuki stage was considered as imaging findings. Surgical options were summarized into direct revascularization or non-direct revascularization. After the participants had fasted for over 12 h in the morning, their blood samples were collected. The level of Hcy was extracted from medical records, and the plasma level of Hcy was determined by enzymatic cycling assay. An increase exceeding 15 μmol/L in serum Hcy level was diagnosed as HHcy. All patients accepted routine postoperative therapy and follow-up, including DSA 6 months postoperatively. The postoperative angiogenesis was evaluated independently by 2 senior physicians according to the Matsushima classification (14). In short, the surgical bypass covered the area including (A) more than two-thirds of the middle cerebral artery (MCA) distribution; (B) between two-thirds and one-third; and (C) only one cortical branch or no collateral circulation. Grade A and grade B were all considered to be good postoperative angiogenesis.

Surgical Procedures

The 3 kinds of surgical procedures were used for MMD treatment, such as direct, indirect, and combined revascularization of the two (2). The detailed procedures refer to our previous work. In short, the direct bypass was the anastomosis of the cortical branch of the MCA and the superficial temporal artery (STA). In indirect bypass, the STA branch was isolated and placed on the cortical surface. Both direct and indirect bypass performed on the same hemisphere was called combined bypass.

Statistical Analysis

The SPSS software (version 25.0) and R software (4.0.5) were used to perform the statistical analyses. The Pearson's chi-square test was used to compare the categorical variables. For continuous variables, the *t*-test and Mann–Whitney *U* test were utilized. The study employs logistic regression to investigate the independent factors. The 95% confidence intervals (*CIs*) and odds ratios (*ORs*) were calculated for potential risk factors related to poor postoperative angiogenesis. It was statistically significant when the $p < 0.05$ (two-sided).

RESULTS

Characteristics and Laboratory Examinations of Postoperative Angiogenesis

A total of 79 subjects were analyzed in this study. The characteristics and laboratory examinations of postoperative angiogenesis were summarized in **Table 1**. A total of 37 (46.8%) subjects were in the poor postoperative angiogenesis group, such as 21 men and 16 women. The proportion of men was

TABLE 1 | Baseline characteristics and laboratory examinations of postoperative angiogenesis.

Variables	All patients ($n = 79$)	Postoperative angiogenesis		*P*-value
		Poor ($n = 37$)	Good (n = 42)	
Age, y, median (IQR)	37 (29–47)	40 (32–48)	34 (26–44)	0.01
Sex (%)				0.006
Male	32 (40.5)	21 (65.6)	11 (26.2)	
Female	47 (59.5)	16 (43.2)	31 (73.8)	
Primary symptom (%)				0.731
Infarction	54 (68.4)	26 (70.3)	28 (66.7)	
Non-infarction	25 (31.6)	11 (29.7)	14 (33.3)	
Medical history (%)				
Hypertension	10 (12.7)	6 (16.2)	4 (9.5)	0.372
Diabetes	6 (7.6)	2 (5.4)	4 (9.5)	0.491
Hyperlipidemia	0 (0)	0 (0)	0 (0)	*
Thyroid disease	0 (0)	0 (0)	0 (0)	*
Smoking	10 (12.7)	5 (13.5)	5 (11.9)	0.83
Drinking	4 (5.1)	2 (5.4)	2 (4.8)	0.896
Clinical feature, median (IQR)				
Heart rate, bpm	78 (74–80)	78 (72–80)	78 (75.5–80)	0.928
SBP, mmHg	127 (115–137)	127 (115–138)	122 (114.75–135)	0.297
DBP, mmHg	80 (75–90)	84 (76–94)	78 (74–90)	0.073
BMI, kg/m^2	24.14 (22.64–26.83)	25.35 (23.26–27.68)	22.88 (20.63–25.84)	0.005
Surgical option (%)				0.042
Indirect bypass	46 (58.2)	26 (70.3)	20 (47.6)	
Non-indirect bypass	33 (41.8)	11 (29.7)	22 (52.4)	
Laboratory results, median (IQR)				
WBC count, 109/L	5.60 (5.10–6.60)	5.72 (5.18–6.64)	5.58 (5.08–6.46)	0.883
PLT, 109/L	229 (203–275)	232 (198–272)	228 (213–278)	0.680
Glucose, mmol/L	4.45 (4.18–4.8)	4.45 (4.03–4.87)	4.46 (4.18–4.80)	0.791
Creatinine, μmol/L	53.4 (45.3–64.1)	61.8 (48.85–71.50)	49.4 (43.50–56.98)	<0.001
Uric acid, μmol/L	288.3 (240.4–390.0)	318.8 (264.6–469.4)	281.7 (235.0–376.8)	0.036
Albumin, g/L	41.9 (40.0–44.5)	42.1 (39.8–43.9)	41.4 (40.0–44.9)	0.806
Triglyceride, mmol/L	1.35 (0.92–1.84)	1.78 (1.03–2.03)	1.14 (0.82–1.53)	0.001
Total cholesterol, mmol/L	4.09 (3.58–4.77)	4.24 (3.77–4.86)	3.88 (3.36–4.73)	0.116
HDL-C, mmol/L	1.08 (0.87–1.36)	0.94 (0.82–1.24)	1.23 (1.05–1.41)	0.001
LDL-C, mmol/L	2.58 (2.13–3.17)	2.81 (2.29–3.72)	2.21 (1.95–2.81)	0.009
ApoA, g/L	1.23 (1.07–1.43)	1.16 (1.00–1.26)	1.39 (1.10–1.47)	0.022
ApoB, g/L	0.84 (0.73–0.98)	0.89 (0.81–1.00)	0.77 (0.68–0.93)	0.013
Hcy, μmol/L	14.1 (11.26–16.41)	14.97 (12.01–17.60)	12.65 (8.77–14.68)	0.004
HHcy (%)	27 (34.2)	18 (48.6)	9 (21.4)	0.011
Suzuki stage (%)				0.165
I	1 (1.3)	0 (0)	1 (2.4)	
II	21 (26.6)	13 (35.1)	8 (19.0)	
III	41 (51.9)	16 (43.2)	25 (59.5)	
IV	11 (13.9)	7 (18.9)	4 (9.5)	
V	5 (6.3)	1 (2.7)	4 (9.5)	
VI	0 (0)	0 (0)	0 (0)	
PCA (%)	14 (17.7)	6 (16.2)	8 (19.0)	0.742

*SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; Hcy, homocysteine; HHcy, hyperhomocysteinemia; SD, standard deviation; IQR, interquartile range. Suzuki staging and posterior circulation involvement were defined on the operative side. *P < 0.05, significant difference.*

TABLE 2 | Clinical characteristics of patients according to hyperhomocysteinemia (Hhcy).

Variables	Hyperhomocysteinemia		*P*-value
	Absent ($n = 52$)	Present ($n = 27$)	
Age, y, mean (SD)	34 (28.3–44)	44 (32–47)	0.046
Sex (%)			0.003
Male	15 (28.8)	17 (63.0)	
Female	37 (71.2)	10 (37.0)	
Primary symptom (%)			0.005
Infarction	30 (57.7)	24 (88.9)	
Non-infarction	22 (42.3)	3 (11.1)	
Medical history (%)			
Hypertension	4 (7.7)	6 (22.2)	0.082
Diabetes	4 (7.7)	2 (7.4)	1
Hyperlipidemia	0 (0)	0 (0)	*
Thyroid disease	0 (0)	0 (0)	*
Smoking	6 (11.5)	4 (14.8)	0.728
Drinking	2 (3.8)	2 (7.4)	0.603
Clinical feature, mean (SD)			
Heart rate, bpm	78 (73–80)	80 (78–80)	0.135
SBP, mmHg	121 (114–135)	134 (118–140)	0.020
DBP, mmHg	78 (74–90)	90 (79–98)	0.025
BMI, kg/m^2	23.19 (20.70–24.84)	26.67 (23.83–27.68)	0.001
Surgical option (%)			0.539
Indirect bypass	29 (55.8)	17 (63.0)	
Non-indirect bypass	23 (44.2)	10 (37.0)	
Laboratory results, median (IQR)			
WBC count, 109/L	5.45 (4.97–6.40)	6.22 (5.60–7.10)	0.003
PLT, 109/L	229 (212–277)	230 (173–272)	0.304
Glucose, mmol/L	4.47 (4.18–4.97)	4.4 (3.91–4.72)	0.363
Creatinine, μmol/L	51.15 (43.9–60.8)	57.2 (47.9–71.4)	0.013
Uric acid, μmol/L	275.5 (234.9–339.1)	332.1 (287.9–469.0)	0.004
Albumin, g/L	41.4 (39.5–44.1)	43.0 (40.7–45.1)	0.111
Triglyceride, mmol/L	1.21 (0.78–1.69)	1.66 (1.03–2.09)	0.006
Total cholesterol, mmol/L	3.91 (3.45–4.78)	4.22 (3.67–4.60)	0.984
HDL-C, mmol/L	1.22 (0.91–1.36)	0.94 (0.81–1.25)	0.033
LDL-C, mmol/L	2.49 (2.12–3.23)	2.66 (2.21–3.17)	0.788
ApoA, g/L	1.26 (1.10–1.47)	1.16 (1.00–1.35)	0.175
ApoB, g/L	0.85 (0.72–0.98)	0.84 (0.77–1.00)	0.291
Suzuki stage (%)			0.825
I	1 (1.9)	0 (0)	
II	15 (28.8)	6 (22.2)	
III	27 (51.9)	14 (51.9)	
IV	6 (11.5)	5 (18.5)	
V	3 (5.8)	2 (7.4)	
VI	0 (0)	0 (0)	
PCA (%)	8 (15.4)	6 (22.2)	0.450

*SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; Hcy, homocysteine; HHcy, hyperhomocysteinemia; SD, standard deviation; IQR, interquartile range. Suzuki staging and posterior circulation involvement were defined on the operative side. *P < 0.05, significant difference.*

significantly higher in the poor postoperative angiogenesis group (56.8%) than in the good postoperative angiogenesis group (26.2%) ($p = 0.006$). In this cohort, the mean age was 40 ± 8 years. Between groups, subjects were significantly older in the poor postoperative angiogenesis group ($p = 0.001$). As for clinical features, BMI was significantly higher in the poor postoperative angiogenesis group ($p = 0.005$). There was no significant difference in the proportion of infarction as a primary symptom

TABLE 3 | Univariate analysis of risk factors for patients with poor postoperative angiogenesis.

Variables	Univariate analysis		
	OR	95%CI	*P*-value
Age	0.948	0.902–0.996	0.033
Sex			
Female	3.699	1.435–9.532	0.007
Male	*	*	*
Primary symptom (%)			
Non-infarction	1.182	0.456–3.066	0.731
Infarction	*	*	*
Clinical feature, mean (SD)			
Heart rate, bpm	0.992	0.928–1.060	0.813
SBP, mmHg	0.975	0.943–1.008	0.143
DBP, mmHg	0.961	0.918–1.006	0.085
BMI, kg/m^2	0.809	0.693–0.945	0.007
Surgical option (%)			
Indirect bypass	0.385	0.152–0.974	0.044
Non-indirect bypass	*	*	*
Suzuki stage	1.249	0.732–2.132	0.414
Laboratory results, median			
WBC count, 109/L	1.108	0.793–1.548	0.547
LY	0.992	0.429–2.292	0.984
PLT, 109/L	1.004	0.995–1.014	0.387
Glucose, mmol/L	0.825	0.542–1.255	0.368
Creatinine, μmol/L	0.927	0.887–0.969	0.001
Uric acid, μmol/L	0.995	0.991–1.000	0.031
Albumin, g/L	1.026	0.896–1.176	0.708
Triglyceride, mmol/L	0.281	0.122–0.674	0.003
Total cholesterol, mmol/L	0.765	0.440–1.332	0.344
HDL-C, mmol/L	16.142	2.468–105.592	0.004
LDL-C, mmol/L	0.553	0.286–1.068	0.078
ApoA, g/L	10.2	1.202–86.578	0.033
ApoB, g/L	0.047	0.003–0.747	0.03
Hcy	0.905	0.834–0.982	0.016
PCA	1.216	0.379–3.898	0.742

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; Hcy, homocysteine; OR, odds ratio.
Suzuki staging and posterior circulation involvement were defined on the operative side.
**P < 0.05, significant difference.*

($p = 0.731$), while it was 70.3% in the poor postoperative angiogenesis group and 66.7% in the good postoperative angiogenesis group (66.7%). In surgical options, 70.3% of subjects received indirect bypass in the poor postoperative angiogenesis group compared with 47.6% of subjects in the good postoperative angiogenesis group ($p = 0.042$). Those with poor postoperative angiogenesis showed a higher level of Hcy ($p = 0.004$), Cr ($p < 0.001$), UA ($p = 0.036$), TG ($p = 0.001$), HDL-C ($p = 0.001$), LDL-C ($p = 0.009$), ApoA ($p = 0.022$), and ApoB ($p = 0.013$). Meanwhile, the occurrence of HHcy in patients with poor postoperative angiogenesis was also significantly higher ($p = 0.011$).

TABLE 4 | Multivariate analysis on the risk of poor postoperative angiogenesis.

Variables*	Multivariate analysis		
	OR	95%CI	*P*-value
Creatinine, μmol/L	0.881	0.783–0.992	0.037
Hcy	0.817	0.707–0.944	0.006

**Variables not finally entered into the model: age (P = 0.366), sex (P = 0.440), primary symptom (P = 0.186), BMI (P = 0.517), glucose (P = 0.069), uric acid (P = 0.142), albumin (P = 0.496), triglyceride (P = 0.164), total cholesterol (P = 0.214), HDL-c (P = 0.945), LDL-c (P = 0.147), ApoA (P = 0.758), ApoB (P = 0.779), WBC (P = 0.835), LY (P = 0.484), PLT (P = 0.403), surgical option (P = 0.156). BMI, body mass index; Hcy, homocysteine; OR, odds ratio. *P < 0.05, significant difference.*

Clinical Features of Patients According to HHcy

Clinical features of subjects according to HHcy are summarized in **Table 2**. The mean age in the HHcy group was significantly older than in the normal Hcy group ($p = 0.046$). Men in the HHcy group (63.0%) were also more than that in the normal Hcy group (28.8%), which was statistically significant ($p = 0.003$). As for primary symptoms, subjects with infarction as primary presentation in the HHcy group (88.9%) were significantly more than in the normal Hcy group (57.7%) ($p = 0.005$). In clinical features, the level of SBP, DBP, and BMI is statistically significant ($p < 0.05$). In laboratory examinations, the level of WBC ($p = 0.003$), CR ($p = 0.013$), UA ($p = 0.004$), HDL-C ($p = 0.033$), and TG ($p = 0.006$) in the poor postoperative angiogenesis group was significantly higher than in the good postoperative angiogenesis.

Logistic Analysis of Potentially Related Factors Associated With Poor Postoperative Angiogenesis

Potentially related factors for poor postoperative angiogenesis in adult MMD subjects were analyzed. The univariate analysis showed that age ($p = 0.033$), female ($p = 0.007$), BMI ($p = 0.007$), indirect bypass ($p = 0.044$), UA ($p = 0.031$), Cr ($p = 0.001$), TG ($p = 0.003$), HDL-C ($p = 0.004$), ApoA ($p = 0.033$), ApoB ($p = 0.03$), and Hcy ($p = 0.016$) were associated with poor postoperative angiogenesis in univariate logistic analysis (**Table 3**). After adjusting for all potential covariables, the results showed that creatinine ($OR = 0.881$, 95% $CI = 0.783$–0.992, $p = 0.037$) and Hcy ($OR = 0.817$, 95% $CI = 0.707$–0.944, $p = 0.006$) were independent factors related to the poor postoperative angiogenesis (**Table 4**). We also found that the HHcy was significantly associated with poor postoperative angiogenesis.

Nomogram

To establish a predictive model of poor postoperative angiogenesis, we constructed a nomogram based on related factors in multivariate analysis, such as creatinine and Hcy. The nomogram achieved a c-index of 0.779, which reflects good predictive performance. The nomogram is shown in **Figure 1**. We also generated a calibration curve for the nomogram, which

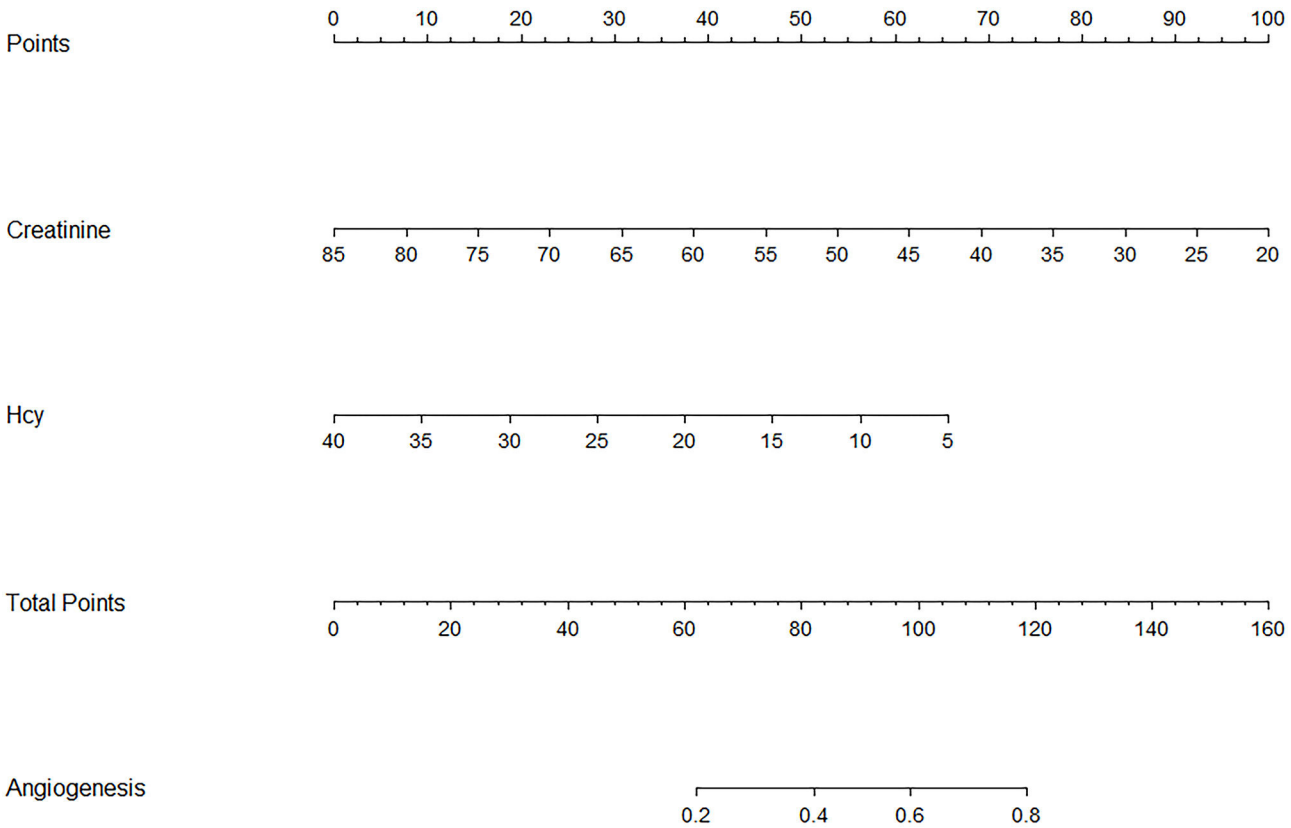

FIGURE 1 | Nomogram of related factors influencing postoperative angiogenesis. Hcy represents homocysteine. Angiogenesis represents the possibility of good postoperative angiogenesis in adult moyamoya disease (MMD) patients.

is shown in **Supplementary Figure 1**. The mean absolute error reached 0.019. Then, we performed the Hosmer–Lemeshow goodness-of-fit test, which indicated that the model was well calibrated ($\chi^2 = 9.1299$, $p = 0.3315$).

Hcy Inhibits Proliferation, Migration, Tube Formation in HBMEC Which Is Reversed by VEGFA

To further study the biological function of Hcy in the angiogenesis of human cerebral vessels, we utilized human brain microvascular endothelial cells (HBMECs) to perform *in vitro* experiments. In the Edu assay, we found that the proliferation was significantly decreased by Hcy and was reversed by vascular endothelial growth factor (VEGF) treatment (**Figure 2A**). In the CCK-8 assay, we further confirmed that the proliferation rate was significantly decreased when treated with Hcy for 72 h ($p < 0.01$), and such effect could be reversed by VEGF (**Figure 2B**). Migration assay and tube formation assay were also performed to explore the influence of Hcy on vessel formation, and the results revealed HBMECs treated with Hcy were significantly decreased in migration ($p < 0.01$) and tube formation (**Figures 2C,D**). After being treated with VEGF, the effect of Hcy on HBMECs was reversed. The results indicated that Hcy inhibits proliferation, migration, and tube formation in HBMECs, and VFGF may become a potential treatment target for those patients with poor postoperative angiogenesis.

DISCUSSION

In this study, we prospectively enrolled adult MMD patients and investigated the potentially related factors of poor postoperative angiogenesis and found the increased level of Hcy ($p = 0.004$) and HHcy ratio ($p = 0.011$) were significantly associated with poor postoperative angiogenesis patients. It suggested that the Hcy plays a vital role in poor postoperative angiogenesis in MMD patients. We also established a nomogram and found patients with lower Hcy level is correlated with a better postoperative angiogenesis in MMD patients. Furthermore, we utilized HBMECs to conduct proliferation, migration, and tube formation assays and showed the inhibition effect of Hcy on cerebral angiogenesis can be reversed by VEGF.

The previous literature reported the link between Hcy and acute ischemic events, such as acute myocardial infarction (15). In this study, we revealed the potential effect of Hcy on long-term postoperative angiogenesis in adult MMD patients. Recently, HHcy was also reported to be associated with brain disorders, such as stroke (16). Our previous studies showed

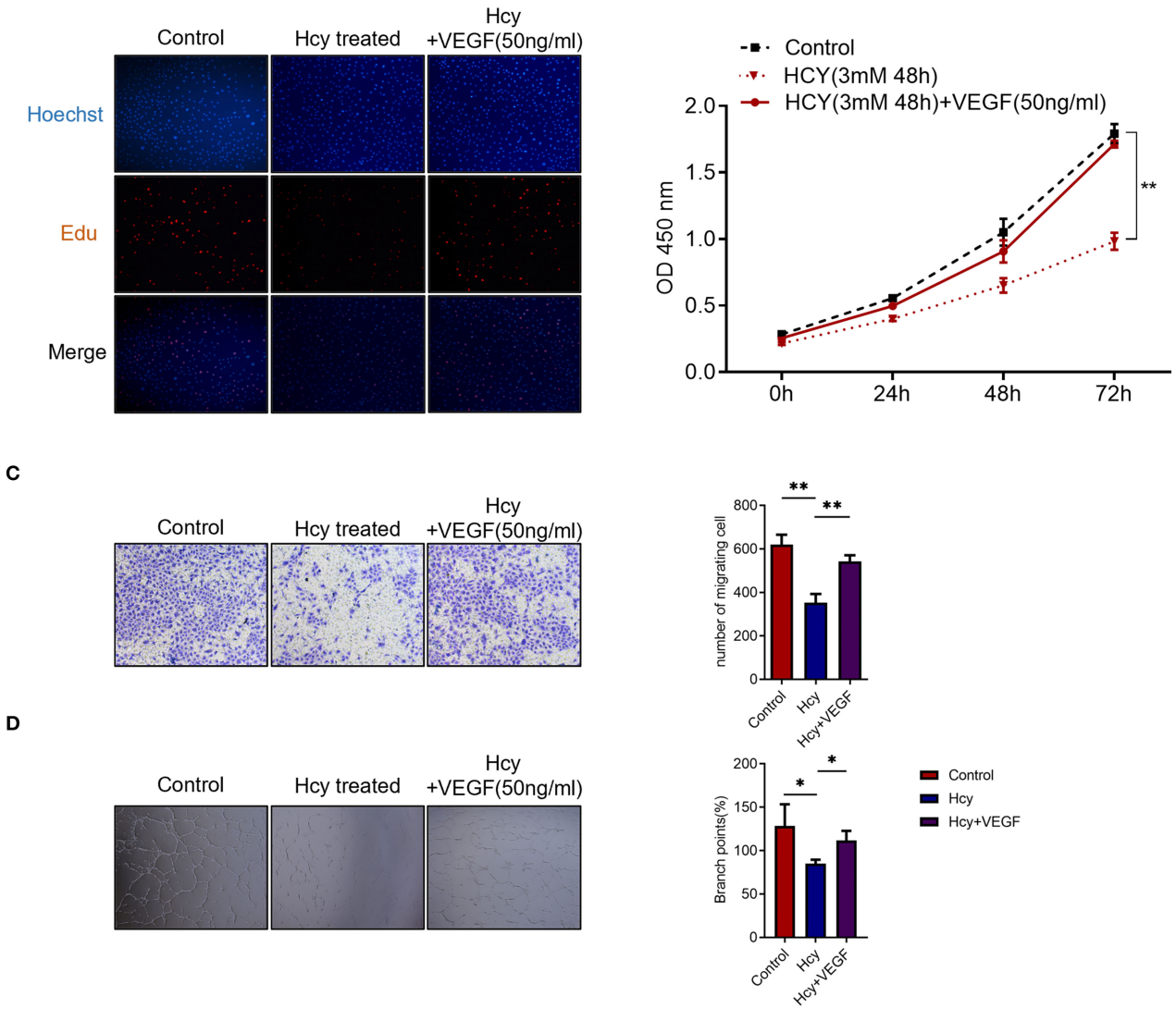

FIGURE 2 | Homocysteine (Hcy) inhibits the HBMECs proliferation, migration, and tube formation, which is reversed by VEGF. **(A,B)** Edu assay and CCK-8 assay of HBMECs treated with Hcy and VEGF165. **(C,D)** Transwell migration assay and tube formation assay of HBMECs treated with Hcy and VEGF165. *$p < 0.05$, **$p < 0.01$.

that the HHcy was associated with a higher risk of MMD and was correlated with postoperative acute ischemia within 7 days (3, 17). However, the relationship between poor cerebral postoperative angiogenesis and HHcy is not well-understood.

The Hcy, a key metabolite of methionine, is thought to participate in a variety of biological processes (18–20). Although how Hcy is involved in the pathogenesis of MMD is unclear, several possible mechanisms were reported in diseases. Recently, advances have shown that increased Hcy in serum level is a primary cause of cardiovascular diseases, diabetes, neurodegenerative diseases, and so on. Hcy was also involved in the initiation and progression of atherosclerosis by inhibiting the expression of miR-195-3p and in turn, enhancing the inflammation through IL-31 (21). In cardiovascular diseases, Hcy was reported to cause endothelial dysfunction through ENaC, or the toxicity related to iron containing proteins. Some studies reported that the Hcy could induce cell injury *via* Akt/eNOS pathway (22, 23). However, the possible role of Hcy on cerebral endothelial cells needs further research.

In MMD patients, the key mechanism leading to HHcy was thought to be associated with the mutation in MTHFR, which can interrupt the Hcy metabolism (24). And studies elucidated that Hcy may be involved in the pathogenesis of MMD by increasing MMP-9 in the vascular wall to induce inflammation (25, 26), but how Hcy is involved in postoperative cerebral angiogenesis remains unclear. Sato et al.

reported that in STA–MCA bypass operations, HHcy is a risk factor for unsuccessful revascularization because it causes hypercoagulation (27). It also suggested that postoperative vitamin and folic acid replacement therapy contributes to an improved success rate of bypass surgery in patients with MMD, and postoperative replacement therapy may be beneficial in these patients (28–30). Recently, it was reported that Hcy can induce peripheral vessel apoptosis *in vitro* by modulating mitochondrial dysfunction, and autography *via* MIF/mTOR signaling (31–34). To study the effect of Hcy on postoperative cerebral angiogenesis, we conduct proliferation, migration, and tube formation experiments and found a significant decrease when HBMECs were treated with Hcy, and the effect of Hcy on HBMEC was further reversed by VEGF. The results confirmed that the Hcy can directly inhibit the brain angiogenesis to affect the long-term prognosis, and VEGF is a potential treatment for patients with poor postoperative angiogenesis.

In past studies on MMD, researchers focused on perioperative complications. However, studies on long-term follow-up and metabolism factors are limited. We utilized laboratory examinations to predict potential risk factors and further explore the mechanisms of Hcy on cerebral vessels. The logistic regression confirmed that the Hcy was an independent risk factor of poor postoperative angiogenesis. *In vitro* experiments confirmed the inhibition effect of Hcy on HBMECs. This indicated that postoperative angiogenesis can be worsened by HHcy in MMD patients. Interestingly, creatinine was also found to be significantly associated with the outcome of this study. Considering creatinine was at normal levels in whole patients enrolled in the study, this difference may be due to metabolites rather than pathological change, which may need further exploration. The results revealed the important biological role of HHcy on poor postoperative angiogenesis in MMD patients. For postoperative MMD patients, the level of Hcy should be monitored and well-controlled. Further, for patients with poor postoperative angiogenesis, HHcy potentially can be a therapeutic target and VEGF can be considered to be some kind of treatment. However, there were still limitations in our study. First, the sample size in the study was limited to a single-center study. Second, we did not include children MMD patients, which may be unaffected by HHcy due to the continuously high expression of PI3K/AKT pathway which can activate the cerebral vascular proliferation. Third, a follow-up up to several years is needed. Finally, the comprehensive mechanism and therapeutic drugs which may improve postoperative angiogenesis in MMD patients need further research.

CONCLUSION

The study found that HHcy was significantly associated with poor postoperative angiogenesis in adult MMD patients. Hcy significantly inhibits HBMECs proliferation, migration, and tube formation. Furthermore, VEGF could reverse the inhibition effect induced by Hcy. Therefore, a new perspective that HHcy can act as a potential indicator and target is provided, and VEGF becomes a potential therapeutic drug to promote postoperative angiogenesis in MMD patients. Lowering the level of Hcy may be beneficial for postoperative MMD patients. In the future, focusing on the underlying mechanism of HHcy might help to guide postoperative clinical management.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Beijing Tiantan Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XY, XL, and JW collected data. RW, YZ, and DZ supervised the data collection. QH analyzed the results, performed *in vitro* experiments, and wrote the manuscript. PG made the statistical comparison. PG and JZ designed the study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.902474/full#supplementary-material

REFERENCES

1. Kuroda S, Houkin K. Moyamoya disease: current concepts and future perspectives. *Lancet Neurol.* (2008) 7:1056–66. doi: 10.1016/S1474-4422(08)70240-0
2. Acker G, Fekonja L, Vajkoczy P. Surgical management of moyamoya disease. *Stroke.* (2018) 49:476–82. doi: 10.1161/STROKEAHA.117.018563
3. Ge P, Zhang Q, Ye X, Liu X, Deng X, Wang J, et al. Modifiable risk factors associated with moyamoya disease: a case-control study. *Stroke.* (2020) 51:2472–9. doi: 10.1161/STROKEAHA.120.030027
4. Mertens R, Graupera M, Gerhardt H, Bersano A, Tournier-Lasserve E, Mensah MA, et al. The genetic basis of moyamoya disease. *Transl Stroke Res.* (2022) 13:25–45. doi: 10.1007/s12975-021-00940-2

5. Kleinloog R, Regli L, Rinkel GJ, Klijn CJ. Regional differences in incidence and patient characteristics of moyamoya disease: a systematic review. *J Neurol Neurosurg Psychiatry*. (2012) 83:531–6. doi: 10.1136/jnnp-2011-301387
6. Kim T, Oh CW, Bang JS, Kim JE, Cho WS. Moyamoya disease: treatment and outcomes. *J Stroke.* (2016) 18:21–30. doi: 10.5853/jos.2015.01739
7. Jakubowski H. Homocysteine modification in protein structure/function and human disease. *Physiol Rev.* (2019) 99:555–604. doi: 10.1152/physrev.00003.2018
8. Hasan T, Arora R, Bansal AK, Bhattacharya R, Sharma GS, Singh LR. Disturbed homocysteine metabolism is associated with cancer. *Exp Mol Med.* (2019) 51:1–13. doi: 10.1038/s12276-019-0216-4
9. Qin X, Li Y, Sun N, Wang H, Zhang Y, Wang J, et al. Elevated homocysteine concentrations decrease the antihypertensive effect of angiotensin-converting enzyme inhibitors in hypertensive patients. *Arterioscler Thromb Vasc Biol.* (2017) 37:166–72. doi: 10.1161/ATVBAHA.116.308515
10. Han L, Wu Q, Wang C, Hao Y, Zhao J, Zhang L, et al. Homocysteine, ischemic stroke, and coronary heart disease in hypertensive patients: a population-based, prospective cohort study. *Stroke.* (2015) 46:1777–86. doi: 10.1161/STROKEAHA.115.009111
11. Christine CW, Auinger P, Joslin A, Yelpaala Y, Green R, Parkinson Study Group DI. Vitamin B12 and homocysteine levels predict different outcomes in early Parkinson's disease. *Mov Disord.* (2018) 33:762–70. doi: 10.1002/mds.27301
12. Elias MF. Reclaiming the importance of homocysteine as a marker of cardiovascular and neurologic disease. *J Intern Med.* (2021) 290:1098–9. doi: 10.1111/joim.13305
13. Research Committee on the P Treatment of Spontaneous Occlusion of the Circle of W Health Labour Sciences Research Grant for Research on Measures for Infractable D. Guidelines for diagnosis and treatment of moyamoya disease (spontaneous occlusion of the circle of willis). *Neurol Med Chir.* (2012) 52:245–66. doi: 10.2176/nmc.52.245
14. Matsushima T, Inoue T, Suzuki SO, Fujii K, Fukui M, Hasuo K. Surgical treatment of moyamoya disease in pediatric patients–comparison between the results of indirect and direct revascularization procedures. *Neurosurgery.* (1992) 31:401–5. doi: 10.1097/00006123-199209000-00003
15. Borowczyk K, Piechocka J, Glowacki R, Dhar I, Midtun O, Tell GS, et al. Urinary excretion of homocysteine thiolactone and the risk of acute myocardial infarction in coronary artery disease patients: the wenbit trial. *J Intern Med.* (2019) 285:232–44. doi: 10.1111/joim.12834
16. Maron BA, Loscalzo J. The treatment of hyperhomocysteinemia. *Annu Rev Med.* (2009) 60:39–54. doi: 10.1146/annurev.med.60.041807.123308
17. Li J, Ge P, Zhang Q, Lin F, Wang R, Zhang Y, et al. Hyperhomocysteinemia is a risk factor for postoperative ischemia in adult patients with moyamoya disease. *Neurosurg Rev.* (2021) 44:2913–21. doi: 10.1007/s10143-021-01482-9
18. Roth W, Mohamadzadeh M. Vitamin B12 and gut-brain homeostasis in the pathophysiology of ischemic stroke. *EBioMedicine.* (2021) 73:103676. doi: 10.1016/j.ebiom.2021.103676
19. Smith AD, Refsum H. Homocysteine - from disease biomarker to disease prevention. *J Intern Med.* (2021) 290:826–54. doi: 10.1111/joim.13279
20. Murray LK, Jadavji NM. The role of one-carbon metabolism and homocysteine in Parkinson's disease onset, pathology and mechanisms. *Nutr Res Rev.* (2019) 32:218–30. doi: 10.1017/S0954422419000106
21. Xiong J, Ma F, Ding N, Xu L, Ma S, Yang A, et al. Mir-195-3p alleviates homocysteine-mediated atherosclerosis by targeting Il-31 through its epigenetics modifications. *Aging Cell.* (2021) 20:e13485. doi: 10.1111/acel.13485
22. Chen J, Huang Y, Hu X, Bian X, Nian S. Gastrodin prevents homocysteine-induced human umbilical vein endothelial cells injury via Pi3k/Akt/Enos and Nrf2/are pathway. *J Cell Mol Med.* (2021) 25:345–57. doi: 10.1111/jcmm.16073
23. Borkowska A, Ziolkowski W, Kaczor K, Herman-Antosiewicz A, Knap N, Wronska A, et al. Homocysteine-induced decrease in huvec cells' resistance to oxidative stress is mediated by Akt-dependent changes in iron metabolism. *Eur J Nutr.* (2021) 60:1619–31. doi: 10.1007/s00394-020-02360-8
24. Moll S, Varga EA. Homocysteine and Mthfr mutations. *Circulation.* (2015) 132:e6–9. doi: 10.1161/CIRCULATIONAHA.114.013311
25. Korai M, Kitazato KT, Tada Y, Miyamoto T, Shimada K, Matsushita N, et al. Hyperhomocysteinemia Induced by excessive methionine intake promotes rupture of cerebral aneurysms in ovariectomized rats. *J Neuroinflammation.* (2016) 13:165. doi: 10.1186/s12974-016-0634-3
26. Liu Y, Song JH, Hou XH, Ma YH, Shen XN, Xu W, et al. Elevated homocysteine as an independent risk for intracranial atherosclerotic stenosis. *Aging.* (2019) 11:3824–31. doi: 10.18632/aging.102019
27. Sato K, Morofuji Y, Horie N, Izumo T, Anda T, Matsuo T. Hyperhomocysteinemia causes severe intraoperative thrombotic tendency in superficial temporal artery-middle cerebral artery bypass. *J Stroke Cerebrovasc Dis.* (2020) 29:104633. doi: 10.1016/j.jstrokecerebrovasdis.2019.104633
28. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med.* (1999) 340:1449–54. doi: 10.1056/NEJM199905133401901
29. Lee M, Hong KS, Chang SC, Saver JL. Efficacy of homocysteine-lowering therapy with folic acid in stroke prevention: a meta-analysis. *Stroke.* (2010) 41:1205–12. doi: 10.1161/STROKEAHA.109.573410
30. Wang X, Qin X, Demirtas H, Li J, Mao G, Huo Y, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet.* (2007) 369:1876–82. doi: 10.1016/S0140-6736(07)60854-X
31. Fan X, Wang E, He J, Zhang L, Zeng X, Gui Y, et al. Ligustrazine protects homocysteine-induced apoptosis in human umbilical vein endothelial cells by modulating mitochondrial dysfunction. *J Cardiovasc Transl Res.* (2019) 12:591–9. doi: 10.1007/s12265-019-09900-6
32. Wang X, Wang Y, Zhang L, Zhang D, Bai L, Kong W, et al. L-Cystathionine protects against homocysteine-induced mitochondria-dependent apoptosis of vascular endothelial cells. *Oxid Med Cell Longev.* (2019) 2019:1253289. doi: 10.1155/2019/1253289
33. Zhang Z, Wei C, Zhou Y, Yan T, Wang Z, Li W, et al. Homocysteine induces apoptosis of human umbilical vein endothelial cells via mitochondrial dysfunction and endoplasmic reticulum stress. *Oxid Med Cell Longev.* (2017) 2017:5736506. doi: 10.1155/2017/5736506
34. Zhang Y, Zhang Y, Tang J, Zhao S, Li C, Huang YP, et al. Oxymatrine inhibits homocysteine-mediated autophagy via mif/mtor signaling in human umbilical vein endothelial cells. *Cell Physiol Biochem.* (2018) 45:1893–903. doi: 10.1159/000487912

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The Monocyte-to-Lymphocyte Ratio Predicts Acute Kidney Injury After Acute Hemorrhagic Stroke

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Objectives: Acute kidney injury (AKI) is a serious complication of acute hemorrhagic stroke (AHS). Early detection and early treatment are crucial for patients with AKI. We conducted a study to analyze the role of the monocyte-to-lymphocyte ratio (MLR) in predicting the development of AKI after AHS.

Methods: This retrospective observational study enrolled all subjects with AHS who attended the neurosurgical intensive care unit (NSICU) at the First Affiliated University of South China between 2018 and 2021. Patient demographics, laboratory data, treatment details, and clinical outcomes were recorded.

Results: Of the 771 enrolled patients, 180 (23.3%) patients developed AKI. Compared to patients without AKI, those with AKI had a higher MLR and the neutrophil-lymphocyte ratio (NLR) at admission ($P < 0.001$). The MLR and the NLR at admission were associated with an increased AKI risk, with odds ratios (ORs) of 8.27 (95% CI: 4.23, 16.17, $p < 0.001$) and 1.17 (95% CI: 1.12, 1.22, $p < 0.001$), respectively. The receiver operating characteristic curve (ROC) analysis was conducted to analyze the ability of the MLR and NLR to predict AKI, and the areas under the curve (AUCs) of the MLR and the NLR were 0.73 (95% CI: 0.69, 0.77, $p < 0.001$) and 0.67 (95% CI: 0.62, 0.72, $p < 0.001$), with optimal cutoff values of 0.5556 and 11.65, respectively. The MLR and the NLR at admission were associated with an increased in-hospital mortality risk, with ORs of 3.13 (95% CI: 1.08, 9.04) and 1.07 (95% CI: 1.00, 1.14), respectively. The AUCs of the MLR and the NLR for predicting in-hospital mortality were 0.62 (95% CI: 0.54, 0.71, $p = 0.004$) and 0.52 (95% CI: 0.43, 0.62, $p = 0.568$), respectively. The optimal cutoff value for the MLR was 0.7059, with a sensitivity of 51% and a specificity of 73.3%.

Conclusions: MLR and NLR measurements in patients with AHS at admission could be valuable tools for identifying patients at high risk of early AKI. The MLR was positively associated with in-hospital mortality and the NLR showed a weak ability for the prediction of in-hospital mortality.

Keywords: monocyte-to-lymphocyte ratio, acute kidney injury, acute hemorrhagic stroke, prediction, in-hospital mortality

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INTRODUCTION

Acute stroke is a common and serious complication that increases mortality and severe disability worldwide and imposes a substantial socioeconomic burden (1, 2). It has been reported that the prevalence rate of survival after stroke in the elderly population is 4.94% in China (3). Stroke can be classified into two main types: ischemic stroke, which accounts for 85% of all acute strokes, and acute hemorrhagic stroke (AHS), which accounts for 15% of all acute hemorrhagic strokes and has a high mortality rate (4). Stroke is often associated with multiple complications, such as infection, malnutrition, and thrombosis (5). Acute kidney injury (AKI) refers to a rapid decline in renal function within hours to days, which relates to high mortality and affects prognosis (6). An increasing number of studies on AKI after stroke have been conducted recently, and it has been reported that the incidence of AKI ranges from 6.8 to 26.7% after acute hemorrhagic stroke (7, 8). Previous studies have suggested that AKI increases the risk of in-hospital mortality and severe disability following stroke (7, 9, 10). Therefore, the early identification of AKI has become a focus in the clinical setting. Currently, the diagnosis of AKI is still based on the changes in urine volume and serum creatine (sCr), which are recognized as insensitive in early diagnosis (11). In recent decades, many specialists have attempted to identify ideal biomarkers to predict AKI, and an increasing number of novel biomarkers, such as kidney injury molecular-1 and cystatin C, have been investigated for their value in early AKI detection (12–14). However, for some reason, they have not yet been widely applied in clinical practice. Inflammatory mediators are involved in the onset and progression of AKI (15). Patients with AKI present with changes in the morphology and the function of vascular endothelial cells (16, 17). The monocyte-to-lymphocyte ratio (MLR) and the neutrophil-to-lymphocyte ratio (NLR) are reliable inflammatory biomarkers that are calculated from complete blood counts (18, 19). Previous studies have demonstrated that the NLR is associated with hospital mortality in patients diagnosed with acute stroke (19, 20). Ultimately, the relationship between the MLR and the NLR in AKI is still unclear. Therefore, we sought to explore the association of the MLR and the NLR with AKI in patients diagnosed with AHS.

METHODS

Study Design

We extracted the data of 929 subjects diagnosed with AHS who attended the neurosurgical intensive care unit (NSICU) of the First Affiliated University of South China from July 2018 to August 2021 in the hospital medical record. The First Affiliated University of South China is a teaching hospital with 2,300 beds, and there are 23 beds in the NSICU. In this study, the population comprised patients older than 18 years. Patients were excluded if they had AKI before admission; were admitted to the NSICU for $<$24 h; had preexisting chronic renal dysfunction requiring renal replacement therapy (RRT); had a tumor or rheumatism; had undergone kidney transplantation; had a second AHS attack; or had missing routine blood test or renal function data within 7 days after admission to the NSICU.

Data Collection

The following variables were collected: sex, age, preexisting clinical conditions, inflammatory markers, blood biochemistry, complete blood count, and Glasgow coma scale (GCS) score. The MLR and the NLR were defined as the ratios of the monocyte count and neutrophil count to the lymphocyte count, respectively, such counts were calculated from a peripheral blood sample on admission by fluorescent flow cytometry (18, 20). AHS was defined as the onset of symptoms and the evidence on cranial CT images. AKI was defined as the rapid decrease of kidney function within a few hours or days. AKI was diagnosed with the Kidney Disease: Improving Global Outcomes (KDIGO) criteria as follows: (a) a rise in sCr of $\geq$0.3 mg/dL (26.5 μmol/L) within 48 h; (b) an increase in sCr to $\geq$1.5 times in the past 7 days; or (c) a urine volume of $\leq$0.5 ml/kg/h for 6 h (21). Patients with sCr increase 1.5 to 1.9 times baseline or increase to $\geq$0.3 mg/dl ($\geq$26.5 μmol/l) or with urine output $<$0.5 ml/kg/hour for 6 to 12 h are classified as being at stage 1; patients with sCr increased 2.0 to 2.9 times baseline or increase to $<$0.5 ml/kg/hour for $\geq$12 h are classified as being at stage 2; stage 3 was marked for patients with Scr increased 3.0 times baseline or increase to $\geq$4.0 mg/dl ($\geq$353.6 μmol/l) or initiation of renal replacement therapy, or in patients $<$18 years, a decrease in estimated baseline glomerular filtration (eGFR) to $<$35 ml/min per 1.73 m^{-2},$<$0.3 ml/kg/hour for $\geq$24 h or anuria for $\geq$12 h (21). The lowest value of sCr measured in the general ward or emergency clinic before attending the NSICU was taken as the baseline creatinine value. When the value was missing, the sCr level was calculated using the modifications of the diet in the renal disease method, assuming a normal glomerular filtration rate of 75 ml·min^{-1}·1.73 m^{-2} (22). The eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation (22).

Statistical Analysis

SPSS 16 software was used to analyze all data (Chicago, IL, USA). Continuous data are presented as medians with interquartile ranges, while categorical variables are shown as frequency counts (percent). The Chi-square tests were used to compare categorical variables between groups. Comparisons between continuous variables were made using *t*-tests. Spearman's correlation was applied to analyze the association of the MLR with other variables. The relationships of the MLR and the NLR with AKI and prognosis were subjected to multivariable logistic regression, and the results are given as odds ratios (ORs). The model of AKI was adjusted for age, sex, eGFR, baseline Scr, blood urea nitrogen, diabetes mellitus, coronary artery disease, hypertension, the Glasgow coma scale (GCS) score, use of angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), and contrast agents. The model of hospital mortality was adjusted for sex (men), age, hypertension, coronary artery disease, diabetes mellitus and hemoglobin, triglyceride, cholesterol, albumin, and the GCS score. The receiver operating characteristic (ROC) curves were applied to evaluate the predictive usefulness of the MLR and the NLR for the development of AKI and in-hospital

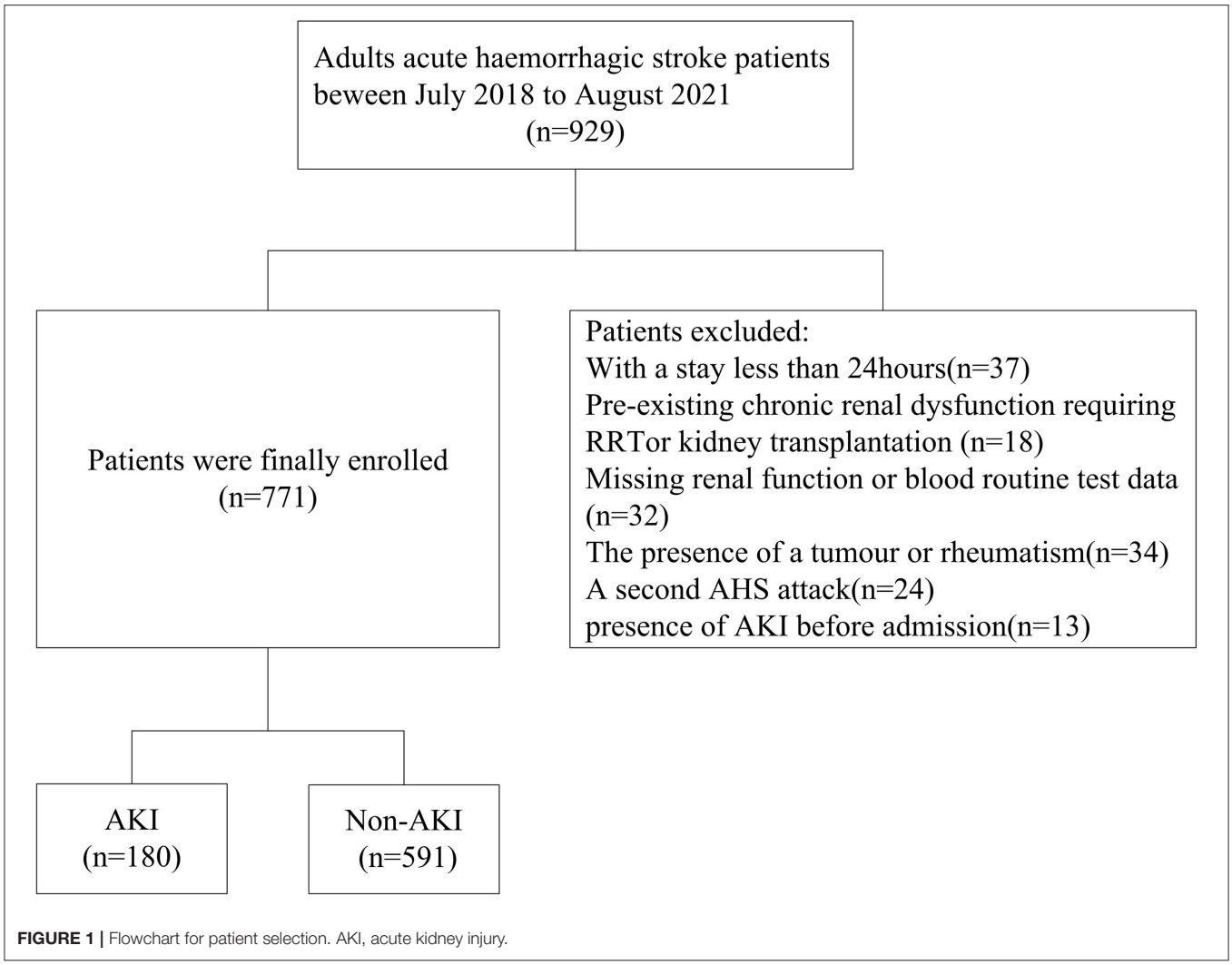

FIGURE 1 | Flowchart for patient selection. AKI, acute kidney injury.

mortality. The Youden index was used to calculate cutoff values as well as the sensitivity and specificity of the parameters. For the study, a two-tailed $p < 0.05$ indicated statistical significance.

RESULTS

Patient Characteristics

Of the 929 patients diagnosed with AHS who attended the NSICU during the screening period, 771 patients were ultimately included (**Figure 1**). A total of 497 (64.5%) men and 274 (35.5%) women were recruited. Of the 180 (23.3%) subjects who developed AKI, 125 (16.2%) had stage 1 AKI, 25 (3.2%) had stage 2 AKI, and 30 (3.9%) had stage 3 AKI. The average age of the patients was 60.53 ± 11.84 years. Male sex, patients with diabetes mellitus or coronary artery disease, and those with a lower eGFR or higher baseline sCr and blood urea nitrogen (BUN) levels had a higher likelihood of developing AKI than their counterparts ($p < 0.05$). Remarkable differences were observed in triglyceride, procalcitonin (PCT), and C-reactive protein (CRP) levels and the GCS scores between the two groups ($p < 0.05$). Patients with AKI had a higher MLR and NLR at admission. Overall, there were 88(11.4%) patients using ACEI, 229 (29.7%) patients using ARB, and 562 (72.9%) patients using contrast agents during the hospital stay. Also, 49 (6.4%) patients died in the hospital and 9 (0.9%) received renal replacement treatment, but the number of patients with assisted ventilation was as high as 412 (53.4%). Moreover, AKI patients had higher rates of mortality, renal replacement therapy (RRT), and assisted ventilation, but no noticeable difference was observed in the length of hospital stay. Compared to the non-AKI groups, the AKI group did not have a higher rate of using contrast agents, ARB, or ACEI (**Table 1**).

The Relationship of the MLR With Other Variables at Baseline

Male sex, baseline sCr, BUN, CRP, and PCT levels, the NLR, the GCS scores, and the PLR displayed a moderate correlation with the MLR ($p < 0.05$), while age, albumin, use of ventilation, and triglyceride levels had no relationship with the MLR ($p > 0.05$) (**Table 2**).

TABLE 1 | Baseline characteristics and outcomes of the patients.

Variables	ALL ($n = 771$)	AKI ($n = 180$)	Non-AKI ($n = 591$)	*p* value
Sex, male (%)	497 (64.5%)	143 (79.4%)	37 (6.3%)	<0.001*
Age (years)	60.53 ± 11.84	61.52 ± 11.36	60.23 ± 11.98	0.200
Primary disease				
Cerebrovascular malformation	144 (18.7%)	35 (19.44%)	109 (18.44%)	0.763
Hypertension	645 (83.7%)	155 (86.1%)	25 (4.2%)	0.310
Diabetes mellitus	91 (11.8%)	39 (21.7%)	52 (8.8%)	<0.001*
Coronary artery disease	35 (4.5%)	15 (8.3%)	20 (3.4%)	0.005
eGFR (ml/min/1.73 m^2)	84.13 ± 25.28	74.94 ± 30.54	86.93 ± 22.74	<0.001*
Laboratory index at NSICU admission				
Baseline sCr (umol/L)	87.09 ± 33.71	104.76 ± 47.29	81.71 ± 26.08	<0.001*
BUN (mmol/L)	5.59 ± 2.75	6.72 ± 3.61	5.24 ± 4.42	<0.001*
Albumin (g/L)	42.8 ± 4.2	42.49 ± 4.42	42.89 ± 4.13	0.271
Triglyceride (mmol/L)	1.64 (0.81, 1.86)	1.78 ± 1.08	1.18 (0.81, 1.86)	<0.001*
Cholesterol (mmol/L)	4.27 ± 0.97	4.36 ± 0.97	4.25 ± 0.97	0.201
PCT (ng/mL)	0.56 (0.04, 0.75)	1.24 (0.80, 0.72)	0.09 (0.043, 0.21)	0.003*
White blood count (mm^3)	11.58 ± 4.24	12.12 ± 4.78	11.41 ± 4.47	0.047*
CRP (mg/L)	24.43 ± 4.89	25.78 (17.02, 131.59)	3.60 (0.73, 22.36)	<0.001*
Potassium (mmol/L)	3.67 ± 0.49	3.69 ± 0.57	3.67 ± 0.46	0.545
Hemoglobin (g/L)	133.59 ± 47.19	138.06 ± 92.52	132.23 ± 13.36	0.147
GCS score	10.27 ± 3.58	10.64 ± 3.47	9.07 ± 3.67	<0.001*
PLR	224.61 ± 165.53	223.56 ± 131.75	224.93 ± 174.63	0.923
MLR	0.5374 ± 0.3001	0.7584 ± 0.2610	0.4701 ± 0.2765	<0.001*
NLR	9.10 ± 4.76	11.67 ± 5.67	8.31 ± 4.02	<0.001*
Medication during admission				
ACEI	88 (11.4%)	19 (10.56%)	69 (11.67%)	0.674
ARB	229 (29.7%)	58 (32.2%)	171 (28.9%)	0.398
Contrast agent	562 (72.9%)	13 (72.2%)	432 (73.1%)	0.817
Outcome				
Hospital mortality	49 (6.4%)	27 (15%)	22 (3.7%)	<0.001*
Renal replacement treatment	9 (0.9%)	8 (4.4%)	1 (0.16%)	<0.001*
Ventilation	412 (53.4%)	132 (73.3%)	280 (47.38%)	<0.001*
Hospital stay	23.87 ± 18.43	22.34 ± 19.57	24.33 ± 18.06	0.203*

*eGFR, estimate baseline glomerular filtration rate; BUN, blood urea nitrogen; sCr, serum creatinine; PCT, procalcitonin; MLR, monocyte-to-lymphocyte ratio; CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; ARB, angiotensin- receptor blocker; GCS, Glasgow coma score; ACEI, angiotensin-converting enzyme inhibitor. *$p < 0.05$.*

The MLR Predicts the Incidence of AKI

According to the multivariable logistic regression model, after adjustment for age, sex (male), baseline sCr level, diabetes mellitus, coronary artery disease, GCS score, and the use of contrast agents, ARB and ACEI, the MLR and NLR at admission were associated with an increased AKI risk, with ORs of 8.27 (95% CI: 4.23, 16.17, $p < 0.001$) and 1.17 (95% CI: 1.12, 1.2, $p < 0.001$), respectively. The ORs for AKI were 1.00 (95% CI: 0.99, 1.01, $p = 0.251$) for CRP and 1.04 (95% CI: 0.96, 1.01, $p = 0.341$) for PCT. The OR for AKI was 2.95 (95%CI: 1.92, 4.53, $p < 0.001$) for ventilation (**Table 3**).

Ability of the MLR and NLR to Predict AKI

The area under the curve (AUC) for the ability of the MLR at admission to predict the development of AKI was 0.73 (95% CI: 0.69, 0.77, $p < 0.001$), with a cutoff value of 0.5556, a sensitivity of 77.8%, and a specificity of 61.3%. The AUC of the NLR for predicting AKI was 0.67 (95% CI: 0.62, 0.72, $p < 0.001$), with a cutoff value of 11.65, which best distinguished the occurrence of AKI; the sensitivity was 47.8%, and the specificity was 82.7% (**Figure 2**).

The Association Between the MLR and In-hospital Mortality

To determine the value of the MLR and the NLR in predicting in-hospital mortality, a multivariable logistic regression was performed (**Table 4**). After adjusting for age, sex (male), diabetes mellitus, coronary artery disease, hypertension, hemoglobin, triglyceride, cholesterol, and albumin levels, and the GCS scores, the MLR and the NLR at admission

TABLE 2 | Associations with the baseline MLR and other factors.

Variable	r	*p*-value
Age (years)	0.06	0.083
Sex (male)	0.15	<0.001*
Baseline sCr (mmol/L)	0.15	<0.001
BUN (mmol/L)	0.12	0.001*
Albumin (g/L)	0.03	0.921
Triglycerides (mmol/L)	0.03	0.451
CRP (mg/L)	0.18	<0.001*
PCT (ng/mL)	0.19	<0.001*
GCS score	0.14	<0.001*
NLR	0.35	<0.001*
PLR	0.25	<0.001*
Ventilation	0.06	0.077

*MLR, monocyte-to-lymphocyte ratio; CRP, C-reactive protein; sCr, serum creatinine; BUN, blood urea nitrogen; PCT: procalcitonin; NLR, neutrophil-to-lymphocyte ratio; GCS, Glasgow coma score. PLR, platelet-to-lymphocyte ratio. *p < 0.05.*

TABLE 3 | The value of the MLR and NLR for the prediction of AKI analyzed by the multivariable logistic regression analysis.

Variable	Unadjusted		After adjustment*	
	OR(95% CI)	*p* value	OR(95% CI)	*p* value
MLR	10.51 (5.70, 19.38)	<0.001	8.27 (4.23, 16.17)	<0.001*
NLR	1.15 (1.11, 1.19)	<0.001	1.17 (1.12, 1.22)	<0.001*
CRP (mg/L)	1.01 (1.00, 1.01)	0.016	1.00 (0.99,1.01)	0.251
PCT (ng/mL)	1.10 (1.00, 1.20)	0.04	1.04 (0.96, 1.11)	0.341
Ventilation	3.05 (2.12,4.41)	<0.001	2.95 (1.92,4.53)	<0.001*
Sex (male)	2.59 (1.38, 3.85)	<0.001		
GCS	0.88 (0.84, 0.93)	<0.001		
Age (years)	1.01 (0.99, 1.03)	0.09		
Hypertension	1.03 (0.66, 1.76)	0.908		
Diabetes mellitus	2.29 (1.38, 3.81)	0.001		
Coronary artery disease	2.49 (1.12, 5.49)	0.024		
eGFR (ml/min/1.73 m^2)	1.02 (1.00, 1.03)	0.019		
BUN (mmol/L)	1.20 (1.13,1.28)	<0.001		
Baseline Scr (mmol/L)	1.02 (1.01,1.03)	<0.001		
ACEI	0.89 (0.52,1.53)	0.674		
ARB	1.17 (0.82,1.67)	0.398		
Contrast agents	0.96 (0.66,1.39)	0.817		

*Adjusted for age, sex, eGFR, baseline Scr level, blood urea nitrogen level, diabetes mellitus, coronary artery disease, and hypertension, GCS score, and the use of ACEIs, ARBs, and contrast agents. MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; PCT, procalcitonin; OR, odds ratio; CI, confidence interval; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin-receptor blockers; ssCr, serum creatinine. *p < 0.05.*

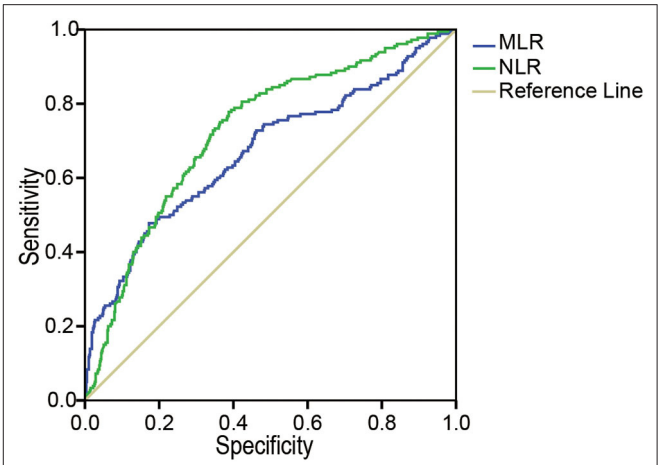

FIGURE 2 | The ROC analysis of the MLR and the NLR for AKI. The area under the ROC of the MLR and NLR at admission to predict the development of AKI were 0.73 (95% CI: 0.69, 0.77, $p < 0.001$) and 0.67 (95% CI: 0.62, 0.72, $p < 0.001$), respectively. MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; AKI, acute kidney injury, ROC, receiver operating characteristics.

TABLE 4 | The association of the MLR and NLR with in-hospital mortality.

Variable	Unadjusted		After adjustment	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
AKI	2.17 (1.65, 2.85)	<0.001	5.28 (2.57, 10.84)	<0.001
MLR	3.38 (1.52, 7.53)	0.003	3.13 (1.08, 9.04)	0.035
NLR	1.04 (0.98, 1.10)	0.168	1.07 (1.00, 1.14)	0.043
PCT (ng/mL)	1.00 (0.90, 1.13)	0.920	1.00 (0.90, 1.11)	0.997
CRP (mg/L)	1.00 (0.99, 1.01)	0.399	1.01 (0.99, 1.01)	0.147
Age (years)	1.04 (1.01, 1.07)	0.003		
Sex (male)	1.57 (0.82, 3.01)	0.177		
Hypertension	0.86 (0.41, 1.82)	0.692		
Diabetes mellitus	3.35 (1.73, 6.50)	<0.001		
Coronary artery disease	1.41 (0.42, 4.77)	0.584		
Hemoglobin (g/L)	1.00 (0.99, 1.01)	0.849		
Triglycerides (mmol/L)	0.96 (0.78, 1.19)	0.719		
Cholesterol (mmol/L)	0.99 (0.72, 1.38)	0.993		
Albumin (g/L)	0.64 (0.34, 1.20)	0.164		
GCS	0.82 (0.75, 0.90)	<0.001		

*Adjusted for sex (male), age, hypertension, coronary artery disease, diabetes mellitus and hemoglobin, triglyceride, cholesterol, and albumin levels, and GCS score. MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; PCT, procalcitonin; OR, odds ratio; CI, confidence interval; GCS, Glasgow coma score; AKI: acute kidney injury. *p < 0.05.*

were associated with increased in-hospital mortality risk, with ORs of 3.13 (95% CI, 1.08, 9.04) and 1.067 (95% CI, 1.00, 1.14), respectively. Meanwhile, the OR of AKI was 5.28 (95% CI 2.57, 10.84) for in-hospital mortality. No remarkable difference was observed between the CRP and PCT levels for in-hospital mortality.

The Prediction of In-hospital Mortality

The AUCs of AKI, the MLR, and the NLR for predicting in-hospital mortality were 0.68 (95% CI: 0.60, 0.78, $p < 0.001$), 0.62 (95% CI: 0.54, 0.71, $p = 0.004$), and 0.52 (95% CI: 0.43, 0.62, $p = 0.568$), respectively. The optimal cutoff value for the MLR was 0.7059, with a sensitivity of 51% and a specificity of 73.3% (**Figure 3**).

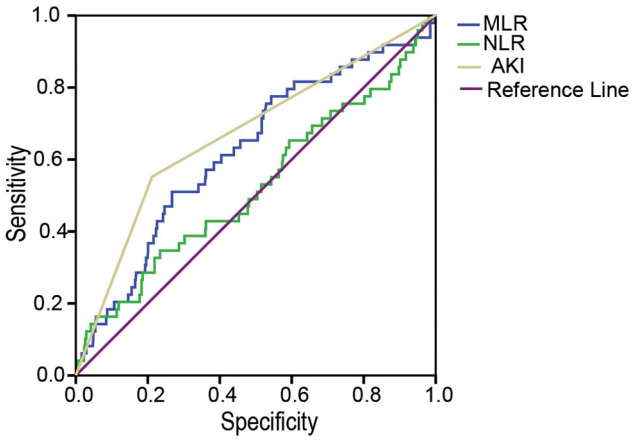

FIGURE 3 | The ROC analysis of the MLR, the NLR, and AKI for in-hospital mortality. The area under the ROC of AKI, the MLR, and the NLR for predicting in-hospital mortality were 0.68 (95% CI: 0.60, 0.78, $p < 0.001$), 0.62 (95% CI: 0.54, 0.71, $p = 0.004$) and 0.52 (95% CI: 0.43, 0.62, $p = 0.568$), respectively. MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; AKI, acute kidney injury; ROC, receiver operating characteristics.

DISCUSSION

In this single-center retrospective study of patients diagnosed with AHS, we assessed the role of the MLR and the NLR at admission in the prediction of the development of AKI and found that a higher MLR and NLR were associated with an increased risk of AKI; the MLR was superior to the NLR in predicting AKI. Meanwhile, the MLR and AKI showed the ability to predict in-hospital mortality.

The incidence of AKI in our study was 23.3%, but in a meta-analysis conducted by Zorrilla-Vaca et al. (7), the pooled prevalence rate of AKI after AHS was 19.0%. The higher incidence rate of AKI may be explained by the study population, which included patients admitted in NSICU. In our study, patients with AKI were predominantly male patients who had a lower eGFR and had diabetes mellitus or coronary artery disease. In contrast to previous studies, our results showed that PCT and CRP levels were not independent predictors of AKI (23, 24). However, unlike the results of previous studies, hypertension did not increase the risk of AKI progression in the current study (8).

To our knowledge, this study is the first to highlight the role of the MLR in the prediction of AKI in patients diagnosed with AHS. The results of this study provide evidence of the MLR as an independent predictive biomarker of AKI. A higher MLR was related to a 3.65-fold increased risk for AKI, and the AUC of the MLR was 0.73. This could be demonstrated by the idea that both immunological changes and inflammation lead to AKI (15, 17). In hemorrhagic stroke, brain tissue injuries, and internal injuries cause blood vessels to rupture, causing an abnormal accumulation of blood within the brain and leading to the activation of monocytes that induced more severe brain cell death and cerebral tissue damage. Increased monocytes and neutrophils are responsible for the higher levels of the MLR and the NLR. Neuroinflammation, blood–brain barrier dysfunction, and the interruption of blood flow are the main mechanisms (2, 25, 26).

The MLR has been demonstrated to be an inflammatory marker (18, 27). Our study also suggested that the MLR was positively related to some inflammatory factors, such as PCT and CRP levels. In the study conducted by Hao-Ran Cheng, patients with stroke-associated pneumonia (SAP) had a higher MLR than non-SAP patients after AIS (28).

Previous studies have demonstrated that ischemia-reperfusion and inflammation play critical roles in the pathophysiology of AKI (18, 19). The MLR and NLR have been proposed as predictive factors for prognosis in patients diagnosed with ischemic stroke (28, 29). The NLR has been demonstrated to be a predictor of AKI in different populations (30, 31). In this study, we found that the MLR and the NLR were predictive of an increased rate of kidney damage in AHS patients. Therefore, the MLR and NLR may be easy, convenient, and cost-effective tools for the prediction of AKI in clinical practice.

In addition, we explored the association of the MLR and the NLR as well as that of AKI with in-hospital mortality and found that both the MLR and AKI were positively related to in-hospital mortality, with associated ORs of 2.09 and 2.95, respectively. However, in this study, in contrast to earlier studies, the NLR did not show an association with in-hospital mortality (29). The reason may be that the research endpoint was in-hospital mortality, not 3-month mortality. The population was patients with AHS in the NSICU, and most patients were critically ill. In the study, only 9 (0.9%) patients received RRT, and there were significant differences between patients with AKI and patients with no AKI. Although, in the study, most patients with AKI in stage 1 and stage 2 were patients who received timely treatment with good prognoses.

Some limitations should be acknowledged in the study. First, our investigation was a single-center retrospective observational study in which confounding factors and selective biases existed. Second, because diuretics can impact urine production and the patients were admitted to the NSICU, some patients may have experienced altered urine production and weight. Histological confirmation was not always performed in the clinic, and the diagnosis of AKI was based on the sCr level. As a result, the true prevalence of AKI may have been underestimated. Third, we only analyzed the MLR at the NSICU admission. We acknowledged that a dynamic measurement of the MLR and NLR could be more accurate for predicting AKI. Lastly, the study only looked at short-term prognoses, but future studies should focus on the long-term monitoring of subjects with AKI. Larger multicenter prospective studies are needed to confirm the value of such biomarkers in AHS-associated AKI.

CONCLUSIONS

Our findings support that MLR and NLR measurements in patients diagnosed with AHS at admission could be valuable

tools for identifying patients at high risk of early AKI. The MLR was also positively associated with in-hospital mortality and the NLR showed a weak ability to predict in-hospital mortality.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FJ and BY were involved in drafting the manuscript, conceptualization, design, involved in the interpretation of data, the critical revision of the manuscript for important intellectual content, and funding resource acquisitions. JL, XY, RZ, RL, and JR obtained all patient data. XL and SZ were involved in the data analysis. All authors gave their final approval for the submission.

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REFERENCES

1. Prasanna T, Forshing L. Acute Stroke. StatPearls. Treasure Island (FL): StatPearls. (2022).
2. Kuriakose D, Zhicheng X. Pathophysiology and Treatment of Stroke: Present Status and Future Perspectives. *Int J Mol Sci.* (2020) 21:7609. doi: 10.3390/ijms21207609
3. Xia X, Yue W, Chao B, Li M, Cao L, Wang L, et al. Prevalence and risk factors of stroke in the elderly in Northern China: data from the National Stroke Screening Survey. *J Neurol.* (2019) 266:1449–58. doi: 10.1007/s00415-019-09281-5
4. Dornák T, Král M, Hazlinger M, Herzig R, Veverka T, Burval S, et al. Posterior vs. anterior circulation infarction: demography, outcomes, and frequency of hemorrhage after thrombolysis. *Int J Stroke*. (2015) 10:1224–8. doi: 10.1111/ijs.12626
5. Johnston SC, Mendis S, Mathers CD. Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modelling. *Lancet Neurol.* (2009) 8:345–54. doi: 10.1016/S1474-4422(09)70023-7
6. Bellomo R, Kellum JA, Ronco C. Acute kidney injury. *Lancet.* (2012) 380:756–66. doi: 10.1016/S0140-6736(11)61454-2
7. Zorrilla-Vaca A, Ziai W, Connolly ES Jr, Geocadin R, Thompson R, Rivera-Lara L. cute Kidney Injury Following Acute Ischemic Stroke and Intracerebral Hemorrhage: A Meta-Analysis of Prevalence Rate and Mortality Risk. *Cerebrovasc Dis*. (2018):45:1–9. doi: 10.1159/000479338
8. Jiang F, Su L, Xiang H, Zhang X, Xu D, Zhang Z, et al. Incidence, risk factors, and biomarkers predicting ischemic or hemorrhagic stroke associated acute kidney injury and outcome: a retrospective study in a general intensive care unit. *Blood Purif.* (2019)47:317–326. doi: 10.1159/000499029
9. Khatri M, Himmelfarb J, Adams D, Becker K, Longstreth WT, Tirschwell DL. Acute kidney injury is associated with increased hospital mortality after stroke. *J Stroke Cerebrovasc Dis.* (2014) 23:25–30. doi: 10.1016/j.jstrokecerebrovasdis.2012.06.005
10. Stamatellopoulos K, Laggouranis A, Alevizaki M, Manios E, Antonis L, Vemmos KN. Renal dysfunction in acute stroke: an independent predictor of long-term all combined vascular events and overall mortality. *Nephrol Dial Transplant.* (2014) 24:194–200. doi: 10.1093/ndt/gfn471
11. Cerdá J. A biomarker able to predict acute kidney injury before it occurs. *Lancet.* (2019) 394:448–50. doi: 10.1016/S0140-6736(19)30843-8
12. Zhen XW, Song NP, Ma LH, Ma LN, Guo L, Yang XD. Calprotectin and neutrophil gelatinase-associated lipocalin as biomarkers of acute kidney injury in acute coronary syndrome. *Am J Med Sci*. (2021) 361:736–43. doi: 10.1016/j.amjms.2020.10.028
13. Li Q, Huang Y, Shang W, Zhang Y, Liu Y, Xu G. The Predictive Value of Urinary Kidney Injury Molecular 1 for the Diagnosis of Contrast-Induced Acute Kidney Injury after Cardiac Catheterization: A Meta-Analysis. *J Interv Cardiol*. (2020):4982987. doi: 10.1155/2020/4982987
14. Hidayati EL, Utami MD, Rohsiswatmo R, Tridjaja B. Cystatin C compared to serum creatinine as a marker of acute kidney injury in critically ill neonates. *Pediatr Nephrol.* (2021) 36:181–6. doi: 10.1007/s00467-020-04668-3
15. Rabb H, Griffin MD, McKay DB, Swaminathan S, Pickkers P, Rosner MH, et al. Inflammation in AKI: current understanding, key questions, and knowledge gaps. *J Am Soc Nephrol.* (2016) 27:371–9. doi: 10.1681/ASN.2015030261
16. Mami I, Tavernier Q, Bouvier N, Aboukamis R, Desbuissons G, Rabant M, et al. A novel extrinsic pathway for the unfolded protein response in the kidney. *J Am Soc Nephrol.* (2016) 27:2670–83. doi: 10.1681/ASN.2015060703
17. Jansen MP, Emal D, Teske GJ, Dessing MC, Florquin S, Roelofs JJ. Release of extracellular DNA influences renal ischemia reperfusion injury by platelet activation and formation of neutrophil extracellular traps. *Kidney Int.* (2017) 91:352–64. doi: 10.1016/j.kint.2016.08.006
18. Gijsberts CM, Ellenbroek GHJM, Ten Berg MJ, Huisman A, van Solinge WW, Lam CS, et al. Effect of monocyte- to-lymphocyte ratio on heart failure characteristics and hospitalizations in a coronary angiography cohort. *Am J Cardiol.* (2017) 120: 911–6. doi: 10.1016/j.amjcard.2017.06.020
19. Gens R, Ourtani A, De Vos A, De Keyser J, De Raedt S. Usefulness of the neutrophil-to-lymphocyte ratio as a predictor of pneumonia and urinary tract infection within the first week after acute ischemic stroke. *Front Neurol.* (2021) 12:671739. doi: 10.3389/fneur.2021.671739
20. Kakhki RD, Dehghanei M, ArefNezhad R, Motedayyen H. The Predicting role of neutrophil- lymphocyte ratio in patients with acute ischemic and hemorrhagic stroke. *J Stroke Cerebrovasc Dis.* (2020) 29:105233. doi: 10.1016/j.jstrokecerebrovasdis.2020.105233
21. John AK, Norbert L, KDIGO AKI. Guideline Work Group. Kidney Disease: Improving Global Outcomes (KDIGO)Acute Kidney Injury Work Group KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Inter Crit Care.* (2013) 17:204. doi: 10.1186/cc11454
22. Bellomo R, Ronco C, Kellum JA, Paul P. Acute Dialysis Quality Initiative workgroup. Acute Dialysis Quality Initiative workgroup Acute renal failure-definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care*. (2004) 8:R204.
23. Kan WC, Huang YT, Wu VC, Shiao CC. Predictive ability of procalcitonin for acute kidney injury: a narrative review focusing on the interference of infection. *Int J Mol Sci.* (2021) 22:6903. doi: 10.3390/ijms221 36903
24. Fu EL, Franko MA, Obergfell A, Dekker FW, Gabrielsen A, Jernberg T, et al. High-sensitivity C-reactive protein and the risk of chronic kidney disease progression or acute kidney injury in post-myocardial infarction patients. *Am Heart J.* (2019) 216:20–9. doi: 10.1016/j.ahj.2019.06.019

25. Testai FD, Aiyagari V. Acute hemorrhagic stroke pathophysiology and medical interventions: Blood pressure control, management of anticoagulant-associated brain hemorrhage and general management principles. *Neurol Clin*. (2008) 26:963–985. doi: 10.1016/j.ncl.2008.06.001
26. Aronowski J, Zhao X. Molecular pathophysiology of cerebral hemorrhage: Secondary brain injury. *Stroke*. (2011) 42:1781–6. doi: 10.1161/STROKEAHA.110.596718
27. Choi YH, Lee JW, Lee SH, Choi JH, Kang J, Lee BS, et al. A high monocyte-to-lymphocyte ratio predicts poor prognosis in patients with advanced gallbladder cancer receiving chemotherapy. *Cancer Epidemiol Biomarkers Prev*. (2019) 28:1045–51. doi: 10.1158/1055-9965.EPI-18-1066
28. Cheng HR, Song JY, Zhang YN, Chen YB, Lin GQ, Huang GQ, et al. High monocyte- to-lymphocyte ratio is associated with stroke-associated pneumonia. *Front Neurol*. (2020) 11:575809. doi: 10.3389/fneur.2020.575809
29. Wang L, Song Q, Wang C, Wu S, Deng L, Li Y, et al. Neutrophil to lymphocyte ratio predicts poor outcomes after acute ischemic stroke: a cohort study and systematic review. *J Neurol Sci*. (2019) 406:116445. doi: 10.1016/j.jns.2019.116445
30. Bu X, Zhang L, Chen P, Wu X. Relation of neutrophil-to- lymphocyte ratio to acute kidney injury in patients with sepsis and septic shock: a retrospective study. *International Immunopharmacology*. (2019) 70:372–7. doi: 10.1016/j.intimp.2019.02.043
31. Kurtul A, Yarlioglues M, Duran M, Murat SN. Association of neutrophil to- lymphocyte ratio with contrast-induced nephropathy in patients with non-ST-elevation acute coronary syndrome treated with percutaneous coronary intervention. *Heart Lung Circ*. (2016) 25:683–90. doi: 10.1016/j.hlc.2016.01.007

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Association Between High Serum Anion Gap and All-Cause Mortality in Non-Traumatic Subarachnoid Hemorrhage: A Retrospective Analysis of the MIMIC-IV Database

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Background: High serum anion gap (AG) on admission is often correlated with poor outcomes in critically ill patients; however, data in patients with non-traumatic subarachnoid hemorrhage (SAH) are lacking. Herein, we aimed to identify the association between serum AG and all-cause mortality in patients with non-traumatic SAH.

Methods: A retrospective analysis of data from the Medical Information Mart for Intensive Care (MIMIC-IV) database was performed on critically ill patients with non-traumatic SAH. Serum AG was collected on Intensive Care Unit (ICU) admission, and ICU and hospital all-cause mortality were analyzed. The multivariate Cox proportional hazard regression model and Kaplan-Meier survival curve analysis were used to analyze the correlation of serum AG with ICU and hospital all-cause mortality. Furthermore, interaction and subgroup analyses were evaluated for the consistency of these correlations.

Results: A total of 893 patients with non-traumatic SAH were included in this study. The all-cause mortality in ICU and hospital were 14.8% (132/893), and 18.9% (169/893), respectively. Multivariate analysis after adjusting for potential confounders indicated that high serum AG levels (≥16 mmol/L) were associated with increased risk of ICU and hospital all-cause mortality as compared to that with low serum AG levels (<16mmol/L), (hazards ratio (HR): 2.31 [95% CI: 1.58–3.38]) and HR: 1.91 [95% CI: 1.36–2.67)], respectively). Similarly, the Kaplan–Meier (K–M) survival curve also showed that patients with high serum AG levels presented with a lower survival rate. Stratified analyses further showed that depending on the variable testes, an association between higher serum AG levels and hospital all-cause mortality in different subgroups was observed.

Conclusion: Among patients with non-traumatic SAH, high serum AG level at ICU admission was associated with increased ICU and hospital all-cause mortality.

Keywords: non-traumatic subarachnoid hemorrhage, serum anion gap, mortality, MIMIC-IV database, critically ill

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INTRODUCTION

Non-traumatic subarachnoid hemorrhage (SAH) is a potentially devastating disease caused primarily by ruptured intracranial aneurysms, which account for 2–7% of all strokes (1). However, the disease-specific burden of non-traumatic SAH is unusually heavy and may be underestimated. Half of the patients with non-traumatic SAH are reported to be younger than 60 years, of which one-third are reported to expire before arrival at the hospital, while others required Intensive Care Unit (ICU) treatment (1, 2). Even with optimal management in the ICU, non-traumatic SAH still has high in-hospital mortality rates (3). Epidemiological investigations have shown high rates of non-traumatic SAH and in-hospital mortality rates of up to 40% (1). Given the life-threatening risk of non-traumatic SAH, non-invasive and inexpensive tests are needed to identify those at greater risk of death and prevent mortalities.

Acid-base imbalance, which has been mainly reported and investigated in critically ill patients, has been correlated with poor outcomes, especially in cases of persistent acid-base imbalance (4). The serum anion gap (AG) is a useful biochemical indicator for evaluating acid-base balance in clinical practice and is easily obtained (5) using the formula: AG = [Na^+ (mmol/L) + K^+ (mmol/L)] – [Cl^-(mmol/L) + HCO_3^- (mmol/L)]. Our stress response during and after the onset of non-traumatic SAH is complicated and leads to disturbance in the inner environment. Many studies have demonstrated that serum AG was strongly related to mortality in critically ill patients, specifically in individuals with congestive heart failure (6), cardiogenic shock (7), ischemic stroke (8, 9), acute kidney injury (10), acute pancreatitis (11), acute myocardial infarction (12) and aortic aneurysm (13). Although the effect of serum AG on mortality following ischemic stroke has been well described (8, 9); however, data in patients with non-traumatic SAH are lacking.

Therefore, the purpose of this study was to clarify the correlation between serum AG levels and mortality in patients with non-traumatic SAH in the ICU.

METHODS

Data Source

We performed a retrospective cohort study using the Medical Information Mart for Intensive Care (MIMIC-IV) (version 1) (14), which is a large publicly accessed database.

The use of this database has been approved by the Massachusetts Institute of Technology and the Institutional Review Board of Beth Israel Deaconess Medical Center (BIDMC, Boston, MA, USA). One of the authors, Changli Zhong, accomplished the National Institutes of Health's web-based course "Protecting Human Research Participants" (Record ID: 39099161) and was approved to access the database to extract data. To protect patient privacy, all data were de-identified. Thus, informed consent was waived by the ethical committee of the Beth Israel Deaconess Medical Center. This study is described in conformity to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement and was managed to conform to the tenets of the Declaration of Helsinki.

Study Population and Variable Extraction

The total number of patients in the MIMIC-IV included 257,366 individuals from 2008 to 2019, of which 50,048 were admitted to the ICU. Among them, 1,142 patients with non-traumatic SAH were selected based on the record of ICD-9 code 430, and ICD-10 codes I60, I600 to I6012, I6000 to I6002, I6020 to I6022, I6030 to I6032, and I6050 to I6052. Patients >18 years were initially enrolled in this study, and only data for the first ICU stay were collected for patients. Meanwhile, patients without serum AG level after ICU admission who stayed in the ICU for <24 h and had a survival time of <0 h, were all excluded. Thus, only 893 patients were included in this study (**Figure 1**).

The first test serum AG value after ICU admission was extracted as the interest variable and the major exposure factor in this study. All variables in this study were extracted from the MIMIC-IV database using Structured Query Language (SQL) with PostgreSQL. Demographic variables, including age, sex, and ethnicity, were obtained. Clinical severity on admission was examined using the Glasgow Coma Score (GCS) and Simplified Acute Physiology Score II (SAPS II). Vital signs in this study also included, such as systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), temperature, heart rate, respiratory rate (RR), and percutaneous oxygen saturation (SpO_2). Comorbidities, including hypertension, diabetes, congestive heart failure, chronic pulmonary disease, sepsis, renal failure, liver diseases, and malignancy, were also included for analysis based on the recorded ICD-9 and ICD-10 codes from the database. Laboratory variables, including white blood cell (WBC) count, platelet count, hemoglobin, glucose, sodium, potassium, chloride, creatinine, blood urea nitrogen (BUN), and bicarbonate, were obtained within the first test after ICU admission. For the missing dataset in the data, we used the predicted mean matching method to impute the missing values. The details of the missing value are shown in **Table 1**.

Outcomes

The primary endpoint was hospital all-cause mortality, and ICU all-cause mortality was regarded as the secondary endpoint, which was defined by patient survival status at the time of hospital discharge.

Statistical Analysis

Continuous variables were described as means ± standard deviation (SD) or as median interquartile ranges (IQR), and categorical variables were described as percentages. We used Fisher's exact and Chi-square tests or the Kruskal-Wallis test to examine the statistical differences between two groups—the low serum AG (AG < 16 mmol/L) and high serum AG (AG ≥ 16 mmol/L) groups. Restricted cubic spline analysis was used to describe the non-linear association between serum AG and ICU and hospital all-cause mortality with non-traumatic SAH subjects. We then used multivariate Cox proportional hazard models to evaluate the relationship of serum AG level with ICU

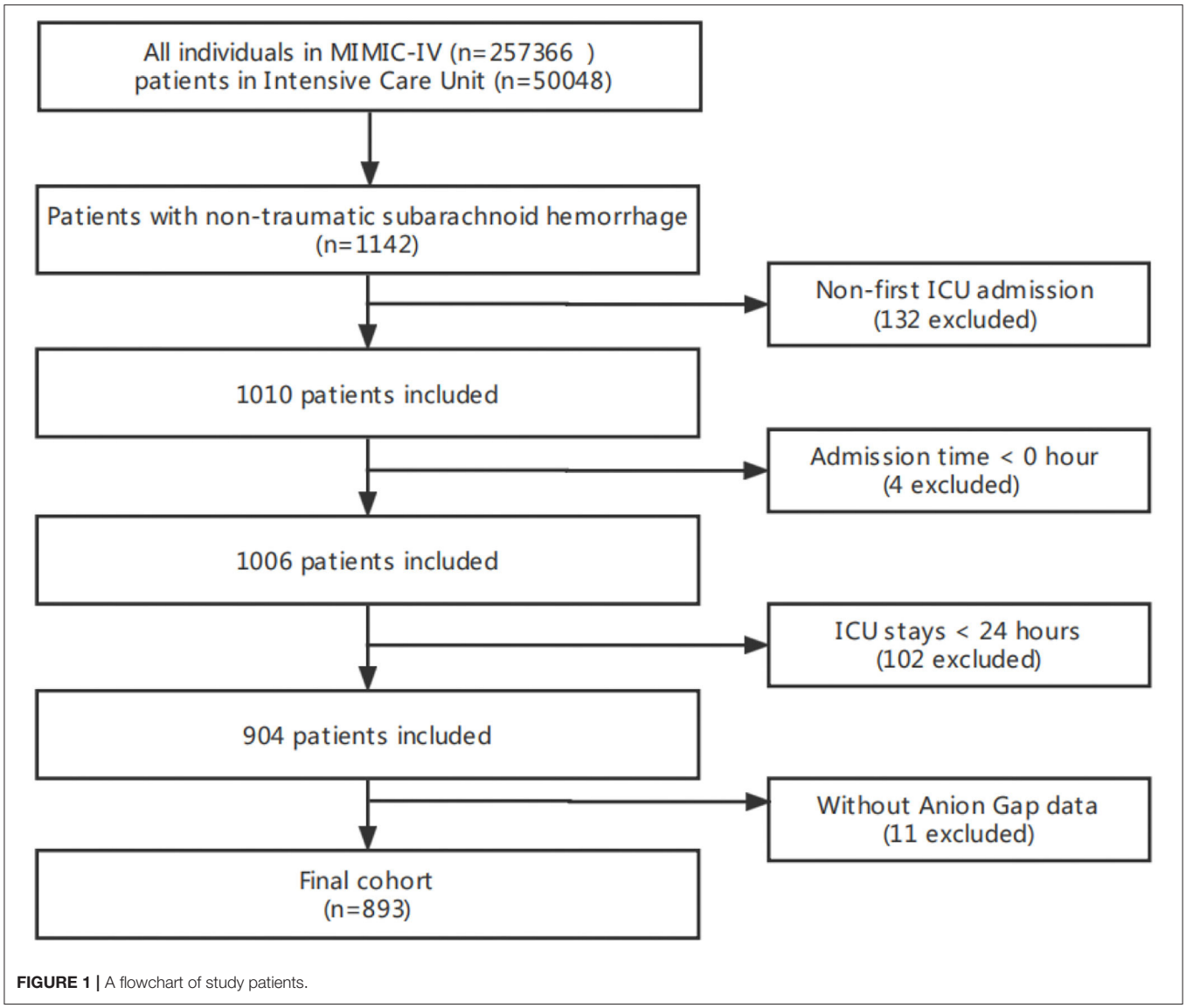

FIGURE 1 | A flowchart of study patients.

TABLE 1 | Details of missing values.

Variables	The number of missing values	The percent of missing values (%)
Respiratory rate	2	0.2
Bicarbonate	6	0.7
BUN	1	0.7
Creatinine	1	0.7
Chloride	6	0.7
Hemoglobin	6	0.7
Platelet	1	0.1
WBC	1	0.1

WBC, white blood cell; BUN, blood urea nitrogen.

and hospital mortality. Baseline variables that were considered clinically relevant or had a change in effect estimate of >10% were chosen as confounders. In Model I, the covariates were adjusted for age, sex, and ethnicity, whereas in Model II, SBP, DBP, RR, heart rate, GCS, diabetes, sepsis, renal failure, chronic pulmonary disease, vasopressor, embolization of aneurysm, clipping of aneurysm, WBC, hemoglobin, platelet, glucose, BUN, and creatinine were also included in addition to the covariates of Model I. The Kaplan–Meier (K–M) curve was also utilized to visualize these relationships. Furthermore, interactions and stratified analyses were conducted using hypertension, diabetes, congestive heart failure, chronic pulmonary disease, renal failure, sepsis, GCS ($\geq$ 8 and $<$ 8), and SAPS II ($\geq$ 45 and $<$ 45), as previously described. For the missing data, we used the predicted mean matching method to fill in the missing values (15). All results were expressed as hazard ratios (HR) with a 95% CI, and a p-value of <0.05 was considered significant. All analyses were performed using the statistical software packages R 3.3.2 (http://www.R-project.org, The R Foundation) and Free Statistics software version 1.4 (Beijing, China).

TABLE 2 | The clinical characteristics of critically ill patients with non-traumatic SAH.

Characteristics	Serum Anion Gap (mmol/L)				*p*-value
	Total (*n* = 893)	Tertile1(<13) (*n* = 212)	Tertile2(≥13, <16) (*n* = 372)	Tertile3(≥16) (*n* = 309)	
Age, years	61.2 ± 14.9	62.1 ± 15.0	60.1 ± 14.6	62.0 ± 15.2	0.172
Sex, *n* (%)					0.791
Male	402 (45.0)	99 (46.7)	163 (43.8)	140 (45.3)	
Female	491 (55.0)	113 (53.3)	209 (56.2)	169 (54.7)	
Ethnicity, *n* (%)					0.005
White	541 (60.6)	138 (65.1)	238 (64)	165 (53.4)	
Asian	31 (3.5)	5 (2.4)	17 (4.6)	9 (2.9)	
Black	69 (7.7)	17 (8)	30 (8.1)	22 (7.1)	
other	252 (28.2)	52 (24.5)	87 (23.4)	113 (36.6)	
SBP, mmHg	125.1 ± 13.0	124.1 ± 12.8	125.4 ± 13.1	125.5 ± 13.1	0.445
DBP, mmHg	64.2 ± 9.0	62.6 ± 7.9	64.7 ± 9.0	64.7 ± 9.5	0.011
MBP, mmHg	82.4 ± 8.7	81.2 ± 7.9	82.8 ± 9.1	82.7 ± 8.8	0.078
HR, beats/min	78.6 ± 13.1	76.4 ± 11.9	77.1 ± 12.6	82.0 ± 13.9	<0.001
RR, beats/min	18.2 ± 3.3	17.6 ± 2.6	17.7 ± 3.2	19.3 ± 3.6	<0.001
Temperature, °C	37.0 ± 0.5	37.0 ± 0.4	37.0 ± 0.4	37.0 ± 0.7	0.788
SpO2, (IQR)	97.6 (96.1, 98.9)	97.7 (96.4, 99.0)	97.6 (96.0, 98.8)	97.6 (96.0, 98.9)	0.433
ICU length of stay, days	9.2 ± 8.5	8.4 ± 8.3	9.8 ± 9.1	9.0 ± 8.0	0.123
Hospital length of stay, days	14.7 ± 12.4	13.8 ± 11.6	15.6 ± 13.0	14.1 ± 12.2	0.149
ICU mortality, *n* (%)	132 (14.8)	25 (11.8)	25 (6.7)	82 (26.5)	<0.001
Hospital mortality, *n* (%)	169 (18.9)	30 (14.2)	44 (11.8)	95 (30.7)	<0.001
Surgery, *n* (%)					0.229
Embolization of aneurysm	36 (4.0)	6 (2.8)	20 (5.4)	10 (3.2)	
Clipping of aneurysm	786 (88.0)	184 (86.8)	323 (86.8)	279 (90.3)	
Vasopressor, *n* (%)	38 (4.3)	5 (2.4)	10 (2.7)	23 (7.4)	0.003
Scoring systems					
GCS	10.6 ± 4.1	11.2 ± 3.8	10.8 ± 4.0	9.8 ± 4.3	<0.001
SAPS II	32.4 ± 13.0	30.8 ± 11.6	30.4 ± 11.6	35.9 ± 14.8	<0.001
Comorbidities, *n* (%)					
Hypertention	446 (49.9)	96 (45.3)	187 (50.3)	163 (52.8)	0.243
Diabetes	127 (14.2)	27 (12.7)	39 (10.5)	61 (19.7)	0.002
Congestive heart failure	74 (8.3)	17 (8)	27 (7.3)	30 (9.7)	0.507
Chronic pulmonary disease	128 (14.3)	46 (21.7)	41 (11)	41 (13.3)	0.002
Sepsis	441 (49.4)	95 (44.8)	170 (45.7)	176 (57)	0.004
Renal failure	63 (7.1)	13 (6.1)	22 (5.9)	28 (9.1)	0.233
Liver diseases	14 (1.6)	3 (1.4)	5 (1.3)	6 (1.9)	0.802
Malignancy	35 (3.9)	5 (2.4)	15 (4)	15 (4.9)	0.35
Laboratory tests					
WBC (10^9/L)	12.6 ± 5.7	11.4 ± 5.7	12.3 ± 5.3	13.9 ± 5.9	<0.001
Hemoglobin (g/dL)	12.8 ± 2.1	12.3 ± 2.2	12.9 ± 2.0	13.0 ± 2.2	<0.001
Platelet (10^9/L)	227.1 ± 89.8	221.8 ± 82.6	229.9 ± 90.7	227.4 ± 93.4	0.573
Glucose(mmol/L)	150.1 ± 58.3	134.7 ± 36.2	148.1 ± 56.9	163.2 ± 68.7	<0.001
Sodium (mmol/L)	138.9 ± 4.0	139.2 ± 4.0	139.0 ± 3.9	138.5 ± 4.2	0.169
Potassium (mmol/L)	4.1 ± 0.8	4.1 ± 0.8	4.1 ± 0.8	4.1 ± 0.8	0.857
Chloride (mmol/L)	103.3 ± 4.8	104.6 ± 4.7	103.5 ± 4.5	102.1 ± 5.0	<0.001
BUN (mg/dL)	17.5 ± 11.7	16.5 ± 8.8	16.4 ± 8.6	19.6 ± 15.7	<0.001
Creatinine (mg/dL)	1.0 ± 0.8	0.8 ± 0.4	0.9 ± 0.3	1.1 ± 1.3	<0.001
Bicarbonate (mmol/L)	22.9 ± 3.7	24.2 ± 3.7	23.3 ± 3.5	21.6 ± 3.4	<0.001

SAH, subarachnoid hemorrhage; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; RR, respiratory rate; HR, heart rate; SpO2, percutaneous oxygen saturation; SAPS II, simplified acute physiology score II; GCS, Glasgow Coma Score; Chronic pulmonary disease, chronic obstructive pulmonary disease; WBC, white blood cell; BUN, blood urea nitrogen.

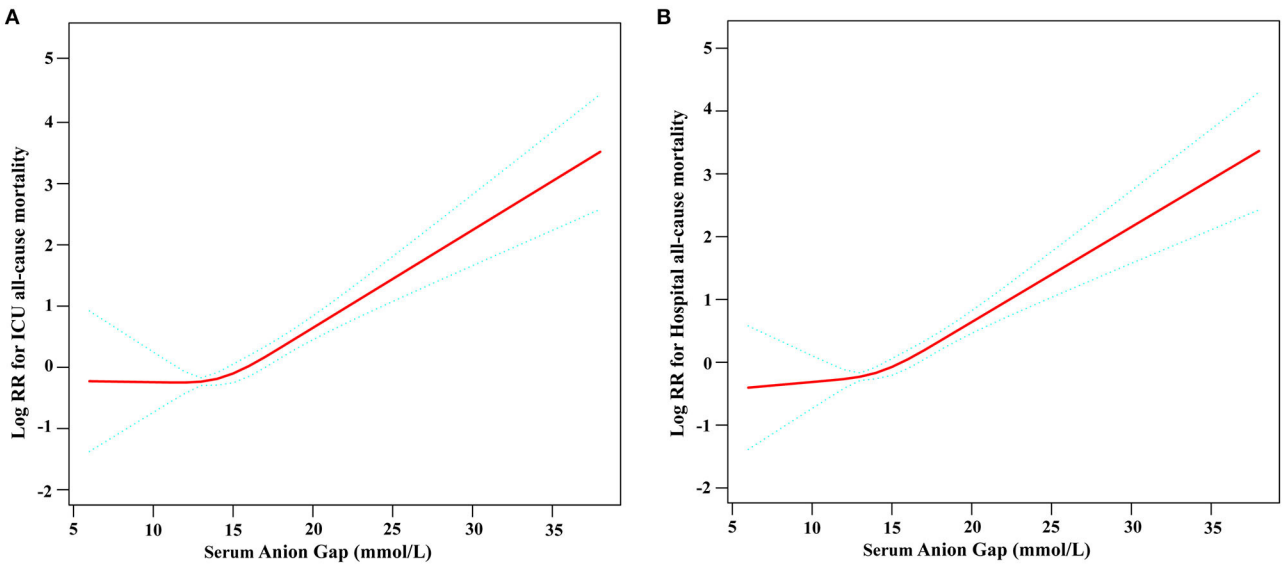

FIGURE 2 | Construction of smooth curve describing the risk of mortality against serum Anion Gap using a restricted cubic spline model. **(A)** ICU all-cause mortality; **(B)** Hospital all-cause mortality. The solid red line represents the smooth curve fit between variables. Blue bands present the 95% confidence interval. Data were adjusted for age, sex, ethnicity, SBP, DBP, respiratory rate, GCS, diabetes, sepsis, renal failure, chronic pulmonary disease, vasopressor, embolization of aneurysm, clipping of aneurysm, WBC, platelet, hemoglobin, glucose, BUN, and creatinine.

RESULTS

Baseline Characteristics of Subjects

In total, 893 of the 1,142 non-traumatic SAH patients who received ICU treatment were included in this study (**Figure 1**). Among them, there were 402 males and 491 females, and the mean age was 61.2 ± 14.9. The distribution of the baseline population characteristics according to serum AG levels in tertiles is described in **Table 2**. The patients with high serum AG levels (≥16 mmol/L) were found to have a lower GCS score, a higher SAPS II score and mortality, received more vasopressor treatment, and had more commodities, such as diabetes, sepsis, and chronic pulmonary disease.

Association Between Serum AG and All-Cause Mortality in Non-Traumatic SAH

Restricted cubic spline analysis revealed a non-linear relationship between serum AG and ICU all-cause mortality in subjects with non-traumatic SAH, which is consistent with hospital all-cause mortality. Serum AG level (<16 mmol/L) was not significantly associated with ICU and hospital all-cause mortality with non-traumatic SAH. There is an increase in ICU and hospital all-cause mortality with non-traumatic SAH as serum AG level (≥16 mmol/L) increases, as shown in **Figure 2**. **Table 3** provides the unadjusted and adjusted analyses for serum AG level and all-cause mortality in patients with non-traumatic SAH using Cox proportional hazards models. When used as a continuous variable, serum AG was associated with an increased risk of ICU (HR: 1.09 [95% CI: 1.04–1.14]) and hospital all-cause mortality (HR: 1.08 [95% CI: 1.03–1.13]). The ICU and hospital all-cause mortality of non-traumatic SAH increased with a per 1-unit increase in serum AG. Serum AG was evaluated as a categorical variable with ICU and hospital all-cause mortality, wherein the lower serum AG level (<16 mmol/L) was considered the reference. In the crude model, high serum AG level was associated with increased risk of ICU (HR: 3.17 [95% CI: 2.23–4.50]) and hospital all-cause mortality (HR: 2.67 [95% CI: 1.96–3.63]), respectively. In Model I, with adjustments for age, gender, ethnicity, and high serum AG level was associated with increased risk of ICU (HR: 2.88 [95% CI: 2.02–4.11]) and hospital all-cause mortality (HR: 2.40 [95% CI: 1.76–3.28]), respectively. Furthermore, Model II, which adjusted for age, sex, ethnicity, SBP, DBP, RR, heart rate, GCS, diabetes, sepsis, renal failure, chronic pulmonary disease, vasopressor, embolization of aneurysm, clipping of aneurysm, WBC, platelet, hemoglobin, glucose, BUN and creatinine, the higher serum AG still remained significantly associated with an increase in ICU (HR: 2.31 [95% CI: 1.58–3.38]) and hospital all-cause mortality (HR: 1.91 [95% CI: 1.36–2.67]) with the low serum AG group as reference. Regarding the sensitivity analysis, serum AG levels were both assessed as a continuous and categorical variable with ICU and hospital all-cause mortality, yielding consistent results."

In addition, the KM survival curve demonstrated that patients with high serum AG levels (≥16 mmol/L) on admission were associated with a lower risk of ICU and hospital survival rate ($p < 0.0001$) in **Figure 3**.

Subgroup Analysis

Subgroup analyses were performed to evaluate the association between high serum AG levels and hospital all-cause mortality (**Figure 4**). Based on the variables tested, an association between higher serum AG level and hospital all-cause mortality

TABLE 3 | Multivariable cox regression models evaluating the association between serum Anion Gap and ICU and hospital all-cause mortality.

Variable	Crude		Model I		Model II	
	HR(95%CI)	*p*-value	HR(95%CI)	*p*-value	HR(95%CI)	*p*-value
ICU all-cause mortality						
Serum AG <16 mmol/L	1 (Ref)		1 (Ref)		1 (Ref)	
Serum AG ≥16 mmol/L	3.17 (2.23, 4.5)	<0.001	2.88 (2.02,4.11)	<0.001	2.31 (1.58,3.38)	<0.001
Serum AG, 1 mmol/L	1.14 (1.11, 1.18)	<0.001	1.15 (1.11, 1.19)	<0.001	1.09 (1.04, 1.14)	<0.001
Hospital all-cause mortality						
Serum AG <16 mmol/L	1 (Ref)		1 (Ref)		1 (Ref)	
Serum AG ≥16 mmol/L	2.67 (1.96, 3.63)	<0.001	2.40 (1.76, 3.28)	<0.001	1.91 (1.36,2.67)	<0.001
Serum AG, 1 mmol/L	1.14 (1.10, 1.18)	<0.001	1.14 (1.11, 1.18)	<0.001	1.08 (1.03,1.13)	<0.001

***Crude model**: adjusted for none.*
***Model I**: adjusted for age, sex, and ethnicity.*
***Model II**: adjusted for **Model I**+SBP, DBP, respiratory rate, heart rate, GCS, diabetes, sepsis, renal failure, chronic pulmonary disease, vasopressor, embolization of aneurysm, Clipping of aneurysm, WBC, platelet, hemoglobin, glucose, BUN, creatinine.*

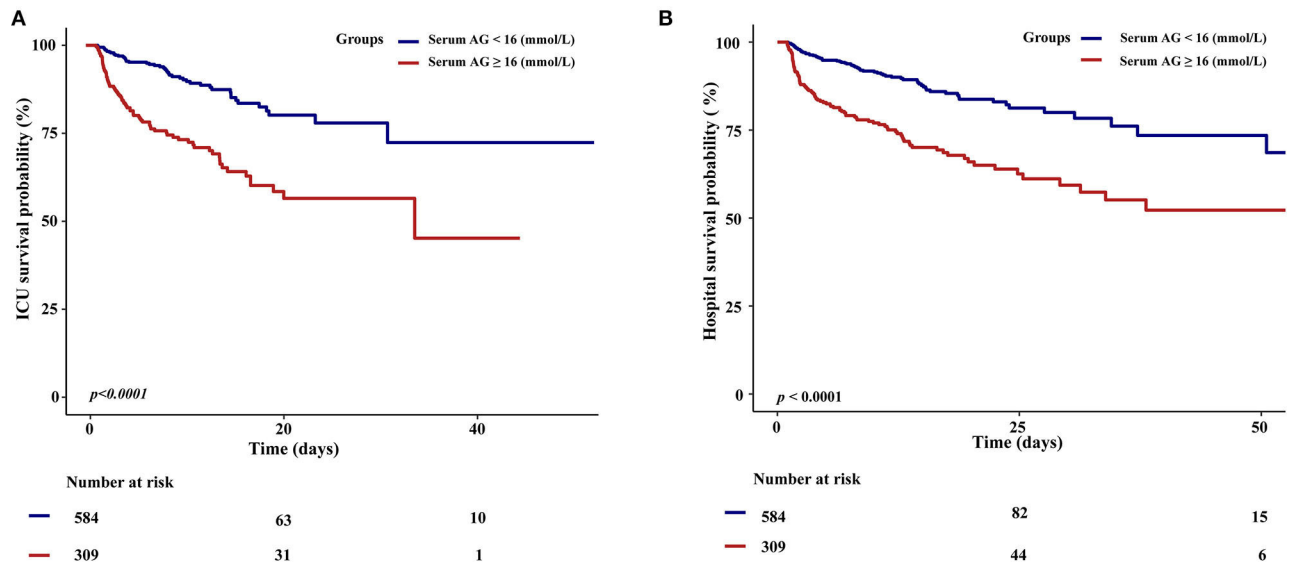

FIGURE 3 | Kaplan–Meier survival curves for critically ill patients with non-traumatic SAH based on serum Anion Gap. **(A)** ICU all-cause mortality; **(B)** Hospital all-cause mortality. *x*-Axis: survival time (days). *y*-Axis: survival probability.

was observed in different subgroups (hypertension, diabetes, congestive heart failure, chronic pulmonary disease, renal failure, sepsis, GCS, and SAPS II) ($p > 0.05$ for all).

DISCUSSION

This study revealed that subjects with high serum AG levels presented with a lower survival rate and shorter survival time, and that high serum AG level on admission was a significant risk for ICU and hospital all-cause mortality in critically ill patients with non-traumatic SAH. Specifically, patients with high serum AG levels (≥16 mmol/L) had a 2.31- and 1.91-fold higher risk of ICU and hospital all-cause mortality, respectively, than those with low serum AG levels. Moreover, we showed that different subgroups had no interactions upon correlating serum AG levels with hospital all-cause mortality.

Serum AG elevation is generally caused by the overproduction of organic acids or the reduced excretion of anions (5). In the clinical setting, serum AG is beneficial due to its simple calculation and because it does not require arterial access, which is why it is routinely determined in all patients admitted to the ICU (13). As such, many studies have explored the relationship between serum AG levels and clinical outcomes of critically ill patients. Recent studies using data from the MIMIC database found that elevated serum AG levels were correlated with an increased risk of all-cause mortality among patients with acute ischemic stroke (8). One study also showed that elevated serum AG levels were significantly associated with mortality in patients with cerebral infarction (9). Similarly,

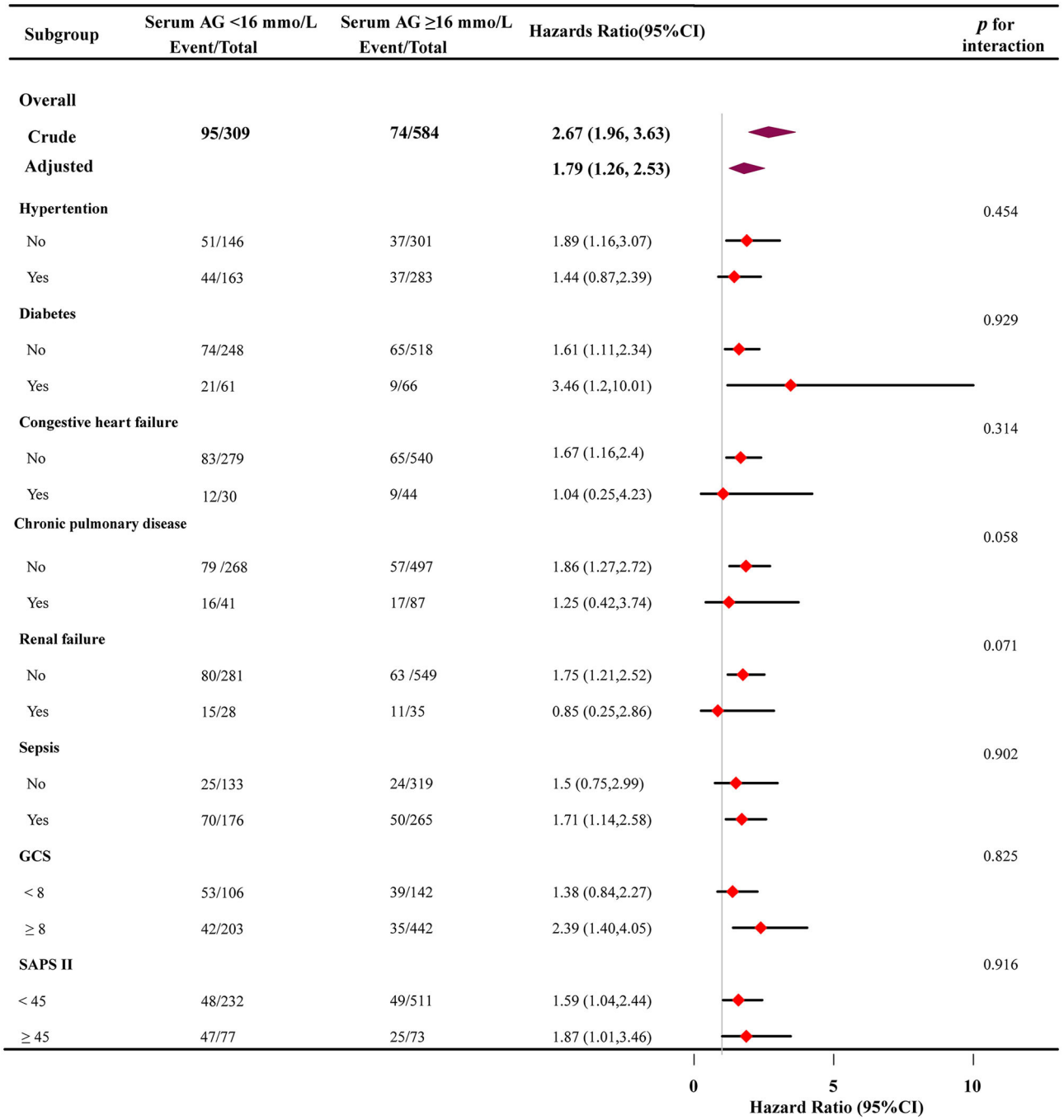

FIGURE 4 | Subgroup analyses of the effect of on hospital all-cause mortality. adjusted for age, sex, and ethnicity, SBP, DBP, respiratory rate, GCS, diabetes, sepsis, renal failure, chronic pulmonary disease, vasopressor, embolization of aneurysm, Clipping of aneurysm, WBC, platelet, hemoglobin, glucose, BUN, creatinine. In each case, the model was not adjusted for the stratification variable.

our study results were consistent with previous studies. After adjusting for confounding factors, patients with high serum AG levels ≥ 16 mmol/L) had a 2.31- and 1.91-fold higher risk of ICU and hospital all-cause mortality, respectively, than those with low serum AG levels. Furthermore, a recent systematic review and meta-analysis indicated that serum AG might be a viable tool for assessing the prognosis of critically ill patients, especially in areas with poor medical resources (16). Studies also showed that hypertension, diabetes, congestive heart failure, chronic pulmonary disease, renal failure, and sepsis are common comorbidities in patients with non-traumatic SAH. Although these comorbidities have been associated with poor outcomes,

our stratified and subgroup analyses did not change our overall results.

Interestingly, Tang et al. (12) and Cheng et al. (10) reported a U-shaped relationship between serum AG and mortality in critically ill patients with congestive heart failure and acute kidney injury, respectively. Gong et al. (11) also reported this U-shaped relationship between serum AG and ICU mortality in patients with acute pancreatitis. However, these findings may have obscured the relationship considering their insufficient sample size and that the low anion gap was assumed as a laboratory error (17).

It is difficult to clarify the precise mechanism behind the close correlation between serum AG and all-cause mortality in patients with non-traumatic SAH. Nevertheless, we can propose several potential explanations. One explanation is that an intracranial aneurysm rupture often causes a stress response in the body, leading to a disturbance in the internal environment. In addition, the cerebral blood flow (CBF) becomes unstable and brain metabolism is disturbed, leading to changes in ion concentration. Another explanation is that elevated serum AG levels usually manifest as a mild hemodynamic disturbance with inadequate tissue perfusion, which predisposes the patient to cerebral vasospasm (18). A third possibility is that elevated serum AG levels result in increased blood lactate levels, and acidosis due to increased organic acids can aggravate tissue ischemia and hypoxia (19). Once epilepsy is induced by massive bleeding, it can further aggravate ischemia and hypoxia, and tissue hypoxia caused by many factors can cause deterioration of the condition and even lead to death (20). Furthermore, high serum AG levels have been shown to be associated with high levels of inflammatory biomarkers (21). The influence of blood in the subarachnoid space initiates the rapid activation of inflammatory cascades, and the neuroinflammatory response has been found to play an important role in the outcome of patients with non-traumatic SAH (22).

Despite these findings, several limitations of our study should be noted. First, given the retrospective design of the study, data had already been collected. Second, due to the limitations of the MIMIC database, missing information that could have affected the model was not collected, such as albumin, lactate, pH, and Hunt & Hess grades. However, it should be noted that the potential results from these variables would bias toward the null, resulting in an undervaluation of the connection between serum AG levels and all-cause mortality. Third, we were only able to provide the association between serum AG levels and mortality rather than establish a causal relationship. Nevertheless, the relationship between high serum AG levels and all-cause mortality was clearly revealed.

In summary, this retrospective observational study revealed that high serum AG levels were a significant risk for ICU and hospital all-cause mortality in patients with non-traumatic SAH. Further prospective studies with larger sample sizes should be performed to assess the causality between high serum AG levels and ICU and hospital all-cause mortality.

DATA AVAILABILITY STATEMENT

The data analyzed in this study was obtained from Medical Information Mart for Intensive Care IV (MIMIC-IV, https://mimic-iv.mit.edu), the following licenses/restrictions apply: To access the files, you must be a credentialed user, finish required training and sign the data use agreement for the project. Requests to access these datasets should be directed to PhysioNet, https://physionet.org/, doi: 10.13026/s6n6-xd98.

ETHICS STATEMENT

The studies involving human participants were examined and approved by Beth Israel Deaconess Medical Center. To protect patient privacy, all data were de-identified; therefore, the Ethical Committee of the Beth Israel Deaconess Medical Center waived the requirement for informed consent.

AUTHOR CONTRIBUTIONS

JL and CZ designed the study and collected the data. JL, CZ, MY, and LH interpreted the result and interpreted the result. JL wrote the first draft of the manuscript. CZ contributed to the refinement of the manuscript. The final manuscript has been read and approved by all authors.

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REFERENCES

1. Nieuwkamp DJ, Setz LE, Algra A, Linn FH, de Rooij NK, Rinkel GJ. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: a meta-analysis. *Lancet Neurol.* (2009) 8:635–42. doi: 10.1016/S1474-4422(09)70126-7
2. Johnston S C, Selvin S, Gress D R. The burden, trends, and demographics of mortality from subarachnoid hemorrhage. *Neurology.* (1998) 50:1413–8. doi: 10.1212/WNL.50.5.1413
3. Samuels O B, Sadan O, Feng C, et al. Aneurysmal subarachnoid hemorrhage: trends, outcomes, and predictions from a 15-year perspective of a single neurocritical care unit. *Neurosurgery.* (2021) 88:574–83. doi: 10.1093/neuros/nyaa465
4. Adrogue H J, Madias *N* E. Management of life-threatening acid-base disorders. First of two parts. *N Engl J Med.* (1998) 338:26–34. doi: 10.1056/NEJM199801013380106
5. Kraut J A, Madias *N* E. Serum anion gap: its uses and limitations in clinical medicine. *Clin J Am Soc Nephrol.* (2007) 2:162–74. doi: 10.2215/CJN.03020906
6. Tang Y, Lin W, Zha L, et al. Serum anion gap is associated with all-cause mortality among critically ill patients with congestive heart failure. *Dis Markers.* (2020) 2020:8833637. doi: 10.1155/2020/8833637
7. Zhang T, Wang J, Li X. Association between anion gap and mortality in critically ill patients with cardiogenic shock. *Int J Gen Med.* (2021) 14:4765–73. doi: 10.2147/IJGM.S329150

8. Jhou HJ, Chen PH, Yang LY, Chang SH, Lee CH. Plasma anion gap and risk of in-hospital mortality in patients with acute ischemic stroke: analysis from the MIMIC-IV database. *J Pers Med*. (2021) 11:1004. doi: 10.3390/jpm11101004
9. Liu X, Feng Y, Zhu X, et al. Serum anion gap at admission predicts all-cause mortality in critically ill patients with cerebral infarction: evidence from the MIMIC-III database. *Biomarkers*. (2020) 25:725–32. doi: 10.1080/1354750X.2020.1842497
10. Cheng B, Li D, Gong Y, et al. Serum anion gap predicts all-cause mortality in critically ill patients with acute kidney injury: analysis of the MIMIC-III database. *Dis Markers*. (2020) 2020:6501272. doi: 10.1155/2020/6501272
11. Gong F, Zhou Q, Gui C, et al. The relationship between the serum anion gap and all-cause mortality in acute pancreatitis: an analysis of the MIMIC-III database. *Int J Gen Med*. (2021) 14:531–8. doi: 10.2147/IJGM.S293340
12. Xu C, Sun L, Dong M, et al. Serum anion gap is associated with risk of all-cause mortality in critically Ill patients with acute myocardial infarction. *Int J Gen Med*. (2022) 15:223–31. doi: 10.2147/IJGM.S336701
13. Chen Q, Chen Q, Li L, et al. Serum anion gap on admission predicts intensive care unit mortality in patients with aortic aneurysm. *Exp Ther Med*. (2018) 16:1766–77. doi: 10.3892/etm.2018.6391
14. Johnson A E, Pollard T J, Shen L, et al. MIMIC-III, a freely accessible critical care database. *Sci Data*. (2016) 3:160035. doi: 10.1038/sdata.2016.35
15. Zhang Z. Multiple imputation with multivariate imputation by chained equation (MICE) package. *Ann Transl Med*. (2016) 4:30. doi: 10.3978/j.issn.2305-5839.2015.12.63
16. Glasmacher S A, Stones W. Anion gap as a prognostic tool for risk stratification in critically ill patients - a systematic review and meta-analysis. *BMC Anesthesiol*. (2016) 16:68. doi: 10.1186/s12871-016-0241-y
17. Goldstein R J, Lichtenstein *N* S, Souder D. The myth of the low anion gap. *JAMA*. (1980) 243:1737–8. doi: 10.1001/jama.1980.03300430039020
18. Kurisu K, Yenari M A. Therapeutic hypothermia for ischemic stroke; pathophysiology and future promise. *Neuropharmacology*. (2018) 134:302–9. doi: 10.1016/j.neuropharm.2017.08.025
19. Sekine S, Shimodaira C, Uesawa Y, et al. Quantitative structure-activity relationship analysis of cytotoxicity and anti-UV activity of 2-aminotropones. *Anticancer Res*. (2014) 34:1743–50.
20. Rush B, Wiskar K, Fruhstorfer C, et al. Association between seizures and mortality in patients with aneurysmal subarachnoid hemorrhage: a nationwide retrospective cohort analysis. *Seizure*. (2016) 41:66–9. doi: 10.1016/j.seizure.2016.07.008
21. Farwell W R, Taylor E N. Serum anion gap, bicarbonate and biomarkers of inflammation in healthy individuals in a national survey. *CMAJ*. (2010) 182:137–41. doi: 10.1503/cmaj.090329
22. Hong C M, Tosun C, Kurland D B, et al. Biomarkers as outcome predictors in subarachnoid hemorrhage—a systematic review. *Biomarkers*. (2014) 19:95–108. doi: 10.3109/1354750X.2014.881418

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Longitudinal changes in the hypothalamic–pituitary–adrenal axis and sympathetic nervous system are related to the prognosis of stroke

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Background and purpose: This study sought to improve methods to identify biomarkers in the neuroendocrine system related to stroke progression to improve the accuracy of traditional tools for evaluating stroke prognosis.

Methods: Seventy-four stroke patients and 237 healthy controls were prospectively included. We measured urinary epinephrine (E), noradrenaline (NE), dopamine (DA) and cortisol (F) on days 1, 3, and 5 after stroke onset and plasma F, adrenocorticotropic hormone (ACTH), thyrotropin (TSH), prolactin (PRL), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and growth hormone (GH). The correlation between these hormone levels and 90-day prognosis was analyzed, their value in assessing prognosis was compared with lesion volume and National Institutes of Health Stroke Scale (NIHSS) scores using receiver operating characteristic (ROC) curves, and their correlation with conventional clinical variables was assessed.

Results: Levels of F, 24-h urinary free cortisol(UFC), E, NE, DA, and GH on days 1, 3, and 5 were significantly higher in stroke patients than in controls ($P < 0.01$), while ACTH and TSH decreased, gradually approaching normal within 5 days of onset. Levels of E, NE, F, and 24-h UFC were proportional to severity, and all gradually decreased within 5 days of onset in patients with a good prognosis and gradually increased or remained high in those with a poor prognosis. After adjustment for age, sex, NIHSS, or Glasgow Coma Scale (GCS) score, $F > 13.6$ μg/dL, ACTH > 22.02 pg/mL and NE > 123.5 μg/ 24 h were identified as risk factors for a poor prognosis 90 days after stroke ($P < 0.05$). The combination of F, ACTH, NE, white blood cell count (WBC), glucose (Glu), and hemoglobin (Hb) was significantly more accurate than lesion volume (AUC: 0.931 vs. 0.694 $P = 0.019$) and NIHSS score (AUC: 0.931 vs. 0.746 $P = 0.034$) in predicting poor prognosis of stroke 1 day after onset. Hormones and traditional clinical variables were correlated to varying degrees, with NE correlating most strongly with 24-h UFC ($r = 0.54$) and moderately positively with lesion volume ($r = 0.40$) and NIHSS score ($r = 0.45$).

Conclusions: Stroke causes significant time-phased dynamic changes in the hypothalamic–pituitary–adrenal axis and sympathetic nervous system, and plasma F, ACTH, and urinary NE levels can be used to assess stroke severity and prognosis.

Chinese clinical trial registry: Registration Number: ChiCTR1900024992. Registration Date: 2019/8/6.

KEYWORDS

stroke, hormones, catecholamines, sympathetic, severity, prognosis

Stroke is a leading cause of death worldwide (1), with 6.2 million people dying from stroke in 2017, which is nearly two times greater than the number of deaths in 1990 (2); approximately 3 to 4% of health care expenditures in Western countries are spent on stroke (3). Reducing stroke-related mortality to decrease the socioeconomic burden is a global challenge.

Early and accurate assessment of stroke severity and early risk and prognostic analysis are essential for making the right clinical decisions to improve prognosis. The National Institutes of Health Stroke Scale (NIHSS) is commonly used internationally as a standardized and widely used assessment for predicting the 3-month prognosis of acute cerebrovascular events. However, its use requires specific training, and there is observer subjectivity. Other scale assessment methods, such as the European Stroke Scale (ESS), the Canadian Neurological Scale (CNS) and the Scandinavian Stroke Scale (SSS), for assessing the prognosis of stroke have the same limitations. The scale method suffers from interrater inconsistency (4) and underestimates the risk of posterior circulation stroke (5). Therefore, there is a clinical need for simple and objective assessment tools for predicting disease progression, outcome and mortality.

Neuroimaging has made great advances in recent years and provides a more objective and quantitative assessment for understanding the severity and prognosis of stroke. In addition to imaging, biomarker studies related to stroke prognosis are known as hot spots; however, a systematic evaluation found that although these studies involve neuroendocrine aspects, not many (6, 7) involve the detection of central endocrine metabolites, which can be useful in the assessment of stroke. The classic 'stress response' of the body to stroke occurs with hypothalamic–pituitary–adrenal (HPA) stressors. In cerebral ischemia, HPA changes are one of the first measurable endocrine changes and are characterized by increased cortisol levels, decreased thyroid function and deficits in the synthesis of metabolic hormones such as growth hormone (GH) and insulin. Other anterior pituitary axis hormones, such as peripheral thyroid hormone and growth hormone, are also useful in predicting stroke prognosis (8), which is associated with immune dysregulation due to neuroendocrine disruption after stroke. Low T3 syndrome is an independent predictor of survival among acute stroke patients, and even low T3 levels within the normal range have been associated with a poorer prognosis among acute stroke patients (9). Patients with higher levels of growth hormone have a higher mortality rate and a poorer prognosis, which are associated with stress-mediated increases in GH levels (10).

Although the prevalence of neuroendocrine system changes during the acute phase of stroke is being increasingly recognized, the dynamics of key neuroendocrine factors of the organism, other than cortisol, GH and T3, in stroke onset and their relationship with prognosis have been less well studied (8, 11–13). Although some studies have found potential mechanisms for the presence of brain–heart interactions in stroke related to the HPA axis. For example, catecholamines may mediate neurogenic heart damage after brain injury (14), and the HPA axis is associated with poststroke infection (15). However, there is a lack of research on whether these mechanisms are related to each other. In addition, HPA axis modification therapy for stroke has been unsuccessful in translation because of the poor agreement between the therapeutic window of corticotropin-releasing factor antagonism and the pharmacokinetics of the explored antagonists (16). Studies of central noradrenergic agonists for stroke have encountered similar problems (17). Therefore, a comprehensive understanding of endocrine changes in different periods after stroke is essential for the development of new drug targets.

In conclusion, although the central endocrine metabolic pathway reflects, to some extent, the neurotransmitter regulation of brain tissue and the performance of this function is closely related to the number and function of central neuronal cells, the complexity of the regulation and interaction of the central endocrine metabolic pathway, especially the rhythmic nature of endocrine and the variability during different stages of

disease, greatly limit the use of endocrine-related biomarkers. We designed a prospective cohort study to investigate the dynamic expression of these central endocrine metabolites in the plasma of stroke patients under the premise of strictly controlling the endocrine rhythm. In addition, in order to avoid the issue of large fluctuations in plasma catecholamine levels, we selected urine samples with more stable catecholamine levels. We measured serum adrenocorticotropic hormone (ACTH), plasma cortisol (F), 24-h urinary free cortisol (UFC), luteinizing hormone (LH), and urine cortisol on days 1, 3, and 5 after stroke onset. LH, follicle-stimulating hormone (FSH), prolactin (PRL), urinary free epinephrine (E), norepinephrine (NE), dopamine (DA), thyroid-stimulating hormone (TSH), and GH were also quantified. This study aimed to analyze the correlation of these central endocrine metabolites in the early stage of stroke with prognosis at 90 days, to explore their roles in aiding the more commonly used or recognized current clinical tools for stroke severity and prognosis assessment, such as NIHSS scores, Glasgow Coma Scale (GCS) scores and imaging lesion volumes, and to investigate the value of these central endocrine metabolites for prognostic assessment.

Methods

Study population

Patients with stroke who visited the emergency department of the Ruijin Hospital North Campus, Shanghai Jiaotong University School of Medicine, from May 2019 to January 2021 were prospectively selected. The inclusion criteria were as follows: (1) A diagnosis of stroke in accordance with the 2013 update of the American Heart Association/American Stroke Association definition of stroke (18); (2) arrival within 24 h of stroke onset; (3) age >18 years; (4) first onset; (5) GCS score >8; and (6) provision of signed informed consent. The exclusion criteria were (1) the presence of complications of epilepsy, infection, or gastrointestinal bleeding; (2) the use of vasoactive and sympathomimetic active drugs within 1 week before and after admission; (3) exclusion of subarachnoid hemorrhage and intraventricular hemorrhage, taking into account differences in prognostic assessment; (4) a history of psychiatric disease, thyroid disease, pituitary insufficiency, renal disease, severe cardiac insufficiency, respiratory failure, hepatic insufficiency, or malignancy; and (5) surgical treatment. Patients received standard treatment in the emergency stroke unit for hemorrhagic stroke (HS) and ischemic stroke (IS) in accordance with the American Heart Association/American Stroke Association recommended guidelines for the treatment of spontaneous cerebral hemorrhage (2015) (19) and with the American Heart Association/American Stroke Association recommended guidelines for the early treatment of patients with acute ischemic stroke 2018, respectively (20). Individuals who underwent health check-ups at our center during the same period were selected as healthy controls. All patients or family members signed a written informed consent form.

Baseline data acquisition

All patients were evaluated by 2 designated emergency department physicians. The demographic and clinical characteristics included age, sex, body mass index, stroke type, lesion site, GCS score, NIHSS score, vital signs on admission, past medical history, preadmission medication history and the presence of hypertension (HTN), diabetes mellitus (DM), hyperlipidemia (HL), coronary artery disease (CAD), and smoking-related vascular risk factors. The GCS assesses the state of consciousness by eye opening response, speech, and movement. The NIHSS is used to assess the degree of functional impairment caused by stroke and consists of a total of 11 tests with a score range of 0 to 42, with higher scores indicating more severe stroke. Routine hematological investigations, including routine blood tests, hepatic and renal function, coagulation, fasting glucose and lipids, were completed within 24 h of admission and cerebrovascular and cervical angiography within 72 h of admission. CT and diffusion-weighted imaging (DWI) lesion volumes were assessed using MIPAV software (version 11.0) by two experienced radiologists with no knowledge of the clinical and laboratory findings.

Specimen test

Collection of 24-h urine was started immediately after admission. The urine collection bottle was prefilled with 5% glacial acetic acid to decrease the urine pH to < 4.0, the specimen was protected from light, and the 24-h urine volume was recorded. Urine specimens were sent to the Shanghai Institute of Hypertension at 8:00 a.m. on days 1, 3, and 5 after admission for assessment of urinary free E, NE, and DA levels within 2 h by high-performance liquid chromatography (instrument provided by Agilent Technologies, Ltd., with the corresponding reagents); 5 ml of peripheral cubital venous blood was collected from the subjects at 8:00 after an overnight fast on days 1, 3, and 5 after admission and sent to the hospital laboratory within 30 min of collection. Twenty-four-hour UFC, F, PRL, FSH and LH levels were measured by chemiluminescence (instrument: Beckman DXI800, reagents in kit), TSH levels were measured by electrochemiluminescence (instrument: Abbott i2000, reagents in kit), and GH and ACTH levels were measured by electrochemiluminescence (instrument: Roche Cobas 601, reagents in kit).

Outcomes

The event endpoint was functional outcome 90 days after onset. The modified Rankin Scale (mRS) score was obtained by a standardized telephone interview with the patient or a family member by 1 trained doctor 90 days after onset, with a good outcome defined as an mRS score ≤2. Briefly, a Rankin Scale score of 0 indicates no symptoms; a score of 1 indicates no evident disability despite symptoms; a score of 2 indicates slight disability, with an inability to carry out all previous activities; a score of 3 indicates moderate disability, with the need for some help but the ability to walk without assistance; a score of 4 indicates moderately severe disability, with the inability to walk without assistance or to attend to bodily needs without assistance; a score of 5 indicates severe disability, with the patient being bedridden and incontinent and requiring constant nursing care; and a score of 6 indicates death.

Grouping

Patients were divided into groups according to the type of stroke (hemorrhagic stroke and ischemic stroke), the NIHSS score [a light group (<10 points) and a heavy group (≥10 points)], and the mRS score [a good prognosis group (mRS ≤2 points) and a poor prognosis group (mRS >2 points)].

Statistical analysis

Data were statistically analyzed using SPSS 23.0 and MedCalc software. Categorical variables are expressed as frequencies (component ratios) and were compared using the chi-square test. Non-normally distributed variables are presented as the median (interquartile range). The Mann–Whitney *U* test and Kruskal–Wallis test were used to compare two or more sample, and Spearman's correlation was calculated between different variables. Univariate regression models were used to assess the accuracy of biomarkers and other clinical variables in predicting prognosis. We did not perform multivariate analyses due to the limited number of results and the risk of overfitting. A P value < 0.05 indicated a statistically significant difference, and the results were plotted using GraphPad Prism software (version 8.0.2).

Results

Patients

A total of 74 stroke patients were enrolled, excluding 2 cases of postadmission sedation, 3 cases of concurrent infection, 1 case of concurrent gout attack, 1 case of moyamoya disease found after admission and 1 case of postdischarge death due to a traffic accident. Sixty-six patients were included in the analysis (Supplementary Figure S1), comprising 29 patients with hemorrhagic stroke [median age, 55 years (IQR 49–64); 21 males and 8 females] and 37 patients with ischemic stroke [median age, 67 years (IQR 52–74); 22 males and 15 females]. The prognosis was good in 40 cases and poor in 26 cases at the 90-day follow-up, with most patients having one or two risk factors, most commonly hypertension. In the same period, 237 healthy individuals were included in the healthy control group. There was no significant difference in age or sex between the stroke patient and healthy control groups (Table 1).

TABLE 1 Analysis of baseline characteristics of the stroke patient and healthy control groups.

	Stroke patients ($N = 66$)	Healthy controls ($N = 237$)	P-value
Age, *y*	61.1 (± 13.1)	61.0 (± 9.5)	0.980
Male	43 (65.2%)	137 (57.8%)	0.282

Data are presented as means ± SDs for continuous variables and as n (%) for categorical variables.
N indicates the total number of cases in different groups.

Changes in hormone levels at various time points

Compared with those in the healthy control population, the levels of 24-h urinary E, NE and DA in stroke patients increased significantly, and gradually decreased with the passage of time. Among them, the levels of 24-h NE and DA were similar to those in the healthy group [IQR 53.98 (33.23, 120.5) ug/24 h vs. 40.05 (31.43, 52.22) ug/24 h], but the level of 24-h E was still significantly higher than that in the healthy group on the 5th day after onset (Figures 1A–C). F and 24-h UFC levels in the HPA axis pathway were significantly higher in stroke patients, with F levels remaining high for 5 days and 24-h UFC levels gradually decreasing over 5 days, but still significantly higher than those in the healthy control population (Figures 1E,F). ACTH levels decreased slightly on the first day after stroke compared to those in the healthy control group [IQR 29.74 (16.55, 52.54) pg/ml vs. 32.07 (20.10, 46.07) pg/ml], and then gradually increased slightly [IQR 29.74 (16.55, 52.54) pg/ml; 31.08 (24.14, 52.94) pg/ml; 34.06 (19.11, 53.28) pg/ml], but the difference was not statistically significant (Figure 1D). The trend in anterior pituitary hormone levels was inconsistent in the stroke patient population compared to the healthy control population, with GH levels increasing significantly after onset [IQR 0.356 (0.161, 0.738) μg/L vs. 0.137 (0.057, 0.411) μg/L] and remaining high for 5 days, TSH levels decreasing significantly [IQR 0.7833 (0.4215, 1.2340) mIU/L vs. 1.9290 (1.2080, 2.8040)

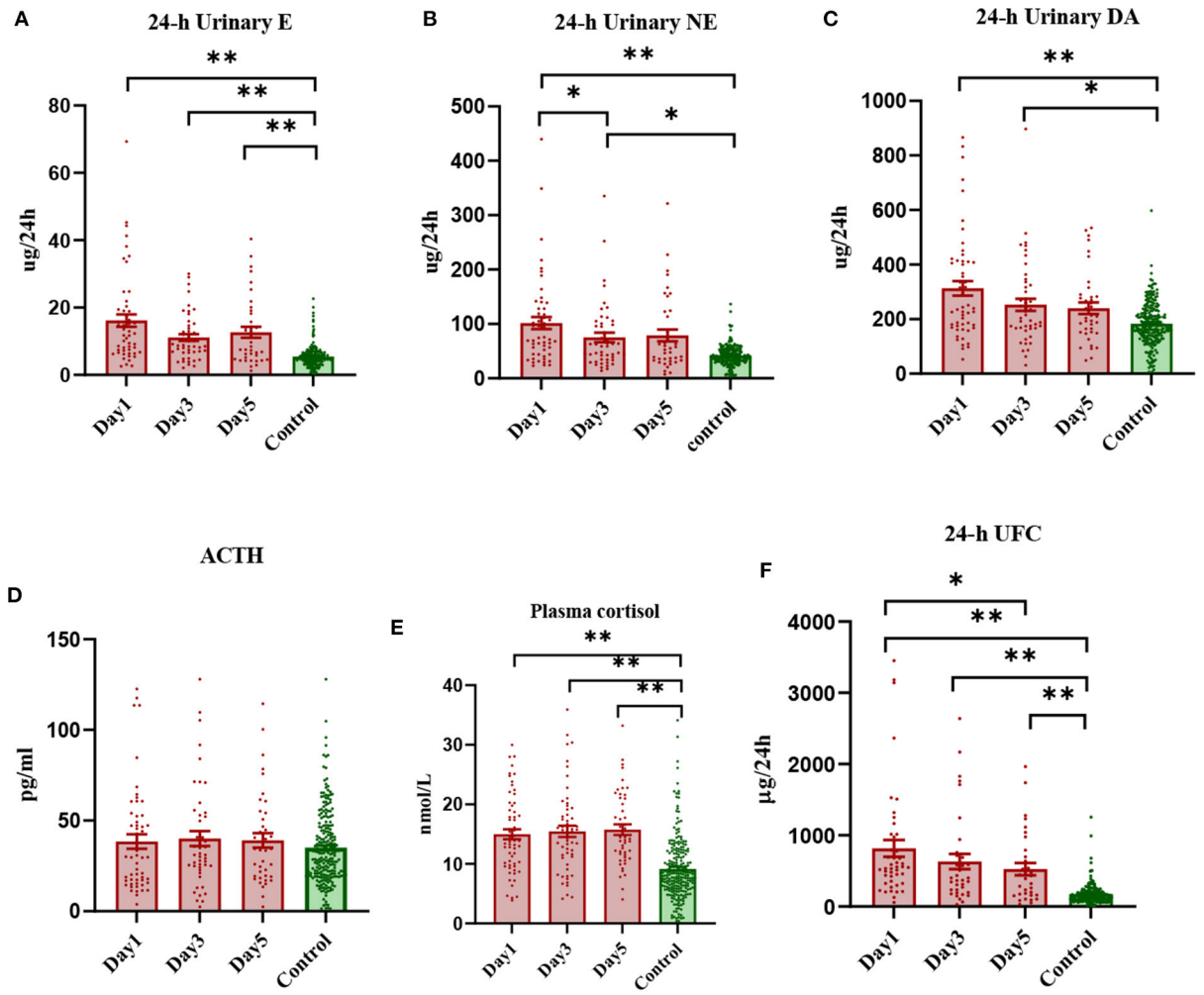

FIGURE 1
Scatterplot of early levels of each hormone in stroke patients vs. healthy controls (median and interquartile range). *Denotes $P < 0.05$, **denotes $P < 0.01$.

mIU/L] and then increasing gradually, and FSH, LH and PRL levels showing no significant change (Supplementary Figure S2).

Baseline characterization

Among the main baseline characteristics of the case group at admission (Table 2), chi-square test and Mann–Whitney *U* test analyses showed that compared with the good prognosis group, the poor prognosis group had higher NIHSS scores (12 vs. 6), lower GCS scores (13 vs. 15), and larger lesion volume (15 cm^2 vs. 4.4 cm^2), WBC was higher (9.03 × $10^9 \cdot L^{-1}$ vs. 7.21 × $10^9 \cdot L^{-1}$), blood glucose was higher (8.9 $mmol \cdot L^{-1}$ vs. 6.3 $mmol \cdot L^{-1}$), hemoglobin level was lower (126 $g \cdot L^{-1}$ vs. 139 $g \cdot L^{-1}$), 24-h NE level was higher (116.71 $\mu g \cdot 24\ h^{-1}$ vs. 68.25 $\mu g \cdot 24\ h^{-1}$), ACTH level was lower (20.20 $pg \cdot ml^{-1}$ vs. 42.72 $pg \cdot ml^{-1}$), FSH level was higher (36.40 $mIU \cdot mL^{-1}$ vs. 8.71 $mIU \cdot mL^{-1}$), and blood cortisol level was higher (15.66 $\mu g \cdot dL^{-1}$ vs. 12.82 $\mu g \cdot dL^{-1}$). There were no significant differences in stroke type, heart rate, blood pressure, site of lesion, risk factors, liver and kidney function, lipids or coagulation, E, DA, LH, PRL, TSH, GH, or 24-h UFC between the prognosis groups.

Comparison of hormone levels between different prognosis and severity groups

Changes in hormone levels after stroke onset differed between the different prognosis and severity groups (Figure 2). On day 1 after the onset of stroke, regardless of prognosis or severity, patients showed increased levels of E, NE, F, and 24-h UFC, and over the next 5 days, E, NE, F, and 24-h UFC

TABLE 2 Baseline characteristics of stroke patients on admission to hospital.

	Total ($n = 66$)	90-day mRS score ≤ 2	90-day mRS score > 2	*P*-value
Baseline characteristic				
NIHSS score, median (IQR)	10 (4.8–13.3)	6 (3.11)	12 (10.16)	0.000
GCS score, median (IQR)	15 (13–15)	15 (14.15)	13 (12.15)	0.000
BMI, median (IQR), $kg{\cdot}m^{-2}$	23.9 (22.0–26.3)	24.9 (22.2–27.0)	23.0 (21.5–25.0)	0.050
HR ($beats{\cdot}min^{-1}$)	80 (75–87)	80 (74–87)	82 (75–88)	0.373
SBP (mmHg)	149 (138–165)	150 (139–163)	149 (134–167)	0.971
DBP (mmHg)	85 (77–95)	87 (80–97)	80 (74–94)	0.218
Stroke type, *n* (%)				
HS	29 (43.9%)	16 (24.2%)	13 (19.7%)	0.424
IS	37 (56.1%)	24 (36.4%)	13 (19.7%)	
Stroke location, *n* (%)				
Basal ganglia	31 (47.0%)	20 (30.3%)	11 (16.7%)	0.803
Thalamus	8 (12.1%)	4 (6.1%)	4 (6.1%)	
Cerebellum	7 (10.6%)	5 (7.6%)	2 (3.0%)	
Occipital lobe	2 (3.0%)	1 (1.5%)	1 (1.5%)	
Frontal lobe	7 (10.6%)	3 (4.5%)	4 (6.1%)	
Parietal lobe	1 (1.5%)	0 (0.0%)	1 (1.5%)	
Temporal lobe	2 (3.0%)	1 (1.5%)	1 (1.5%)	
Cerebellum	1 (1.5%)	1 (1.5%)	0 (0.0%)	
Near tricorn	7 (10.6%)	5 (7.6%)	2 (3.0%)	
Risk factor, *n* (%)				
Hypertension	45 (68.2%)	25 (37.9%)	20 (30.3%)	0.219
Diabetes mellitus	19 (28.8%)	9 (13.6%)	10 (15.2%)	0.162
Hypercholesterolemia	28 (42.4%)	18 (27.2%)	10 (15.2%)	0.599
Smoking	21 (31.8%)	12 (18.2%)	9 (13.6%)	0.694
Imageological examination				
Lesion volume, median (IQR), cm^2	6.6 (1.3–20.0)	4.4 (1.2–7.7)	15.0 (1.2–33.3)	0.026
Routine laboratory inspection				
White blood cells ($10^9{\cdot}L^{-1}$)	8.09 (6.56–9.64)	7.21 (6.17–9.13)	9.03 (6.94–13.89)	0.015
Glucose ($mmol{\cdot}L^{-1}$)	6.9 (5.8–9.6)	6.3 (5.6–8.5)	8.9 (6.6–11.1)	0.004
Hemoglobin ($g{\cdot}L^{-1}$)	137 (125–145)	139 (131–149)	126 (118–139)	0.008
HbA1c (%)	5.9 (5.6–6.6)	5.9 (5.6–6.35)	5.95 (5.35–8.45)	0.925
LDL($mmoI{\cdot}L^{-1}$)	3.06 (2.62–3.77)	3.16 (2.68–3.79)	3.00 (2.49–3.70)	0.399
Platelets ($\times 10^9{\cdot}L^{-1}$)	176 (151–217)	167 (149–202)	191 (172–241)	0.111
Gamma-glutamyl transferase ($IU{\cdot}L^{-1}$)	19 (13–28)	19 (14–26)	21 (13–34)	0.280
Albumin ($g{\cdot}L^{-1}$)	39 (36–43)	39 (37–43)	38 (36–42)	0.609
Creatinine ($\mu moI{\cdot}L^{-1}$)	76 (65–85)	76 (66–83)	73 (63–92)	0.813
INR	1.03 (0.97–1.08)	1.03 (0.98–1.07)	1.03 (0.97–1.12)	0.465
D-dimer ($\mu g{\cdot}mL^{-1}$)	0.14 (0.07–0.32)	0.12 (0.06–0.22)	0.20 (0.08–0.68)	0.060
CRP ($mg{\cdot}dl^{-1}$)	10.0 (10.0–10.8)	10.0 (10.0–10.0)	10.0 (10.0–13.8)	0.081
24-h urinary free catecholamines day 1				
Epinephrine ($\mu g{\cdot}24\ h^{-1}$)	12.04 (6.89–19.32)	10.50 (6.68–18.64)	14.15 (7.47–23.31)	0.347
Norepinephrine ($\mu g{\cdot}24\ h^{-1}$)	74.55 (44.24–128.25)	68.25 (42.21–109.31)	116.71 (65.23–177.26)	0.033
Dopamine ($\mu g{\cdot}24\ h^{-1}$)	239.74 (172.60–411.27)	239.74 (183.87–413.33)	215.58 (160.57–398.10)	0.388

(Continued)

TABLE 2 Continued

	Total ($n = 66$)	90-day mRS score ≤ 2	90-day mRS score > 2	*P*-value
Anterior pituitary hormones day 1				
Adrenocorticotropic ($pg{\cdot}ml^{-1}$)	29.74 (16.55–52.54)	42.72 (18.64–60.51)	20.20 (13.62–46.62)	0.047
Luteinizing hormone ($\mu IU{\cdot}mL^{-1}$)	9.35 (3.93–19.50)	6.54 (3.44–15.87)	14.00 (5.12–26.13)	0.072
Follicle-stimulating hormone ($mIU{\cdot}mL^{-1}$)	14.20 (5.47–48.20)	8.71 (4.70–37.72)	36.40 (8.99–56.99)	0.015
Prolactin ($ng{\cdot}ml^{-1}$)	10.66 (7.58–15.01)	10.74 (7.29–14.03)	10.26 (8.33–15.18)	0.748
Thyroid-stimulating hormone ($mIU{\cdot}L^{-1}$)	0.8102 (0.4245–1.3245)	0.8914 (0.4388–1.5669)	0.7620 (0.4170–1.1996)	0.502
Growth hormone ($\mu g{\cdot}L^{-1}$)	0.356 (0.161–0.738)	0.349 (0.150–0.816)	0.377 (0.169–0.732)	0.802
Cortisol day 1				
Plasma cortisol ($\mu g{\cdot}dL^{-1}$)	13.89 (9.90–19.41)	12.82 (9.22–18.14)	15.66 (13.17–22.42)	0.039
24-h urinary free cortisol ($\mu g{\cdot}24\ h^{-1}$)	548.10 (340.73–994.25)	527.73 (348.65–634.31)	683.13 (324.66–1,527.96)	0.141

Data are presented as n (%) for categorical variables and as medians (interquartile ranges) for continuous variables. The bold values indicate the value of $p < 0.05$.
GCS, Glasgow Coma Scale; NIHSS, National Institutes of Health Stroke Scale; BMI, Body mass index; HR, Heart rate; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HbA1c, Hemoglobin A1c; LDL, Low-density lipoprotein; INR, International normalized ratio; CRP, C-reactive protein.

levels tended to decrease in patients with a good prognosis, while the levels tended to increase gradually or remained high in those with a poor prognosis. The levels of these four hormones were proportional to the severity (Supplementary Figure S3), with patients with severe disease and poor prognosis having the highest levels and those with mild disease and good prognosis having lower levels. The most significant decrease in NE levels was observed in patients with severe disease and good prognosis (2.5-fold on day 1, 1.1-fold on day 3 and 0.2-fold on day 5 compared to healthy controls, $P = 0.012$), while those with mild disease but poor prognosis showed an increasing trend in NE levels (1.2-fold on day 1, 1.5-fold on day 3 and 2.9-fold on day 5 compared to healthy controls). On day 1 after onset, DA levels were higher in the severe group than in the control group and then declined gradually to approach control levels by day 5. In contrast, DA levels in the mild group were comparable to those in the control group on days 1, 3, and 5. On day 1 after onset, ACTH levels were significantly lower in patients with a poor prognosis (0.6-fold) and higher in those with a good prognosis (1.2-fold) compared to the healthy controls and then gradually increased or decreased, approaching healthy control levels on day 5. Patients with a poor prognosis showed a gradual increase in ACTH levels after onset, while those with a good prognosis showed a gradual decrease. FSH and TSH levels were lower, and GH levels were higher in stroke patients than in healthy controls; FSH levels were higher in patients with a poor prognosis on day 1 than in those with a good prognosis, although TSH and GH levels were not significantly different. LH and PRL levels did not differ significantly between stroke and healthy control populations, regardless of prognosis or severity (Supplementary Figures S3, S4).

Creation of receiver operating characteristic curves

To further investigate the value of biomarkers in assessing stroke prognosis, we plotted ROC curves by combining biomarkers measured on day 1 after onset with clinical variables (Supplementary Figure S5). The AUC values for F, NE, and ACTH were found to be similar to WBC, age and lesion volume and lower than the NIHSS score, the GCS score, Glu, and HB. After adjustment for age, sex and the NIHSS score, $F > 13.6$ μg/dL and ACTH > 22.02 pg/mL were found to be risk factors for poor stroke prognosis ($P < 0.05$), and after adjustment for age, sex and the GCS score, NE > 123.5 μg/24 h was identified as a risk factor for poor stroke prognosis ($P < 0.05$). The AUC for the combination of F and ACTH to predict poor stroke prognosis was comparable to that of lesion volume (0.682 vs. 0.682) and smaller than that of the NIHSS score (0.682 vs. 0.768), but the difference was not statistically significant ($P = 0.367$). The AUC (0.677 vs. 0.768) for NE in predicting poor stroke prognosis was comparable to that of lesion volume (0.677 vs. 0.680) and smaller than that of the NIHSS score, but the difference was not statistically significant ($P = 0.260$). The AUC (0.754 vs. 0.682 $P = 0.359$) and sensitivity (58.8% vs. 42.3%) were higher for the combination of F, ACTH, NE, and lesion volume in predicting poor prognosis of stroke than for lesion volume alone. The AUC (0.832 vs. 0.768 $P = 0.190$) and specificity (71.4% vs. 65.0%) were higher for the combination of F, ACTH, NE, and the NIHSS score than for the NIHSS score alone. The accuracy of the combination of F, ACTH, NE, WBC, Glu, and HB in predicting poor prognosis of stroke was significantly higher than that of lesion volume (AUC: 0.931 vs. 0.694 $P =$

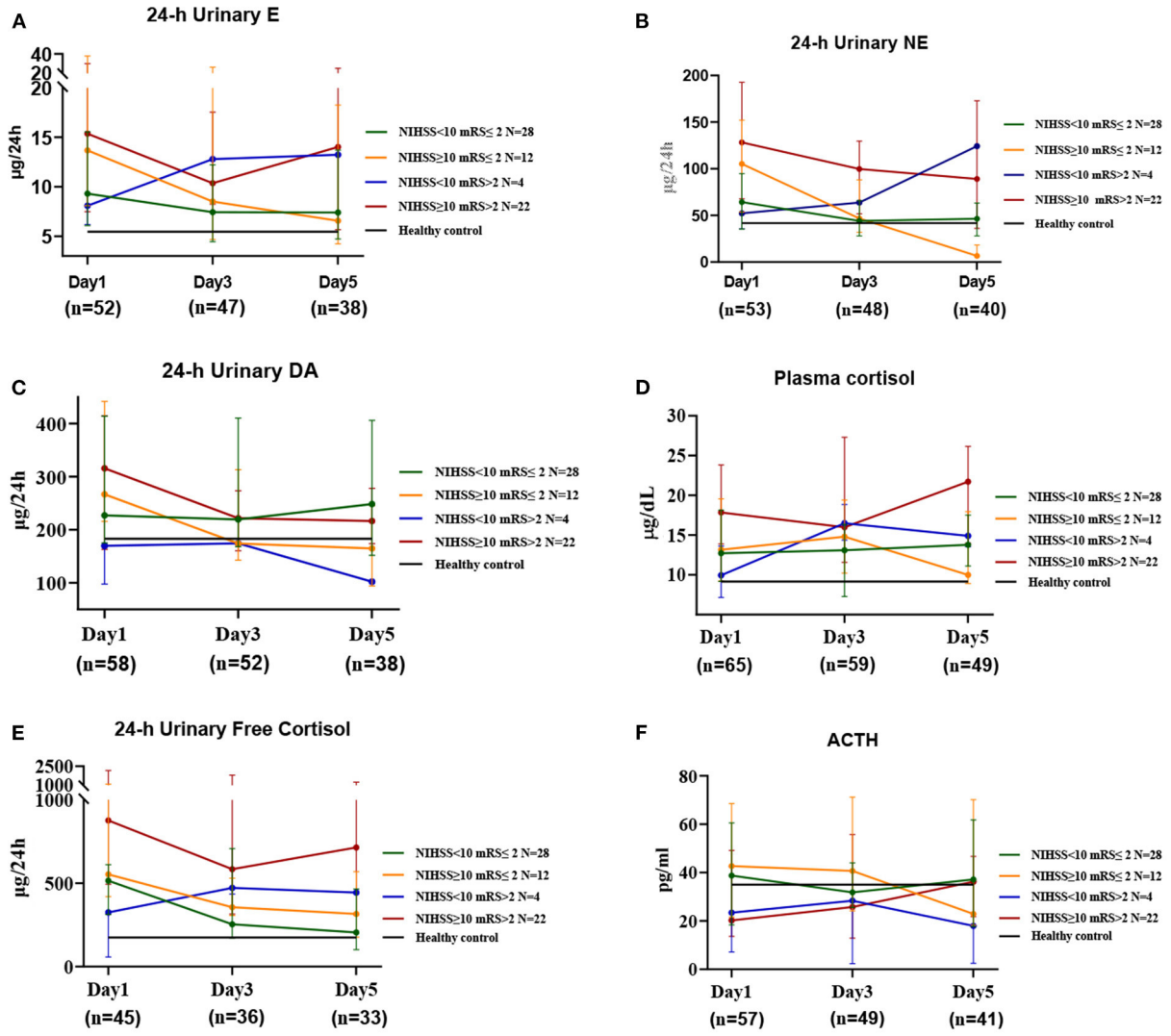

FIGURE 2
The changes in HPA axis hormones and catecholamines in stroke patients at three time periods. This includes the first day (Day 1), the third day (Day 3), and the fifth day after admission (Day 5). Median values for the 3 time periods show good and poor prognoses for patients with mild and severe disease at admission, respectively. Healthy controls show relative mean values, $n = 237$, age = 61.0 (± 9.5) years; *N*′ refers to the number of cases in different prognosis and severity groups; *n*′ refers to the number of specimens in different time periods.

0.019) and the NIHSS score (AUC: 0.931 vs. 0.746 $P = 0.034$) (Figure 3).

The correlation of hormones with clinical variables

We observed varying degrees of correlation of E and NE with F and 24-h UFC on day 1 post-stroke onset, with NE correlating most strongly with 24-h UFC ($r = 0.54$), 24-h UFC correlating negatively with TSH and PRL ($r = -0.56$, $r = -0.43$), and no significant correlation between catecholamines and pituitary hormones. E and NE were moderately positively correlated with lesion volume ($r = 0.47$, $r = 0.40$), and DA was weakly negatively correlated with Glu ($r = -0.37$). E, NE, F, and 24-h UFC were all correlated with the GCS and NIHSS scores to varying degrees, with the strongest correlation being between NE and the NIHSS score ($r = 0.45$). In addition, 24-h UFC was positively correlated with lesion volume and WBC ($r = 0.36$, $r = 0.40$) (Supplementary Figure S5), and NE and UFC were the nodes with a high concentration of 2 connecting lines (7 edges, 9 edges) among the biomarkers (Figure 4, see Supplementary Figure S6 for full information).

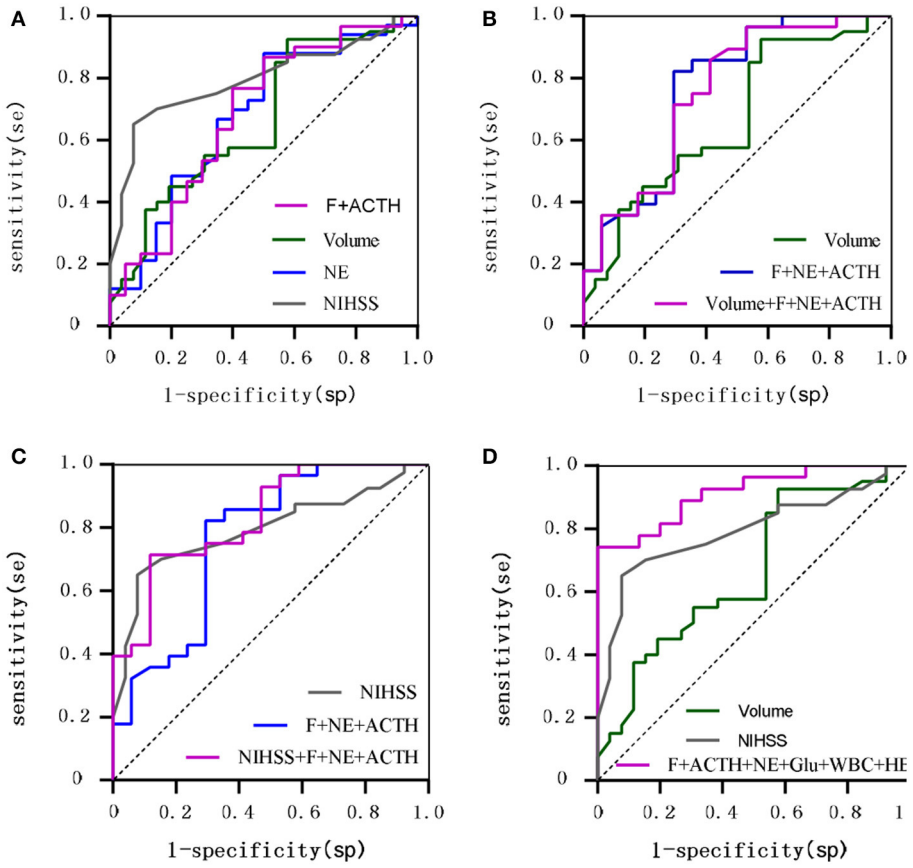

FIGURE 3
ROC curves comparing the value of hormones vs. traditional clinical variables for 90-day prognostic assessment. **(A)** Shows F combined with ACTH, NE, Lesion volume, NIHSS; **(B)** Shows F combined with NE and ACTH compared with Lesion volume; **(C)** Shows F combined with NE and ACTH compared with NIHSS; **(D)** Shows F combined with ACTH and NE and Glu and WBC and HB compared with Lesion volume, NIHSS.

Discussion

In the present study, we simultaneously assessed the dynamics of steroid hormones, catecholamine hormones and gonadotropins at the onset of stroke and their accuracy in predicting functional prognosis and mortality for acute ischemic stroke patients within 90 days of onset by mass spectrometry analysis of targeted metabolomics. Our main finding is that, in agreement with most published results, cortisol is an independent prognostic marker of functional prognosis and mortality in ischemic stroke patients (11). We demonstrated that cortisol levels increased with imaging lesion size and neurological deficits (assessed by the NIHSS), reflecting stroke severity. In contrast, TSH and GH, although also dynamically altered at stroke onset, showed limited variation, possibly because current measures are not sensitive enough to provide meaningful information on prognosis. It is very interesting to note the synergy between HPA hormones, catecholamine hormones and conventional test markers that can be used as an adjunct to imaging and the NIHSS score to assess clinical prognosis and provide important additional predictive information. For example, the accuracy of the combination of F and ACTH in predicting poor prognosis of stroke is comparable to that of lesion volume and second only to the NIHSS score, and the addition of F and ACTH to lesion volume and the NIHSS score increases the accuracy of lesion volume and the NIHSS score in predicting poor prognosis of stroke. Urine tests for catecholamines are also of some value; for example, the 24-h urinary NE level is a better assessor of severity and prognosis within 5 days of stroke onset and has shown similar accuracy to lesion volume in assessing poor stroke prognosis. If lesion volume and the NIHSS score were combined with F, ACTH, and NE markers, they would show higher sensitivity than lesion volume alone and higher specificity than the NIHSS score alone, thus improving the accuracy of prediction.

In addition, our study compared the above neuroendocrine metabolites in urine and plasma and found that 24-h UFC and F levels were significantly higher in stroke patients than in healthy

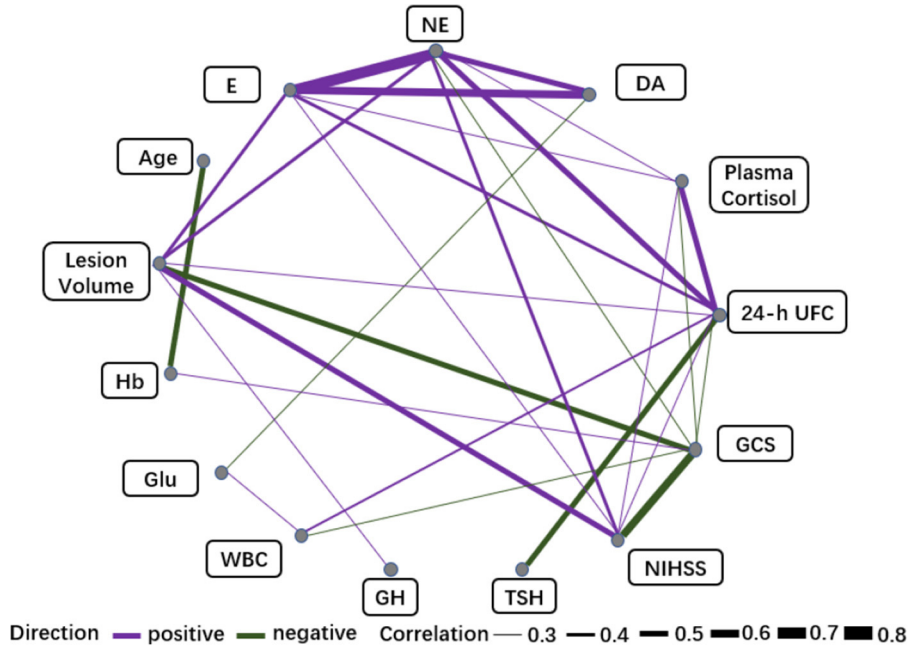

FIGURE 4
Correlation networks for biomarkers (Day 1) and clinical variables. The width of the edge showing stronger or weaker interactions is proportional to the absolute value of biomarker-biomarker correlation (|r|). Edges are shown only when $|r| > 0.3$. A purple edge indicates a positive correlation, and a green edge indicates a negative correlation.

subjects within 5 days of stroke onset, with 24-h UFC and F levels significantly higher in the poor prognosis group than in the good prognosis group and in the high severity group than in the low severity group. Within 5 days of stroke onset, 24-h UFC showed a more pronounced and stable trend than F. Moreover, 24-h UFC showed a similar value to F in assessing prognosis and severity. This is of great value in assessing the trend of disease progression following stroke onset and treatment and in guiding clinical management.

Quantitative stroke neuropathology with substantial quantification of the rate of neural circuit loss in acute ischemic stroke assessed by imaging tests has made possible a more objective and quantitative assessment of brain neuronal loss per unit time in acute brain injury to understand stroke severity and prognosis (21, 22). This study also yielded consistent results, finding that among the indicators tested, E, NE, 24-h UFC, and GH had the best correlation with MRI or CT brain tissue damage volume assessment at stroke onset for prognosis and disease severity, with E correlating most strongly with lesion volume ($r = 0.468$ $P = 0.000$), NE ($r = 0.403$ $P = 0.003$), F ($r = 0.270$ $P = 0.035$), 24-h UFC ($r = 0.364$ $P = 0.014$), and GH ($r = 0.357$ $P = 0.011$). More importantly, our results also used imaging test results as a control parameter for prognostic evaluation of stroke and found that some central endocrine metabolites, such as NE, had an assessment value similar to that of CT or MRI results to assist in the assessment of not only neuroimaging effects but also the dynamic changes in NE and other biomarkers in the acute phase of stroke. A temporal correlation was found for the assessment of stroke progression, treatment effects and prognosis, suggesting that the measurement of these indicators in blood and urine is a more effective adjunct to assessment tools for heterogeneous samples, with its lower cost, easy quantification methods, repeatability in a short period of time and reliable prediction of outcomes. In addition, CT or MRI combined with the measurement of central endocrine metabolites has a more objective and accurate value in improving the prognostic assessment of stroke.

There is growing clinical and experimental evidence of a causal relationship between brain injury and cardiac dysfunction. Most poststroke deaths are attributed to neurological injury, with cardiovascular complications being the second leading cause of death after stroke. Potential mechanisms of brain–heart interactions after stroke, such as the HPA axis, catecholamine surge, and sympathetic and parasympathetic regulation, are relevant, and there are clinical studies directly confirming that supplementation of these hormones to stroke patients has a beneficial effect on stroke. However, few studies have examined urinary levels of E, NE, and DA in the acute and subacute phases following stroke onset. Our study found that urinary E, NE, and DA levels rose significantly on the day of stroke onset and gradually

decreased over the next 5 days but remained high. We therefore suggest that sympathetic arousal may persist in stroke patients for at least 5 days after stroke onset, thereby mediating cardiovascular complications leading to a poor prognosis. However, it is uncertain whether β-blockers and dopamine treatment can improve the outcome of stroke by altering sympathetic drive and increasing dopamine concentrations (23, 24).

The main cause of poor stroke prognosis is poststroke neuroendocrine disruption. Serum cortisol levels increase in proportion to the degree of stress and correlate with stroke severity. The reasons for the poor prognosis of stroke patients with high cortisol levels are related to the following: (1) Excess cortisol has been known to exacerbate ischemic neuronal damage, especially in the hippocampus. (2) Patients with stroke and high cortisol levels are more likely to experience cardiac events (e.g., arrhythmias or myogenic fibrous degeneration), resulting in higher mortality (8). This result of our study is consistent with the results of other studies. Additionally, our study demonstrated the same changes in urinary cortisol levels.

Although in some prospective studies, stroke patients had significantly higher serum GH levels on admission than their normal counterparts, showing a significant correlation with 30-day mortality and 90-day functional recovery, GH levels on admission were considered to be an independent predictor of patient mortality (10). Our study did not show a statistically significant difference, although we also observed a significant increase in GH levels after stroke and a stepwise decrease in GH levels after treatment in patients with prognosis. This may be related to our small sample size and overly specific subgroups, but our results suggest that GH is slightly less sensitive than catecholamines and glucocorticoids in predicting the severity and prognosis of stroke. This may be related to the fact that GH levels are elevated not only in stroke patients but also in critically ill adult patients caused by severe infections of the central nervous system and sepsis, so GH reflects disease severity and patient stress status (25).

Similarly, in our study, TSH was less sensitive than catecholamines in predicting stroke, and although TSH levels decreased significantly after stroke onset and showed a gradual increase within 5 days of stroke onset, TSH did not show value in predicting poor prognosis or assessing severity.

In summary, we suggest that the intensity of the neuroendocrine response may be a potential prognostic marker for brain injury (26), and that these endocrine-related biomarkers combined with imaging and NIHSS scores are valuable in assessing the prognosis of stroke.

Limitations

Stroke is highly heterogeneous, and this study did not include a multifactorial analysis to demonstrate that these biomarkers are independent prognostic indicators due to sample size limitations. To minimize the effect of interference of confounding factors on the results, we set strict inclusion criteria to screen all patients for any comorbidities or any medication use that could affect CA levels, which limited the number of eligible patients in this study and could have led to selection bias. We did not assess patients' basal levels of TSH, GH, and CAs prior to the onset of the disease, such as the presence of subclinical hypothyroidism in patients, which would have affected the results of the study. The secretion of anterior pituitary hormones is rhythmic and is influenced by external environmental factors, such as season, light and darkness, and the sleep-wake cycle [e.g., GH secretion is pulsatile, and TSH levels vary by up to approximately 50% on average from day to day (27)] and by sex and age (28). These factors are inevitable, although there was no difference in the sex ratio between the stroke and control groups and all women were menopausal; we also had a dedicated area with a quiet and normal environment and made efforts to maintain a normal sleep-wake cycle for patients and collect blood and urine specimens at a uniform time.

Conclusion

Although the exact mechanism has not been elucidated, the present study suggests that stroke causes significant temporal and dynamic changes in the HPA axis pathway and sympathetic nervous system (SNS) hormones and that plasma F, ACTH, and urinary NE levels can be used to assess stroke severity and prognosis. These findings need to be confirmed by cohort studies with larger samples and longer follow-up, and future trials are necessary to demonstrate viable options for improving stroke prognosis through treatments that improve the endocrine response.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by ethical approval for the study was obtained from Ethics Committee of Ruijin Hospital North, Shanghai Jiao Tong University School of Medicine (2019-002-2). The patients/participants

provided their written informed consent to participate in this study.

Author contributions

Y-ML and JYe contributed to conception and design of the study. LH, YC, H-WS, LZ, and Z-BL organized the database. HW and X-SW performed the statistical analysis. X-GC and S-YS wrote the first draft of the manuscript. JYu and Y-JZ wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.946593/full#supplementary-material

References

1. Roth GA, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the global burden of disease study 2017. *Lancet.* (2018) 392:1736–88. doi: 10.1016/S0140-6736(18)32203-7

2. Krishnamurthi RV, Ikeda T, Feigin VL. Global, regional and country-specific burden of ischaemic stroke, intracerebral haemorrhage and subarachnoid haemorrhage: a systematic analysis of the global burden of disease study 2017. *Neuroepidemiology.* (2020) 54:171–9. doi: 10.1159/000506396

3. Katan M, Luft A. Global burden of stroke. *Semin Neurol.* (2018) 38:208–11. doi: 10.1055/s-0038-1649503

4. Josephson SA, Hills NK, Johnston SC, NIH Stroke Scale reliability in ratings from a large sample of clinicians. *Cerebrovasc Dis.* (2006) 22:389–95. doi: 10.1159/000094857

5. Kwah LK, Diong J. National institutes of health stroke scale (NIHSS). *J. physiother.* 60:61. doi: 10.1016/j.jphys.2013.12.012

6. Montellano FA, Ungethüm K, Ramiro L, Nacu A, Hellwig S, Fluri F, et al. Role of blood-based biomarkers in ischemic stroke prognosis: a systematic review. *Stroke.* (2021) 52:543–51. doi: 10.1161/STROKEAHA.120.029232

7. Troiani Z, Ascanio L, Rossitto CP, Ali M, Mohammadi N, Majidi S, et al. Prognostic utility of serum biomarkers in intracerebral hemorrhage: a systematic review. *Neurorehabil Neural Repair.* (2021) 35:946–59. doi: 10.1177/15459683211041314

8. Neidert S, Katan M, Schuetz P, Fluri F, Ernst A, Bingisser R, et al. Anterior pituitary axis hormones and outcome in acute ischaemic stroke. *J Intern Med.* (2011) 269:420–32. doi: 10.1111/j.1365-2796.2010.02327.x

9. Alevizaki M, Synetou M, Xynos K, Pappa T, Vemmos KN. Low triiodothyronine: a strong predictor of outcome in acute stroke patients. *Eur J Clin Invest.* (2007) 37:651–7. doi: 10.1111/j.1365-2362.2007.01839.x

10. Zweifel C, Katan M, Schuetz P, Ernst A, Mariani L, Müller B, et al. Growth hormone and outcome in patients with intracerebral hemorrhage: a pilot study. *Biomarkers.* (2011) 16:511–6. doi: 10.3109/1354750X.2011.599074

11. Barugh AJ, Gray P, Shenkin SD, MacLullich AM, Mead GE. Cortisol levels and the severity and outcomes of acute stroke: a systematic review. *J Neurol.* (2014) 261:533–45. doi: 10.1007/s00415-013-7231-5

12. O'Keefe LM, Conway SE, Czap A, Malchoff CD, Benashski S, Fortunato G, et al. Thyroid hormones and functional outcomes after ischemic stroke. *Thyroid Res.* (2015) 8:9. doi: 10.1186/s13044-015-0021-7

13. Brouns R, Van Hemelrijck A, Drinkenburg WH, Van Dam D, De Surgeloose D, De Deyn PP. Excitatory amino acids and monoaminergic neurotransmitters in cerebrospinal fluid of acute ischemic stroke patients. *Neurochem Int.* (2010) 56:865–70. doi: 10.1016/j.neuint.2009.12.014

14. Chen Z, Venkat P, Seyfried D, Chopp M, Yan T, Chen J. Brain-heart interaction: cardiac complications after stroke. *Circ Res.* (2017) 121:451–68. doi: 10.1161/CIRCRESAHA.117.311170

15. Vogelgesang A, Dressel A. Immunological consequences of ischemic stroke: immunosuppression and autoimmunity. *J Neuroimmunol.* (2011) 231:105–10. doi: 10.1016/j.jneuroim.2010.09.023

16. Lichlyter DA, Krumm ZA, Golde TA, Doré S. Role of CRF and the hypothalamic-pituitary-adrenal axis in stroke: revisiting temporal considerations and targeting a new generation of therapeutics. *FEBS J.* (2022). doi: 10.1111/febs.16380

17. Sternberg Z, Schaller B. Central noradrenergic agonists in the treatment of ischemic stroke-an overview. *Transl Stroke Res.* (2020) 11:165–84. doi: 10.1007/s12975-019-00718-7

18. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. An updated definition of stroke for the 21st century. *Stroke.* (2013) 44:2064–89. doi: 10.1161/STR.0b013e318296aeca

19. Hemphill JC, Greenberg SM, Anderson CS, Becker K, Bendok BR, Cushman M, et al. Guidelines for the management of spontaneous intracerebral hemorrhage. *Stroke.* (2015) 46:2032–60. doi: 10.1161/STR.0000000000000069

20. Furie KL, Jayaraman MV. 2018 guidelines for the early management of patients with acute ischemic stroke. *Stroke.* (2018) 49:509–10. doi: 10.1161/STROKEAHA.118.020176

21. Desai SM, Rocha M, Jovin TG, Jadhav AP. High variability in neuronal loss. *Stroke.* (2019) 50:34–7. doi: 10.1161/STROKEAHA.118.023499

22. Saver JL. Time is brain–quantified. *Stroke.* (2006) 37:263–6. doi: 10.1161/01.STR.0000196957.55928.ab

23. Eizenberg Y, Grossman E, Tanne D, Koton S. Pre admission treatment with beta-blockers in hypertensive patients with acute stroke and 3-month outcome-data from a national stroke registry. *J Clin Hypertens.* (2018) 20:568–72. doi: 10.1111/jch.13211

24. Starr JB, Tirschwell DL, Becker KJ. Increased infections with β-blocker use in ischemic stroke, a β(2)-receptor mediated process? *Neurol Sci.* (2017) 38:967–74. doi: 10.1007/s10072-017-2877-x

25. Schuetz P, Müller B, Nusbaumer C, Wieland M, Christ-Crain M. Circulating levels of GH predict mortality and complement prognostic scores in critically ill medical patients. *Euro J Endocrinol.* (2009) 160:157–63. doi: 10.1530/EJE-08-0786

26. El Husseini N, Laskowitz DT. The role of neuroendocrine pathways in prognosis after stroke. *Expert Rev Neurother.* (2014) 14:217–32. doi: 10.1586/14737175.2014.877841

27. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American association of clinical endocrinologists and the American thyroid association. *Thyroid.* (2012) 22:1200–35. doi: 10.1089/thy.2012.0205

28. Ehrenkranz J, Bach PR, Snow GL, Schneider A, Lee JL, Ilstrup S, et al. Circadian and circannual rhythms in thyroid hormones: determining the TSH and free T4 reference intervals based upon time of day, age, and sex. *Thyroid.* (2015) 25:954–61. doi: 10.1089/thy.2014.0589

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The relationship between red blood cell distribution width at admission and post-stroke fatigue in the acute phase of acute ischemic stroke

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Introduction: Post-stroke fatigue (PSF) is a common complication in the patients with acute ischemic stroke (AIS). This prospective study aimed to investigate the relationship between red blood cell distribution width (RDW) at admission and PSF in the acute phase.

Methods: The AIS patients were enrolled in Nantong Third People's Hospital, consecutively. PSF in the acute phase was scored according to the Fatigue Severity Scale. Levels of RDW were measured at admission. The associations were analyzed using multivariate regression and restricted cubic splines (RCS).

Results: From April 2021 to March 2022, a total of 206 AIS patients (mean age, 69.3 ± 10.7 years; 52.9% men) were recruited. After the adjustment for potential confounding factors, RDW at admission remained the independent associated factor with PSF in the acute phase (OR [odds ratio], 1.635; 95% CI [confidence interval], 1.153–2.318; $P = 0.006$). The linear dose-response associations of RDW with PSF in the acute phase were found, based on the RCS model (P for non-linearity = 0.372; P for linearity = 0.037). These results remained significant in other models.

Conclusions: RDW at admission could serve as a novel biomarker of PSF in the acute phase of AIS.

KEYWORDS

acute ischemic stroke, post-stroke fatigue, red blood cell distribution width, restricted cubic spline, biomarker

Introduction

Stroke is one of the vital reasons for global disease burden (1–3). Acute ischemic stroke (AIS) accounts for the vast majority of stroke. Although there are many treatment options for ischemic stroke nowadays (4–6), the AIS patients may undergo several complications during their treatment and rehabilitation. Post-stroke fatigue (PSF), whose

incidence range from 23 to 85%, is a common emotional complication after the onset of AIS (7–11). PSF could lead to poor recovery and prognosis (7–11). Therefore, it is meaningful and fundamental to explore the related factors to PSF.

Red blood cell distribution width (RDW), which could be obtained from routine blood testing, is an easily accessible biomarker. According to previous studies, RDW is able to reflect the variability in volumes of peripheral red blood cells and associated with inflammation (12, 13). In addition, RDW has been shown to be an effective biomarker for many diseases. For instance, RDW may serve as an undesirable prognostic factor in patients treated with hematopoietic stem cell transplantation (14). The levels of RDW might be associated with long-term all-cause mortality in the patients with acute myocardial infarction (15). Moreover, one recent study, which is carried out by Li Y et al., have found that elevated levels of RDW at admission may be able to predict depression after the onset of AIS (16).

Nevertheless, to our knowledge, there is no research about the relationship between the levels of RDW at admission and PSF in AIS patients. Hence, our prospective study was designed to assess the role of RDW on PSF in the acute phase.

Materials and methods

Study subjects

From April 2021 to March 2022, the AIS patients were enrolled in our study from the Nantong Third People's Hospital, consecutively and prospectively.

The inclusion criteria were listed as follows:

- Onset within 7 days;
- Age≥18 years;
- With sufficient cognitive ability;
- With sufficient knowledge of the Mandarin Chinese language and Nantong local dialect.

The exclusion criteria were listed as follows:

- Pre-stroke fatigue;
- Incomplete data;
- Refuse to participate in the research;
- Poor mental state.

This study was registered with China clinical trial registration center (Registration number: ChiCTR2100044165), approved by the ethics committee of Nantong Third People's hospital (Ethics number: EK2021008), and performed according to the principles of the Declaration of Helsinki.

Clinical assessments

The clinical assessments were completed in a separate conversation room. We collected general demographic questionnaires (age and sex), past medical history (hypertension, diabetes, cancer, coronary heart disease, atrial fibrillation, arthritis, previous stroke, alcohol abuse and tobacco use), clinical data (systolic blood pressure, diastolic blood pressure, stroke severity and anxiety severity) and laboratory parameters (fast blood glucose, total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, creatinine and RDW). Stroke severity was assessed by National Institute of Health stroke scale (NIHSS). Anxiety severity was assessed *via* Hamilton Anxiety Scale (HAMA). The levels of RDW at admission were measured within 24 h after admission.

The definition of PSF in the acute phase

PSF was assessed by the Fatigue Severity Scale (FSS) within 2 weeks after onset of ischemic stroke. FSS consists of 9 items, each item according to the patient's evaluation of fatigue severity will gradually transition the result to 1–7 points. The higher the score, the more severe PSF. We took the total score of 36 as the dividing line (7).

Statistical analysis

R software (Version 4.1.3; http://www.r-project.org) was used to conduct statistical analyses. Categorical variables are expressed as n (percentages). Normally distributed variables are expressed as the mean ± SD, and abnormally distributed continuous variables are expressed as medians (interquartile range [IQR]). The differences between PSF group and non-PSF group were identified with the Student's *t*-test, the Wilcoxon W-test, the chi-square test or Fisher's exact test as appropriate. The violin plot was utilized to present the distribution of RDW between the PSF group and the non-PSF group. We explored the relationship between RDW and PSF in different logistic regression models. Model 1 was unadjusted model. Model 2 was adjusted for age and sex. Model 3 was adjusted for age, sex, coronary heart disease and baseline NIHSS score. Model 4 was adjusted for age, sex, coronary heart disease, NIHSS score and HAMA score. What is more, we used restricted cubic splines (RCS) with three knots placed at the 10th, 50th, and 90th percentiles to evaluate the dose-response relationship of RDW with PSF in different models. $P < 0.05$ was considered statistically significant.

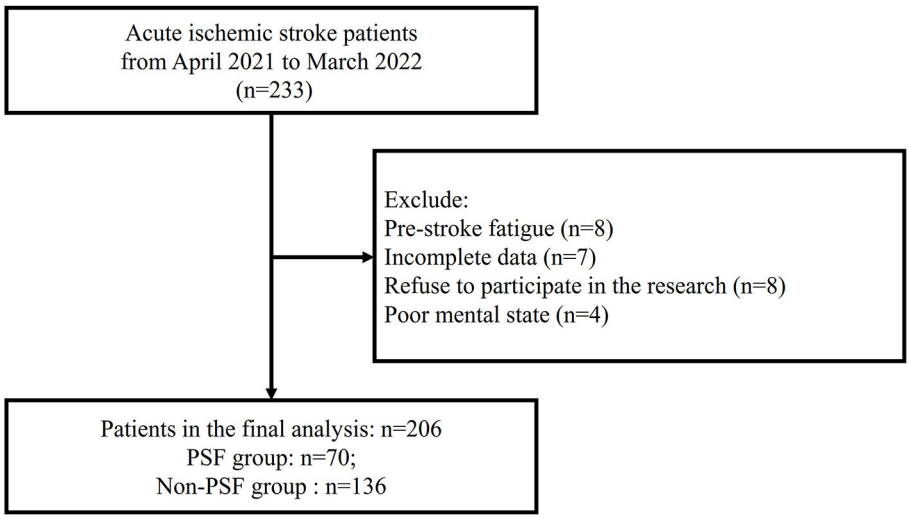

FIGURE 1
Flowchart.

Results

From April 2021 to March 2022, we screened 233 AIS patients, and excluded 27 AIS patients as following reasons: Pre-stroke fatigue ($n = 8$); Incomplete data ($n = 7$); Refuse to participate in the research ($n = 8$); Poor mental state ($n = 4$) (Figure 1). Finally, a total of 206 AIS patients (mean age, 69.3 ± 10.7 years; 52.9% men) were included in the analysis.

Table 1 showed the baseline data of AIS patients stratified by PSF or non-PSF. In our study, the patients in the PSF group ($n = 70$) were more likely to have higher proportion of coronary heart disease ($P = 0.026$), elevated levels of NIHSS score ($P = 0.036$), HAMA score ($P = 0.001$) and RDW ($P = 0.023$). Figure 2 displayed the levels of RDW between the PSF groups and non-PSF groups (13.1% [12.5, 13.9%] vs. 12.8% [12.3, 13.4%], $P = 0.023$).

Figure 3 exhibited the results of logistic regression to explore the relationship between RDW and PSF in the acute phase. In the unadjusted model (model 1), RDW at admission might be related to PSF (OR [odds ratio], 1.545; 95% CI [confidence interval], 1.139–2.098; $P = 0.005$). After the adjustment for age and sex (model 2), RDW might also be the related factor to PSF (OR, 1.536; 95% CI, 1.135–2.080; $P = 0.005$). After the adjustment for age, sex, coronary heart disease and baseline NIHSS score (model 3), the OR of PSF for RDW was 1.517 (95% CI, 1.116–2.060, $P = 0.008$). What is more, in the model 4, which included age, sex, coronary heart disease, NIHSS score and HAMA score, RDW remained the independent associated factor with PSF (OR, 1.635; 95% CI, 1.153–2.318; $P = 0.006$).

Figure 4 manifested the results of multivariable-adjusted spline regression models. In the model including age and sex, the linear dose-response associations of RDW at admission with PSF in the acute phase were found (P for non-linearity = 0.312; P for linearity = 0.019; Figure 4A). Furthermore, the linear dose-response associations of RDW with PSF in the acute phase remained significant in the model including age, sex, coronary heart disease and baseline NIHSS score (P for non-linearity = 0.351; P for linearity = 0.022; Figure 4B) and the model including age, sex, coronary heart disease, NIHSS score and HAMA score (P for non-linearity = 0.372; P for linearity = 0.037; Figure 4C).

Discussion

In this prospective observational study, we have found that RDW at admission could be one independent associated factor with PSF in the acute phase of AIS, according to the results of different logistic regression models. In addition, based on the RCS models, the linear dose-response associations of RDW with PSF in the acute phase were confirmed.

The incidence of PSF in the acute phase is 34.0 % in our study, which is in line with previous studies (7–11). This may be attributed to our rigorous and meticulous assessment about PSF during this prospective study. The incidence also indicated that approximately 1/3 of AIS patients might suffer from PSF in the acute phase. Therefore, the neurologists may

TABLE 1 Baseline data of AIS patients stratified by PSF or non-PSF.

Variable	PSF group ($n = 70$)	Non-PSF group ($n = 136$)	*P*
Demographics			
Age, year	70.8 ± 10.6	68.5 ± 10.7	0.147
Male, *n* (%)	35 (50.0)	74 (54.4)	0.548
Past medical history, *n* (%)			
Hypertension	50 (71.4)	93 (68.4)	0.653
Diabetes	26 (37.1)	46 (33.8)	0.636
Cancer	3 (4.3)	9 (6.6)	0.755
Coronary heart disease	13 (18.6)	11 (8.1)	0.026
Atrial fibrillation	6 (8.6)	10 (7.4)	0.757
Arthritis	2 (2.9)	8 (5.9)	0.500
Previous stroke	21 (30.0)	28 (20.6)	0.133
Tobacco use			0.478
Never	15 (21.4)	28 (20.6)	
Ever	17 (24.3)	24 (17.6)	
Always	38 (54.3)	84 (61.8)	
Alcohol Abuse			0.356
Never	17 (24.3)	46 (33.8)	
Ever	9 (12.9)	17 (12.5)	
Always	44 (62.9)	73 (53.7)	
Clinical data			
SBP, mmHg	144.2 ± 19.9	142.5 ± 17.0	0.518
DBP, mmHg	82.7 ± 12.4	83.2 ± 14.1	0.814
NIHSS, score	2 (1, 3)	1 (1, 2)	0.036
HAMA, score	7 (4, 10)	3 (0, 6)	0.001
Laboratory parameters			
FBG, mmol/l	5.39 (4.79, 7.01)	5.33 (4.83, 6.99)	0.828
TC, mmol/l	4.14 (3.31, 5.00)	4.32 (3.69, 4.94)	0.371
TG, mmol/l	1.39 (0.99, 1.76)	1.49 (1.08, 2.00)	0.193
HDL, mmol/l	1.06 (0.92, 1.19)	1.03 (0.91, 1.19)	0.744
LDL, mmol/l	2.44 (1.87, 3.22)	2.64 (2.23, 3.25)	0.229
Creatinine, μmol/L	73.0 (60.5, 86.5)	69.0 (59.6, 86.4)	0.777
RDW, %	13.1 (12.5, 13.9)	12.8 (12.3, 13.4)	0.023

AIS, Acute ischemic stroke; PSF, Post-stroke fatigue; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; NIHSS, National institute of health stroke scale; HAMA, Hamilton anxiety scale; FBG, Fast blood glucose; TC, Total cholesterol; TG, Triglycerides; HDL, High density lipoprotein; LDL, Low density lipoprotein; RDW, Red blood cell distribution width.

be supposed to pay attention to the management of PSF in the acute phase.

RDW is a readily available laboratory parameter, which could reflect the variability in volumes of red blood cell. Higher levels of RDW mean greater variation in volumes. In normal Chinese population, the levels of RDW range from 11.0 to 16.0%, and may increase under pathological conditions. It is well known that inflammatory response plays a prominent role on the pathophysiology of cerebrovascular disease (17–22). RDW may be associated with C-reactive protein, interleukin −6 and other inflammatory biomarkers (23, 24). The research performed by Semba RD et al. manifested that serum selenium might be an independent predictor of RDW and mediate effects on RDW *via* interleukin-6 (25). These findings showed that RDW might be able to be an inflammatory biomarker and be involved in the pathophysiology of several inflammation-related diseases, for example, cerebrovascular diseases.

Previous clinical studies have revealed the role of RDW on cerebrovascular disease. Vayá A et al. found that RDW >14.0% might increase the risk of cryptogenic stroke (26). The results of one cross-sectional study, which enrolled 432 primary AIS patients, indicated that the AIS patients with carotid artery atherosclerosis could possess higher levels of RDW (27). RDW may also serve as an independent related factors to the prognostic outcomes in AIS patients

treated with intravenous thrombolysis (28). Another research recruited the AIS patients without intravenous thrombolysis or endovascular treatment, and showed that elevated levels of RDW were related to increased risk of hemorrhagic transformation (29). A meta-analysis displayed that the baseline levels of RDW might be a predictor of stroke occurrence and outcome (30). What is more, high RDW levels may increase the risk of hemorrhagic transformation after intravenous thrombolysis in AIS patients (31). Nowadays, the levels of RDW were found to be linked to post-stroke depression (16), another common emotional complication after the onset of AIS. Although there are several studies focusing on the relationship between RDW and cerebrovascular disease, this study is the first prospective study that explore the role of RDW on PSF, and may assist neurologists with managing AIS patients.

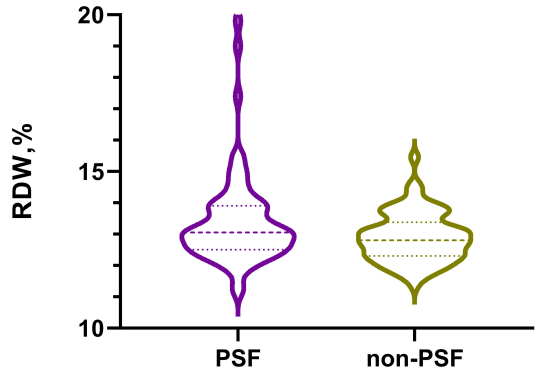

FIGURE 2
The violin plot in distribution of RDW between the PSF group and non-PSF group. RDW, red blood cell distribution width; PSF, post-stroke fatigue.

However, there are still several shortcomings in our prospective observational study. First, we only collected the data about PSF in the acute phase now. Therefore, we are following up the PSF at 6 months in these AIS patients prospectively, and we will explore the relationship between RDW and PSF at 6 months in the future research. Second, the sample size of this study is relatively small. We aim to carry out the study with large sample to provide higher levels of evidence about the association of RDW with PSF. Third, RDW and other laboratory parameters may change during hospitalization, so it might be critical to monitor these parameters, dynamically. In addition, we have not utilized machine learning in this study and collected the data of other inflammatory biomarkers, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).

In short, this prospective observational study is the first study centering on the relationship between RDW at admission and PSF in the acute phase of AIS, as far as we know. Linear dose-response associations of RDW with PSF in the acute phase of AIS have been found. Consequently, RDW at admission may be a novel biomarker

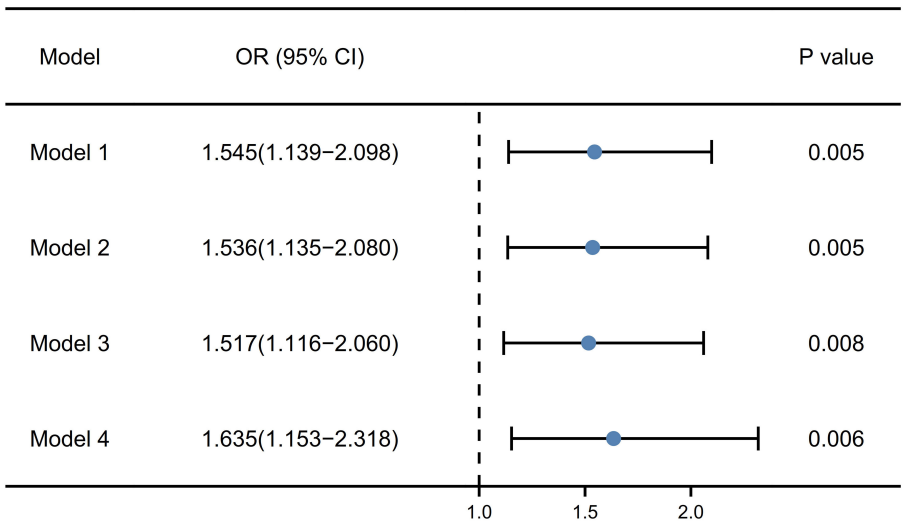

FIGURE 3
Multivariate logistic regression models for the association of RDW with PSF. Model 1, unadjusted model; Model 2, adjusted for age and sex; Model 3, adjusted for age, sex, coronary heart disease and baseline NIHSS score; Model 4, adjusted for age, sex, coronary heart disease, NIHSS score and HAMA score. RDW, red blood cell distribution width; PSF, post-stroke fatigue; NIHSS, National institute of health stroke scale; HAMA, Hamilton anxiety scale.

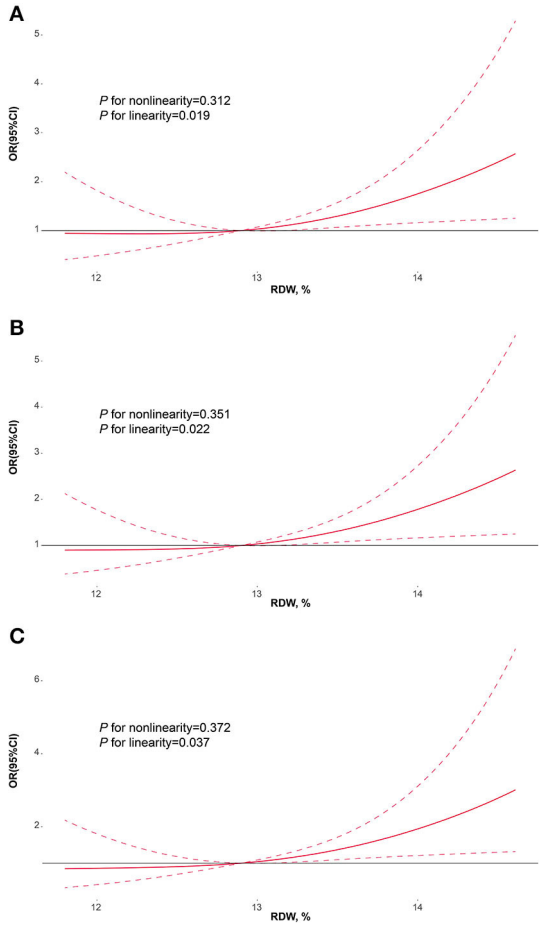

FIGURE 4
Restricted cubic spline regression models for the association of RDW with PSF. Adjusted odds ratios and 95% confidence intervals derived from restricted cubic spline regression, with knots placed at the 10th, 50th, and 90th percentiles of RDW. **(A)** adjusted for age and sex; **(B)** adjusted for age, sex, coronary heart disease and baseline NIHSS score; **(C)** adjusted for age, sex, coronary heart disease, NIHSS score and HAMA score. RDW, red blood cell distribution width; PSF, post-stroke fatigue; NIHSS, National institute of health stroke scale; HAMA, Hamilton anxiety scale.

of PSF in the acute phase, which is helpful for neurologists. Nevertheless, the study with large sample is required in the future, and these conclusions need to be verified in other stroke centers.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Nantong Third People's hospital (Ethics number: EK2021008). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MP wrote the manuscript and performed the statistical analyses. YuC collected the data and assisted with writing the manuscript. YaC, KF, HS, HH, WZ, HZ, and JJ collected the data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Ma Q, Li R, Wang L, Yin P, Wang Y, Yan C, et al. Temporal trend and attributable risk factors of stroke burden in China, 1990-2019: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Health.* (2021) 6:e897–906. doi: 10.1016/S2468-2667(21)00228-0

2. Prevalence i. and mortality of stroke in China: results from a nationwide population-based survey of 480 687 adults. *Circulation.* (2017) 135:759–71. doi: 10.1161/CIRCULATIONAHA.116.025250

3. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T, et al. Heart disease and stroke statistics—2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation.* (2006)113:e85–151. doi: 10.1161/CIRCULATIONAHA.105.171600

4. Wang Y, Meng X, Wang A, Xie X, Pan Y, Johnston SC, et al. Ticagrelor versus clopidogrel in CYP2C19 loss-of-function carriers with stroke or TIA. *N Engl J Med.* (2021) 385:2520–30. doi: 10.1056/NEJMoa2111749

5. Xiong Y, Yan R, Gu H, Wang S, Fisher M, Zhao X, et al. Intravenous thrombolysis in Chinese patients with mild acute ischemic stroke. *Ann Transl Med.* (2021) 9:767. doi: 10.21037/atm-21-40

6. Yang P, Zhang Y, Zhang L, Zhang Y, Treurniet KM, Chen W, et al. Endovascular thrombectomy with or without intravenous alteplase in acute stroke. *N Engl J Med.* (2020) 382:1981–93. doi: 10.1056/NEJMoa2001123

7. Wang J, Li F, Xiao L, Peng F, Sun W, Li M, et al. Depressed TSH level as a predictor of post-stroke fatigue in patients with acute ischemic stroke. *Neurology.* (2018) 91:e1971–78. doi: 10.1212/WNL.0000000000006534

8. Paciaroni M, Acciarresi M. Poststroke fatigue. *Stroke.* (2019) 50:1927–33. doi: 10.1161/STROKEAHA.119.023552

9. Sarfo FS, Berchie P, Singh A, Nichols M, Agyei-Frimpong M, Jenkins C, et al. Prevalence, trajectory, and predictors of poststroke fatigue among ghanaians. *J Stroke Cerebrovasc Dis.* (2019) 28:1353–61. doi: 10.1016/j.jstrokecerebrovasdis.2019.02.002

10. De Doncker W, Dantzer R, Ormstad H, Kuppuswamy A. Mechanisms of poststroke fatigue. *J Neurol Neurosurg Psychiatry.* (2018) 89:287–93. doi: 10.1136/jnnp-2017-316007

11. Alghamdi I, Ariti C, Williams A, Wood E, Hewitt J. Prevalence of fatigue after stroke: a systematic review and meta-analysis. *Eur Stroke J.* (2021) 6:319–32. doi: 10.1177/23969873211047681

12. Kim CH, Park JT, Kim EJ, Han JH, Han JS, Choi JY, et al. An increase in red blood cell distribution width from baseline predicts mortality in patients with severe sepsis or septic shock. *Crit Care.* (2013) 17:R282. doi: 10.1186/cc13145

13. Vayá A, Alis R, Hernández JL, Calvo J, Micó L, Romagnoli M, et al. RDW in patients with systemic lupus erythematosus. Influence of anaemia and inflammatory markers. *Clin Hemorheol Microcirc.* (2013) 54:333–9. doi: 10.3233/CH-131738

14. Jia X, Cheng S, Zhang L, Zheng Y, Zou H, Huang S, et al. Elevated red blood cell distribution width as a poor prognostic factor in patients with hematopoietic stem cell transplantation. *Front Oncol.* (2021) 10:565265. doi: 10.3389/fonc.2020.565265

15. Chen M, Liao L, Yan J, Lin FQ. Predictive value of red blood cell distribution width for 1-year all-cause mortality in critically Ill patients with acute myocardial infarction. *Int J Gen Med.* (2022)15:465–71. doi: 10.2147/IJGM.S345109

16. Li Y, Zhang M, Dong C, Xue M, Li J, Wu G. Elevated red blood cell distribution width levels at admission predicts depression after acute ischemic stroke: a 3-month follow-up study. *Neuropsychiatr Dis Treat.* (2022)18:695–704. doi: 10.2147/NDT.S351136

17. Sun H, Li S, Xu Z, Liu C, Gong P, Deng Q, et al. SNHG15 is a negative regulator of inflammation by mediating TRAF2 ubiquitination in stroke-induced immunosuppression. *J Neuroinflammation.* (2022) 19:1. doi: 10.1186/s12974-021-02372-z

18. Zhang Y, Xing Z, Zhou K, Jiang S. The predictive role of systemic inflammation response index (SIRI) in the prognosis of stroke patients. *Clin Int Aging.* (2021) 16:1997–2007. doi: 10.2147/CIA.S339221

19. Przykaza Ł. Understanding the connection between common stroke comorbidities, their associated inflammation, and the course of the cerebral Ischemia/reperfusion cascade. *Front Immunol.* (2021) 12:782569. doi: 10.3389/fimmu.2021.782569

20. Franx BAA, Van der Toorn A, Van Heijningen C, Vivien D, Bonnard T, Dijkhuizen RM. Molecular magnetic resonance imaging of vascular inflammation after recanalization in a rat ischemic stroke model. *Stroke.* (2021) 52:e788–91. doi: 10.1161/STROKEAHA.121.034910

21. Wu M, Zhang X, Chen J, Zha M, Yuan K, Huang K, et al. A score of low-grade inflammation for predicting stroke recurrence in patients with Ischemic stroke. *J Inflamm Res.* (2021) 14:4605–14. doi: 10.2147/JIR.S328383

22. Esenwa C, Cheng NT, Luna J, Willey J, Boehme AK, Kirchoff-Torres K, et al. Biomarkers of coagulation and inflammation in COVID-19-associated ischemic stroke. *Stroke.* (2021) 52:e706–09. doi: 10.1161/STROKEAHA.121.035045

23. Lappé JM, Horne BD, Shah SH, May HT, Muhlestein JB, Lappé DL, et al. Red cell distribution width, C-reactive protein, the complete blood count, and mortality in patients with coronary disease and a normal comparison population. *Clin Chim Acta.* (2011) 412:2094–9. doi: 10.1016/j.cca.2011.07.018

24. Montagnana M, Cervellin G, Meschi T, Lippi G. The role of red blood cell distribution width in cardiovascular and thrombotic disorders. *Clin Chem Lab Med.* (2011) 50:635–41. doi: 10.1515/cclm.2011.831

25. Semba RD, Patel KV, Ferrucci L, Sun K, Roy CN, Guralnik JM, et al. Serum antioxidants and inflammation predict red cell distribution width in older women: the Women's Health and Aging Study I. *Clin Nutr.* (2010) 29:600–4. doi: 10.1016/j.clnu.2010.03.001

26. Vayá A, Hernández V, Rivera L, Hernández JL, Lago A, España F, et al. Red blood cell distribution width in patients with cryptogenic stroke. *Clin Appl Thromb Hemost.* (2015) 21:241–5. doi: 10.1177/1076029614547262

27. Jia H, Li H, Zhang Y, Li C, Hu Y, Xia C. Association between red blood cell distribution width (RDW) and carotid artery atherosclerosis (CAS) in patients with primary ischemic stroke. *Arch Gerontol Geriatr.* (2015) 61:72–5. doi: 10.1016/j.archger.2015.04.005

28. Turcato G, Cappellari M, Follador L, Dilda A, Bonora A, Zannoni M, et al. Red blood cell distribution width is an independent predictor of outcome in patients undergoing thrombolysis for ischemic stroke. *Semin Thromb Hemost.* (2017) 43:30–5. doi: 10.1055/s-0036-1592165

29. Wang C, Wang L, Zhong D, Deng L, Qiu S, Li Y, et al. Association between red blood cell distribution width and hemorrhagic transformation in acute ischemic stroke patients. *Cerebrovasc Dis.* (2019) 48:193–9. doi: 10.1159/000504742

30. Song SY, Hua C, Dornbors III D, Kang RJ, Zhao XX, Du X, et al. Baseline red blood cell distribution width as a predictor of stroke occurrence and outcome: a comprehensive meta-analysis of 31 studies. *Front Neurol.* (2019) 10:1237. doi: 10.3389/fneur.2019.01237

31. Fan H, Liu X, Li S, Liu P, Song Y, Wang H, et al. High red blood cell distribution width levels could increase the risk of hemorrhagic transformation after intravenous thrombolysis in acute ischemic stroke patients. *Aging.* (2021) 13:20762–73. doi: 10.18632/aging.203465

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Relationship between the mean of 24-h venous blood glucose and in-hospital mortality among patients with subarachnoid hemorrhage: A matched cohort study

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Objective: The aim of this study was to explore the correlation between the mean of 24-h venous blood glucose (BG) and in-hospital mortality and all-cause mortality (ACM) in patients with subarachnoid hemorrhage (SAH).

Methods: Detailed clinical information was acquired from the Medical Information Mart for Intensive IV (MIMIC-IV) database. The best cutoff value of mean BG was calculated using the X-tile program. Univariate and multivariate logistic regressive analyses were utilized to analyze the prognosis significance of mean BG, and survival curves were drawn using the Kaplan-Meier (K-M) approach. To improve the reliability of results and balance the impact of underlying confounders, the 1:1 propensity score matching (PSM) approach was utilized.

Results: An overall of 1,230 subjects were selected herein. The optimal cutoff value of the mean BG for in-hospital mortality was 152.25. In addition, 367 pairs of score-matched subjects were acquired after PSM analysis, and nearly all variables' differences were balanced. K-M analysis showed that patients with mean BG $\geq$ 152.25 mg/dl had significantly higher in-hospital, 3-month, and 6-month mortalities compared with patients with mean BG $<$ 152.25 mg/dl ($p < 0.001$). The multivariable logistic regressive analyses revealed that patients with mean BG $\geq$ 152.25 mg/dl had significantly increased in-hospital mortality compared with patients with mean BG $<$ 152.25 mg/dl after the adjustment for possible confounders (OR = 1.994, 95% CI: 1.321–3.012, $p = 0.001$). Similar outcomes were discovered in the PSM cohort.

Conclusion: Our data suggested that mean BG was related to ACM of patients with SAH. More studies are needed to further analyze the role of the mean of 24-h venous BG in patients with SAH.

KEYWORDS

subarachnoid hemorrhage, mean blood glucose, admission blood glucose, in-hospital mortality, MIMIC-IV database

Introduction

Subarachnoid hemorrhage (SAH) is one of the major health issues, with a 30-day death rate between 18 and 40% as per previously published studies (1, 2). Survivors are usually unable to regain independence from serious disability or have difficulties in communications, retention, or execution. Therefore, discovering prediction factors of the short-term or long-term prognostic results is imperative.

Previous researchers have discovered risky factors related to unsatisfactory prognoses (3–6). BG contents are usually increased on admission posterior to SAH, which might indicate a stress reaction to the bleeding (7–11). High blood glucose (BG) is common in critically ill patients (CPs), and the association between admission high BG and death rate was broadly researched as well. Liu et al. revealed that admission BG > 142.00 mg/dl (7.91 mmol/L) was related to elevated risks of modified 30- and 90-day all-cause mortality (ACM) in CPs

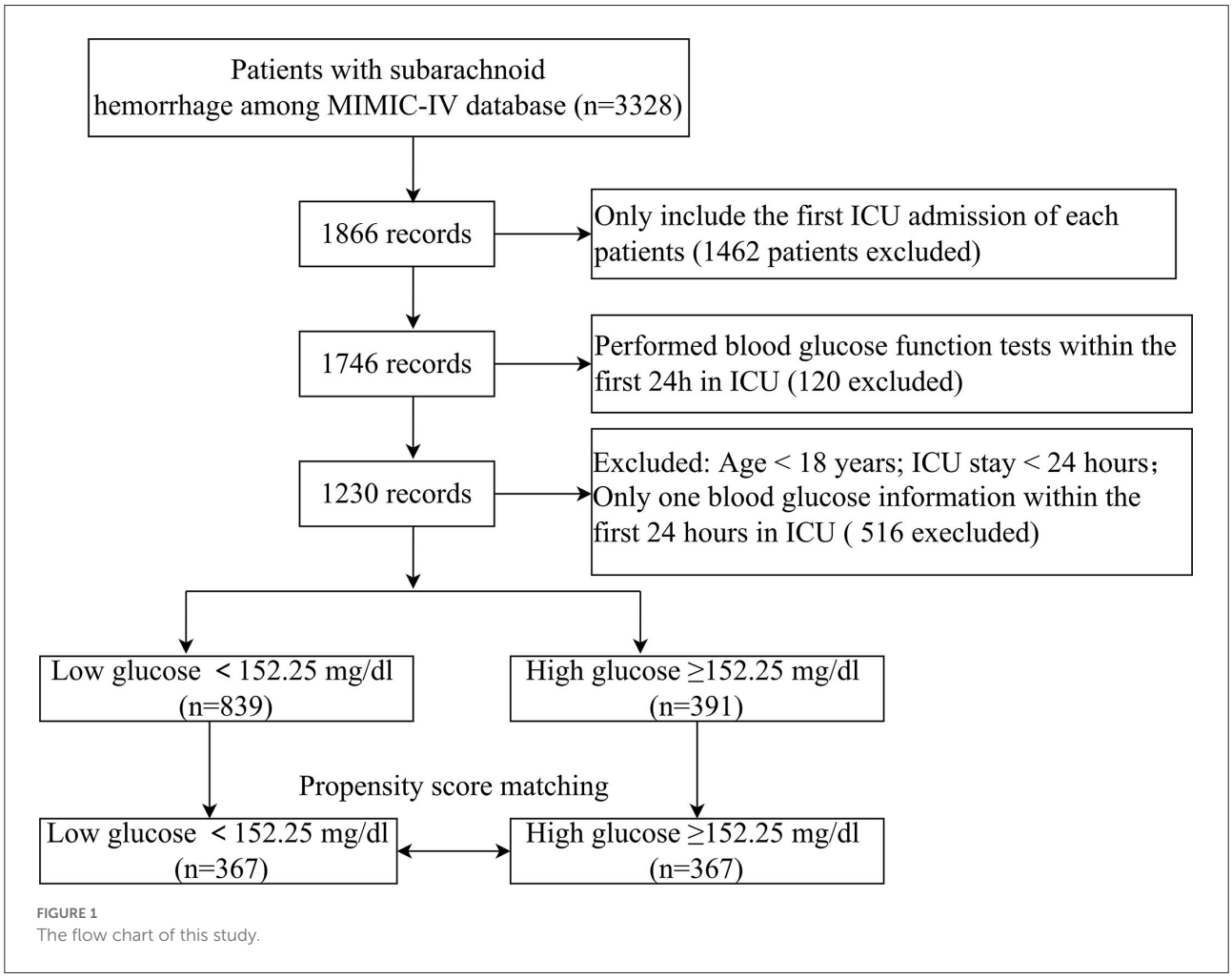

FIGURE 1
The flow chart of this study.

(12). Moreover, statistically, Eagles et al. discovered a remarkable reduction in unsatisfactory prognoses among SAH sufferers maintaining the maximal BG contents lower than a determined best cutoff of 9.2 mmol/L (13). Meanwhile, Okazaki et al. found that minimum BG levels on admission were remarkably related to unsatisfactory neurological results in SAH sufferers (14). However, admission BG contents can change quickly due to stress response and altered nutrition consumption. In addition, BG is often measured clinically when there is aggravation, which might cause sampling bias. The objective of this research was to evaluate whether, in SAH sufferers, the mean of 24-h venous BG levels on ICU admission could be a better prediction factor of in-hospital death than single admission BG level alone.

Materials and methods

Data sources

Herein, data were acquired from a vital public database called MIMIC-IV (15). This database contains the information of sufferers admitted to the Beth Israel Deaconess Medical Center (BIDMC) between 2008 and 2019. Posterior to the completion of the National Institutes of Health (NIH) training course and the Protecting Human Research Participants test, our team acquired relevant information from MIMIC-IV. One researcher J-HW obtained approval to exploit the database. Besides, our research was accepted by the Ethics Board of our institution. The findings herein were reported using the Strengthening the Reporting of Observational Studies in Epidemiology guidelines (16).

Study population

The diagnosis of SAH was on the basis of the International Classification of Disease, Ninth Revision. Sufferers meeting the entire standards were selected for analyses: (1) first admission to ICU; (2) age >18 years; and (3) complete records of BG examination within the first 24 h of ICU admission. The exclusion criteria were as follows: (1) ICU patients with a length of stay <24 h and (2) only one BG information in the first 24 h in ICU.

Abbreviations: HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MDP, mean blood pressure; RR, respiratory rate; SpO2, percutaneous oxygen saturation; DCI, delayed cerebral ischemia; WBC, white blood cell; INR, international normalized ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; GCS, Glasgow coma score; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; WFNS scale, World Federation of Neurological Societies Scale; PSM, propensity score matching; DCI, delayed cerebral ischemia; WFNS scale, World Federation of Neurological Societies Scale.

Data acquisition

The variates stated below were acquired from the aforesaid database for the first day of ICU admission: (1) demographical variates: sex, age, and race; (2) vital signs (refer to the abbreviation list at the end of our thesis): HR, SBP, DBP, RR, temperature, and SpO2; (3) coexisting diseases: myocardium infarction, congestion-related cardiac failure, peripheral vascular illness, cerebral vascular illness, persistent lung illness, mild hepatic illness, diabetic illness, and high blood pressure; and (4) lab events (refer to the abbreviation list at the end of our thesis): WBC, neutrophil count, monocyte count, INR, PT, APTT, and BG were identified in the first 24 h of ICU admission. If a variate was identified more than once in the first 24 h, the average was utilized. (5) Severity at admission was identified *via* the SOFA scoring, the SAPS II, APS III, and GCS. (6) Duration of ICU stay, duration of hospitalization, in-hospital death, 3-month death, and 6-month death was recorded.

Endpoints

In-hospital death, duration of ICU stay, duration of hospitalization, 3-month death, and 6-month death were regarded as endpoints.

Statistics

The continuous variates were displayed as average ± standard deviation (SD) or mid-value (interquartile range). The Student's *t*-test or Mann-Whitney *U*-test was used according to the normality of the distribution. Categorical variates were displayed as case quantity (%), and the chi-square test (or Fisher's exact approach) was used for analyses.

The best cutoff value of mean BG was calculated by receiver operating characteristic (ROC) curve analysis using the highest Youden index for predicting survival status. Patients were separated into two groups according to mean BG, namely, low glucose (<152.25 mg/dl) and high glucose (≥152.25 mg/dl). Our team established a generalized additive model (GAM) to identify the non-linear association between mean BG and in-hospital ACM in CPs with SAH. Moreover, our team visualized the association between mean BG and sufferers' survival *via* the Kaplan-Meier (K-M) analysis and utilized the log-rank test for assumption verification.

The univariate and multivariate regressive analyses were completed to relieve the interference of possible confounding factors in the in-hospital mortality. The screening of confounders was based on: (1) the factor exerted an impact (>10%) on the research variate; (2) certain factors might

TABLE 1 The clinical characteristics in critically ill patients with SAH before and after PSM.

Characteristic	Before PSM				After PSM			
	All patients	Low Glucose <152.25	High Glucose ≥152.25	*p*	All patients	Low Glucose <152.25	High Glucose ≥152.25	*p*
N	1,230	839	391		734	367	367	
Demographic								
Female, *n* (%)	603 (49.0)	390 (46.5)	213 (54.5)	0.011	386 (52.6)	190 (51.8)	196 (53.4)	0.712
Age, years	62.7 ± 17.0	61.7 ± 17.8	65.0 ± 15.0	0.001	65.0 ± 14.7	65.3 ± 14.6	64.8 ± 14.9	0.696
Ethnicity, *n* (%)				0.006				0.023
Asian	42 (3.4)	24 (2.9)	18 (4.6)		26 (3.5)	11 (3)	15 (4.1)	
White	719 (58.5)	518 (61.7)	201 (51.4)		427 (58.2)	234 (63.8)	193 (52.6)	
Black	75 (6.1)	49 (5.8)	26 (6.6)		46 (6.3)	21 (5.7)	25 (6.8)	
Other	394 (32.0)	248 (29.6)	146 (37.3)		235 (32.0)	101 (27.5)	134 (36.5)	
Vital signs								
HR, beats/min	81.2 ± 14.5	79.6 ± 14.2	84.5 ± 14.5	< 0.001	81.9 ± 14.6	79.0 ± 13.9	84.7 ± 14.7	< 0.001
SBP, mmHg	124.1 ± 13.2	123.9 ± 13.2	124.7 ± 13.4	0.329	124.7 ± 13.2	124.8 ± 13.0	124.5 ± 13.4	0.818
DBP, mmHg	64.4 ± 9.6	64.8 ± 9.7	63.6 ± 9.2	0.048	64.3 ± 9.5	64.9 ± 9.8	63.7 ± 9.2	0.093
RR, times/min	18.0 (16.0, 20.0)	18.0 (16.0, 19.5)	19.0 (17.0, 21.0)	< 0.001	18.0 (16.0, 21.0)	18.0 (16.0, 19.0)	19.0 (17.0, 21.0)	< 0.001
Temperature, ° C	37.0 (36.8, 37.3)	37.0 (36.8, 37.3)	37.1 (36.8, 37.4)	0.357	37.0 (36.8, 37.4)	37.0 (36.8, 37.3)	37.1 (36.8, 37.4)	0.147
SpO2, %	98.0 (96.0, 99.0)	98.0 (96.0, 99.0)	98.0 (96.0, 99.0)	0.003	98.0 (96.0, 99.0)	97.0 (96.0, 99.0)	98.0 (96.0, 99.0)	< 0.001
Comorbidities, *n* (%)								
Myocardial infarct	145 (11.8)	93 (11.1)	52 (13.3)	0.305	100 (13.6)	52 (14.2)	48 (13.1)	0.747
Congestive heart failure	86 (7.0)	63 (7.5)	23 (5.9)	0.357	48 (6.5)	27 (7.4)	21 (5.7)	0.455
Peripheral vascular disease	811 (65.9)	531 (63.3)	280 (71.6)	0.005	509 (69.3)	246 (67)	263 (71.7)	0.2
Cerebrovascular disease	44 (3.6)	34 (4.1)	10 (2.6)	0.250	27 (3.7)	20 (5.4)	7 (1.9)	0.019
Chronic pulmonary disease	21 (1.7)	13 (1.5)	8 (2)	0.697	12 (1.6)	5 (1.4)	7 (1.9)	0.085
Mild liver disease	181 (14.7)	54 (6.4)	127 (32.5)	< 0.001	145 (19.8)	27 (7.4)	118 (32.2)	< 0.001
Diabetes	58 (4.7)	18 (2.1)	40 (10.2)	< 0.001	48 (6.5)	9 (2.5)	39 (10.6)	< 0.001
Hypertension	149 (12.1)	99 (11.8)	50 (12.8)	0.689	91 (12.4)	47 (12.8)	44 (12)	0.823
Vasospasm, *n* (%)	48 (4.8)	35 (5.3)	13 (3.8)	0.386	26 (4.2)	14 (4.7)	12 (3.8)	0.692
DCI, *n* (%)	40 (4.0)	27 (4.1)	13 (3.8)	0.983	22 (3.6)	9 (3)	13 (4.1)	0.636
Urinary tract infection, *n* (%)	85 (8.5)	63 (9.6)	22 (6.5)	0.13	47 (7.6)	25 (8.4)	22 (6.9)	0.568
Sepsis, *n* (%)	461 (46.2)	319 (48.4)	142 (42)	0.064	280 (45.5)	145 (49)	135 (42.3)	0.115
Pneumonia, *n* (%)	134 (10.9)	81 (9.7)	53 (13.6)	0.052	86 (11.7)	34 (9.3)	52 (14.2)	0.051
Laboratory events								
Admission glucose, mg/dL	132.0 (110.0, 161.0)	117.0 (104.0, 135.0)	183.0 (154.0, 233.0)	< 0.001	144.5 (118.0, 183.0)	119.0 (104.5, 135.0)	182.0 (154.0, 233.0)	< 0.001

(Continued)

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TABLE 1 Continued

Characteristic	Before PSM				After PSM			
	All patients	Low Glucose <152.25	High Glucose ≥152.25	*p*	All patients	Low Glucose <152.25	High Glucose ≥152.25	*p*
WBC, 10^9/L	199.5 (158.0, 251.8)	196.0 (158.0, 243.0)	207.0 (160.0, 264.5)	0.012	200.0 (156.2, 253.0)	193.0 (151.5, 239.0)	207.0 (160.0, 264.5)	0.004
Monocytes, 10^9/L	26.8 (1.0, 32.7)	26.8 (0.9, 32.1)	26.8 (1.3, 32.9)	0.131	26.8 (1.1, 32.8)	26.8 (0.9, 34.8)	26.8 (1.3, 32.2)	0.090
Neutrophils, 10^9/L	5.2 (0.1, 7.2)	5.2 (0.1, 6.7)	5.2 (0.2, 8.7)	0.021	5.2 (0.1, 7.9)	5.2 (0.1, 7.4)	5.2 (0.2, 8.6)	0.066
INR	1.1 (1.1, 1.3)	1.1 (1.1, 1.3)	1.2 (1.1, 1.4)	< 0.001	1.2 (1.1, 1.3)	1.1 (1.1, 1.2)	1.2 (1.1, 1.4)	< 0.001
PT, s	12.8 (11.8, 14.2)	12.6 (11.7, 14.0)	13.1 (12.2, 14.8)	< 0.001	12.8 (11.9, 14.4)	12.5 (11.6, 13.8)	13.2 (12.2, 14.8)	< 0.001
APTT, s	28.6 (25.9, 32.9)	28.7 (26.2, 32.9)	28.1 (25.5, 32.9)	0.165	28.5 (25.8, 32.9)	28.8 (26.3, 33.1)	28.1 (25.5, 32.9)	0.220
Scores								
GCS	13.0 (8.0, 14.0)	13.0 (8.0, 14.0)	12.0 (7.0, 14.0)	0.067	12.0 (7.0, 14.0)	13.0 (8.0, 14.0)	10.0 (6.0, 14.0)	< 0.001
APSIII	39.0 (28.0, 56.0)	38.0 (27.0, 55.0)	42.0 (28.0, 58.0)	0.027	44.0 (31.0, 63.0)	37.0 (27.0, 51.0)	52.0 (37.0, 74.0)	< 0.001
SAPSII	32.0 (24.0, 40.0)	31.0 (25.0, 39.0)	32.0 (23.0, 40.0)	0.910	34.0 (27.0, 43.0)	32.0 (24.0, 39.0)	37.0 (30.0, 46.0)	< 0.001
SOFA	3.0 (2.5, 3.0)	3.0 (2.2, 3.0)	3.0 (3.0, 3.0)	0.242	3.0 (2.0, 3.0)	3.0 (3.0, 3.0)	3.0 (2.0, 3.0)	0.965
WFNS Scale, *n* (%)				0.082				0.424
I	181 (14.7)	126 (15)	55 (14.1)		104 (14.2)	52 (14.2)	52 (14.2)	
II	415 (33.7)	296 (35.3)	119 (30.4)		230 (31.3)	120 (32.7)	110 (30)	
III	21 (1.7)	14 (1.7)	7 (1.8)		14 (1.9)	7 (1.9)	7 (1.9)	
IV	382 (31.1)	263 (31.3)	119 (30.4)		235 (32.0)	123 (33.5)	112 (30.5)	
V	231 (18.8)	140 (16.7)	91 (23.3)		151 (20.6)	65 (17.7)	86 (23.4)	
Length of ICU stay, days	5.0 (2.0, 11.0)	5.0 (2.0, 10.0)	6.0 (3.0, 13.0)	< 0.001	6.0 (2.0, 12.0)	5.0 (2.0, 10.0)	6.0 (3.0, 13.0)	0.007
Length of hospital stay, days	11.0 (6.0, 18.0)	10.0 (6.0, 17.0)	12.0 (5.0, 20.0)	0.335	11.0 (5.2, 18.8)	11.0 (6.0, 18.0)	12.0 (5.0, 20.0)	0.523
In-hospital mortality, *n* (%)	219 (17.8)	94 (11.2)	125 (32)	< 0.001	158 (21.5)	41 (11.2)	117 (31.9)	< 0.001
3-month mortality, *n* (%)	231 (18.8)	101 (12)	130 (33.2)	< 0.001	166 (22.6)	44 (12)	122 (33.2)	< 0.001
6-month mortality, *n* (%)	233 (18.9)	101 (12)	132 (33.8)	< 0.001	168 (22.9)	44 (12)	124 (33.8)	< 0.001

Values are presented as the mean ± standard deviation, median (interquartile range), or the number of patients (%).
HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MDP, mean blood pressure; RR, respiratory rate; SpO2, percutaneous oxygen saturation; DCI, delayed cerebral ischemia; WBC, white blood cell; INR, international normalized ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; GCS, Glasgow coma score; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; WFNS scale, World Federation of Neurological Societies Scale; PSM, propensity score matching.

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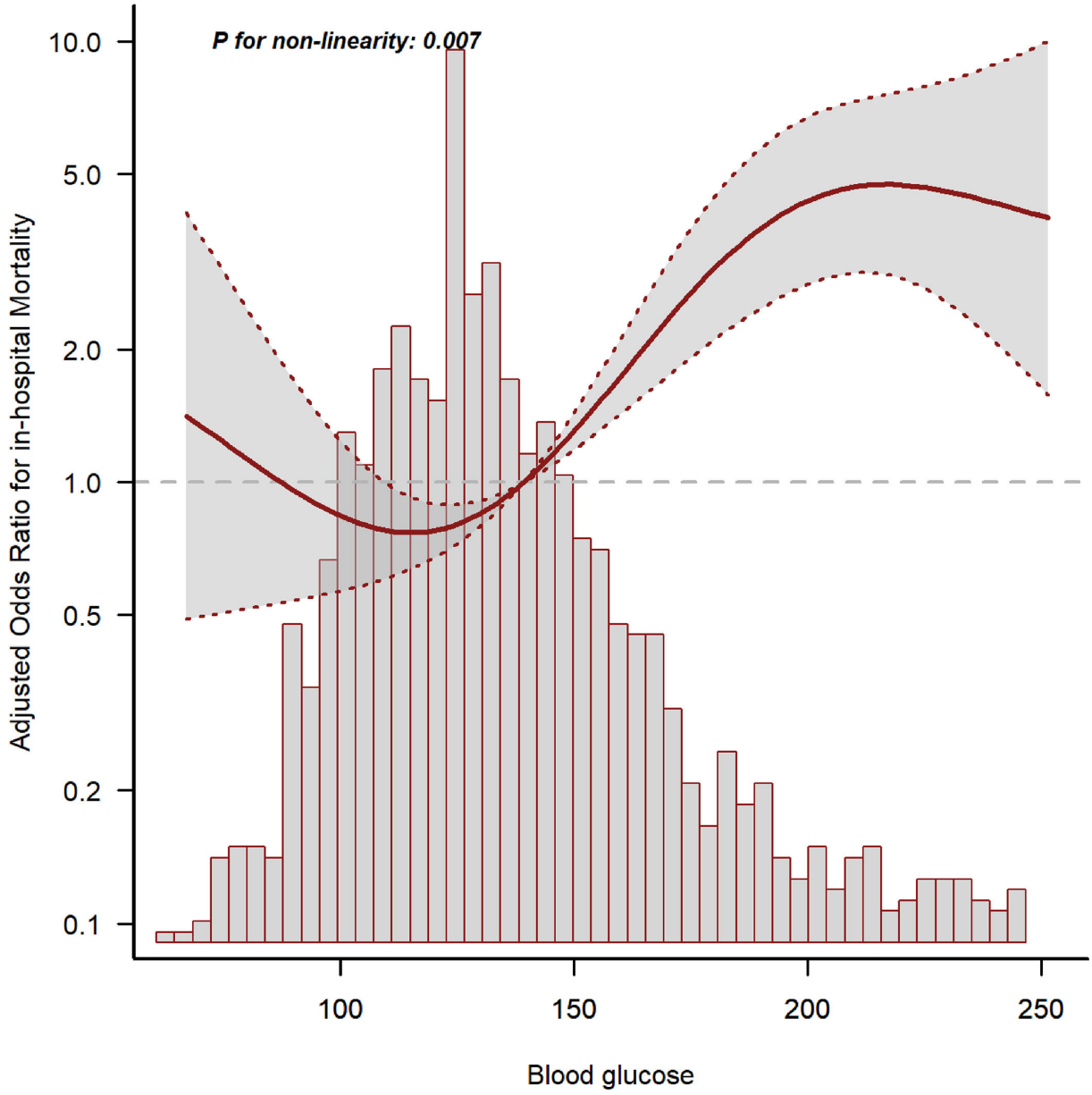

FIGURE 2
The relationship between mean blood glucose (BG) and in-hospital mortality. The solid line shows the smooth curve fit between variables. The red dotted line represents the 95% CI of the fit.

remarkably affect the outcome variate according to past experiences. (3) For univariate analysis, our team modified the variates, of which $p < 0.05$. In the crude model, no variate was modified. In Model I, age, sex, and ethnicity were modified. Model II was modified in terms of age, sex, race, HR, RR, SpO2, PT, and APTT. Based on Model II, our team modified the other four variates, including hypertension, SOFA, GCS, and DBP in our Model III. Based on Model III, our team modified those variates in Model IV, including SAPS II, APS III, diabetes, mild liver disease, INR, vasospasm, DCI, urinary tract infection, sepsis, pneumonia, and WFNS grade.

Given that the sufferer screening standards can hardly be fully stochastic, our team utilized the propensity score matching (PSM) approach to realize the equilibrium of the impact of selection bias and underlying confounders. PSM

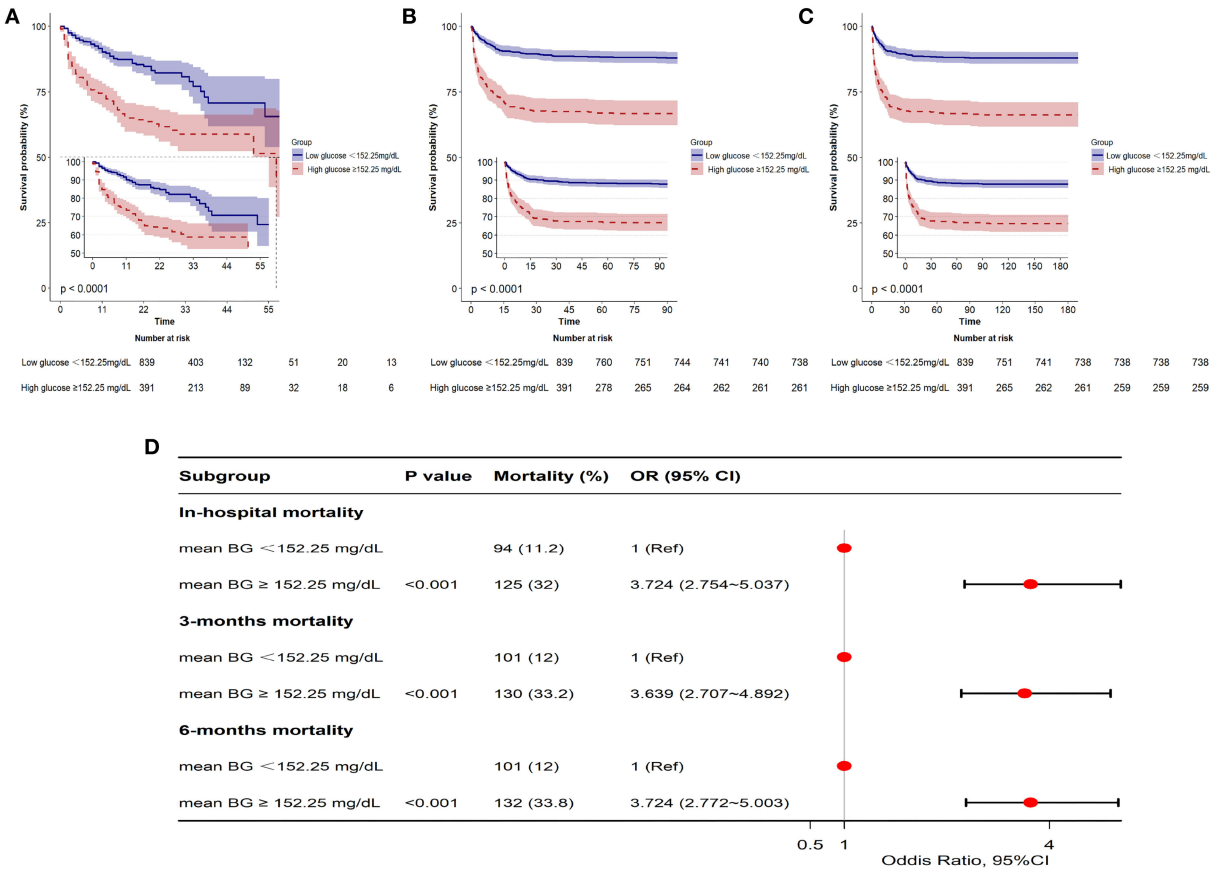

FIGURE 3
Kaplan-Meier (K-M) curves indicate the relationship between the mean BG and in-hospital mortality **(A),** 3-month mortality **(B)**, and 6-month mortality **(C)**. Red line: high glucose ≥152.25 mg/dl; blue line: low glucose<152.25 mg/dl. Forest plot of the results based on logistic regression analysis **(D)**.

analyses were on the basis of the logistic regressive model, and the propensity scoring was computed as per age and gender. The pairs of patients with low glucose (<152.25 mg/dl) and high glucose (≥152.25 mg/dl)were acquired using 1:1 matching with a caliper of 0.01. Ultimately, an overall 734 sufferers were propensity score-matched, and 367 pairs of score-matched sufferers were obtained.

Subgroup analyses were completed through a logistic regressive model as per age (<65 and ≥65 years), sex, myocardium infarction, congestion cardiac failure, peripheral vascular illness, cerebral vascular illness, persistent lung illness, mild hepatic illness, diabetic illness, and high blood pressure. Every test was two-sided, and $p < 0.05$ had significance on statistics.

Every analysis was completed *via* the statistic program packages R 3.3.2 (http://www.R-project.org, The R Foundation) and Free Statistic program 1.1. A two-tailed test was completed, and $p < 0.05$ had significance in statistics.

Results

Baseline features of patients

Overall, 1,230 sufferers meeting the standards were selected (Figure 1). The ROC curve of mean BG was plotted, and its AUC and Youden index were 0.673 (95% CI 0.630–0.715) and 0.308, respectively. The corresponding best cutoff value was 152.25, and the evaluation sensitiveness and specificness were 57.1 and 73.7%, respectively. Based on the cutoff value, 1,230 patients were divided into low glucose (mean BG < 152.25 mg/dl, $n = 839$) and high glucose (mean BG ≥ 152.25 mg/dl, $n = 391$). The demographics, vital signs, coexisting diseases, scoring, lab tests, and other related data between survivor and non-survivor groups are displayed in Supplementary Table 1. Compared with survivors, sufferers in the non-survivor group were older (age mid-value: 66.8 vs. 61.9 years, $p < 0.001$), with greater morbidity of coexisting diseases such as peripheral vascular illness and

TABLE 2 Multivariate logistic regression analyses for in-hospital mortality in patients with SAH before and after PSM.

Characteristic	Non-adjust model		Model I		Model II		Model III		Model IV	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Before PSM										
Mean blood glucose (mg/dL)	1.009 (1.006~1.012)	<0.001	1.009 (1.006~1.012)	<0.001	1.007 (1.004~1.010)	<0.001	1.006 (1.003~1.009)	<0.001	1.008 (1.004~1.012)	0.0002
Low glucose (<152.25mg/dL)	1(Ref)		1(Ref)		1(Ref)		1(Ref)		1(Ref)	
High glucose (≥152.25 mg/dL)	3.724 (2.754~5.037)	<0.001	3.453 (2.528~4.716)	<0.001	2.825 (2.043~3.905)	<0.001	2.618 (1.875~3.656)	<0.001	1.994 (1.321~3.012)	0.001
After PSM										
Mean blood glucose (mg/dL)	1.008 (1.005~1.011)	<0.001	1.008 (1.005~1.011)	<0.001	1.007 (1.004~1.01)	0.0009	1.006 (1.003~1.010)	0.0003	1.010 (1.005~1.015)	0.0001
Low glucose (<152.25mg/dL)	1(Ref)		1(Ref)		1(Ref)		1(Ref)		1(Ref)	
High glucose (≥152.25 mg/dL)	3.307 (2.264~4.83)	<0.001	3.370 (2.302~4.933)	<0.001	2.724 (1.833~4.049)	<0.001	2.621 (1.742~3.945)	<0.001	2.543 (1.485~4.357)	<0.001

Model I, Adjusted for age, gender, and ethnicity.
Model II, Adjusted for age, gender, ethnicity, HR, RR, SpO2, PT, and APTT.
Model III, Adjusted for age, gender, ethnicity, HR, DBP, RR, SpO2, PT, APTT, hypertension, SOFA, and GCS.
Model IV, Adjusted for age, gender, ethnicity, HR, DBP, RR, SpO2, PT, APTT, hypertension, SOFA, GCS, SAPSII, APSIII, diabetes, mild liver disease, INR, vasospasm, DCI, urinary tract infection, sepsis, pneumonia, and WFNS scale.
OR, odds ratio; CI, confidence interval; PSM, propensity score matching; SAH, subarachnoid hemorrhage; HR, heart rate; RR, respiratory rate; SpO2, percutaneous oxygen saturation; PT, prothrombin time; APTT, activated partial thromboplastin time; SOFA, Sequential Organ Failure Assessment; GCS, Glasgow coma score, SAPS II, Simplified Acute Physiology Score II; APSIII, acute physiology score III; INR, international normalized ratio; DCI, delayed cerebral ischemia; WFNS scale, World Federation of Neurological Societies Scale.

pneumonia; higher APS III scores, SAPS II scores, WFNS grade IV, and WFNS grade V; and lower GCS scores, WFNS grade I, and WFNS grade II (all p-values<0.05) (Supplementary Table 1).

The clinical features of CPs with SAH across two groups based on mean BG contents are displayed in Table 1. In this study, the average age of patients was 62.7 ± 17.0 years, and approximately 49.0% of them were women. Remarkable diversities were identified in age, gender, ethnicity, HR, RR, SpO2, peripheral vascular disease diabetes, mild liver disease, INR, and PT between diverse groups ($p < 0.05$). Compared with patients with mean BG<152.25 mg/dl, patients with mean BG ≥ 152.25 mg/dl were at greater risks of longer ICU stay (6.0 vs. 5.0 days, $p < 0.001$), in-hospital death (32 vs. 11.2%, $p < 0.001$), 3-month death (33.2 vs. 12%, $p < 0.001$), and 6-month death (33.8 vs. 12%, $p < 0.001$) (Table 1).

Association between mean bg and all-cause in-hospital mortality in patients with SAH

The GAM analysis revealed a U-shaped relationship between mean BG and in-hospital ACM in SAH sufferers, which revealed that aberrant mean BG might be related to elevated in-hospital death (Figure 2). The K-M curves contrasting the two groups are displayed in Figure 3. Sufferers with mean BG≥152.25 mg/dl had a significantly higher in-hospital mortality rate (Figure 3A), 3-month mortality (Figure 3B), and 6-month mortality (Figure 3C) compared with patients with mean BG < 152.25 mg/dl ($p < 0.001$). The mean BG group (≥152.25 mg/dl) exhibited a remarkably increased risk of in-hospital death (OR, 95% CI: 3.724, 2.754-5.037, $p < 0.001$), 3-month death (OR, 95% CI: 3.639, 2.707–4.892, $p < 0.001$), and 6-month death (OR, 95% CI: 3.724, 2.772–5.003, $p < 0.001$) compared with the mean BG group (< 152.25 mg/dl) (Figure 3D).

Our team utilized logistic proportion risk models to independently analyze the effects of mean BG on the risks of in-hospital death (univariable and multivariable logistic proportion risk models) (Supplementary Table 2; Table 2). In the crude model, the increase in mean BG was related to in-hospital death (OR = 1.009, 95% CI: 1.006–1.012, $p < 0.001$). In multivariate analysis, Model I was modified in terms of age, sex, and ethnicity; Model II was modified in terms of age, sex, ethnicity, HR, RR, SpO2, PT, and APTT; Model III was adjusted for Model II, DBP, hypertension, SOFA, and GCS. Based on Model III, we further modified those variates in Model IV, including SAPS II, APS III, diabetes, mild liver disease, INR, vasospasm, DCI, urinary tract infection, sepsis, pneumonia, and WFNS grade, and the results showed that patients with mean BG≥152.25 mg/dl had significantly higher in-hospital mortality compared with patients with mean

BG<152.25 mg/dl (Model I: OR =3.453, 95% CI: 2.528–4.716, $p < 0.001$; Model II: OR = 2.825, 95% CI: 2.043–3.905, $p < 0.001$; Model III: 2.618, 95% CI: 1.875–3.656, $p < 0.001$; Model IV: OR = 1.994, 95% CI: 1.321–3.012, $p = 0.001$) (Table 2).

Cutoff values of mean BG and admission BG, and their correlation with in-hospital mortality

For the sake of evaluating the underlying prediction merit of the mean BG and admission BG for in-hospital mortality, ROC curve analyses were completed, and the AUC for mean BG and admission BG were 0.673 (95% CI: 0.630–0.715; $p < 0.001$) and 0.652 (95% CI: 0.610–0.694; $p < 0.001$), separately (Figure 4). The optimal cutoff values were 152.25 and 141.5, separately.

Subgroup analysis

Subgroup analyses of age (<65 and ≥65 years), sex, and coexisting diseases were utilized to compare the in-hospital mortality between the two groups, and the outcomes are displayed in Figure 5. The interplay between the mean BG and the entire subgroup factors was studied and no remarkable interplay was identified. ($p > 0.05$).

Prognosis value of mean BG after PSM

Given the imbalanced baseline features between the two groups, our team completed a 1:1 ratio PSM to realize the equilibrium of the latent confounders, and 367 pairs of score-matched sufferers were acquired. The baseline features of sufferers posterior to PSM analysis are displayed in Table 1. Posterior to PSM analysis, remarkable diversities between the two groups were still identified in the duration of ICU stay (6.0 vs. 5.0 days, $p = 0.007$), in-hospital death (31.9 vs. 11.2%, $p < 0.001$), 3-month death (33.2 vs. 12%, $p < 0.001$), and 6-month death (33.8 vs. 12%, $p < 0.001$).

The outcomes of multivariable logistic regressive analyses in sufferers posterior to PSM analysis revealed that mean BG ≥ 152.25 mg/dl was still an independent prediction factor of in-hospital death (Model I: OR = 3.370, 95% CI: 2.302–4.933, $p < 0.001$; Model II: OR = 2.724, 95% CI: 1.833–4.049, $p < 0.001$; Model III: OR = 2.621, 95% CI: 1.742–3.945, $p < 0.001$; and Model IV: OR = 2.543, 95% CI: 1.485–4.357, $p < 0.001$) (Table 2).

Discussion

Our research was completed to analyze the relationship between mean BG and in-hospital death in CPs with SAH. The results showed that mean BG, as a continuous or categorical variate, was remarkably related to in-hospital death in multivariable logistic regressive analyses. In addition, statistically, our team discovered a remarkable elevation in unsatisfactory prognoses among sufferers maintaining mean BG contents higher than a determined best cutoff value of 152.25. Moreover, mean BG had a higher AUC value in the ROC analysis and had a better prognostic performance for in-hospital mortality than admission BG, which had been largely ignored in previous studies.

McIntyre et al. published a single-center retrospective cohort research based on 217 SAH sufferers, which showed that elevated mean BG levels were independently associated with worse outcomes. Higher mean BG could independently serve as a risk factor for the death rate and could best discriminate patients with SAH at risk of death (17). Those outcomes resembled our discoveries. Nevertheless, our research merely selected 217 sufferers and neglected multiple vital confounding factors, such as hypertension (18) and GCS score (19). In this study, we conducted the largest cohort study ($n = 1{,}230$), utilized an extended model strategy to modify the latent confounding factors, and discovered a steady association between mean BG and in-hospital death.

The reasons why we used average BG in this study are as follows. First, previous studies have demonstrated that high BG is common in CPs (12, 20–23). Frontera et al. retrospectively completed a cohort study of 281 SAH sufferers and found that high BG posterior to SAH was related to severe complicating diseases, more ICU stay, and elevated risk of death or serious disability (8). The study finished by Latorre et al. demonstrated that valid GLU management to sustain BG < 140 mg/dl was related to superior neurological results in SAH sufferers (9). However, few studies on the relationship between the mean of 24-h BG and the prognosis of subarachnoid hemorrhage were carried out. Second, admission BG contents can vary quickly due to stress response, altered nutrition consumption, catecholamine, cortisol, and the use of beta-blockers, or insulin. In addition, GLU is usually determined clinically when there is aggravation, which might give rise to sampling bias. The objective of our research was to reveal whether, in sufferers with SAH, the mean of 24-h venous BG levels on ICU admission can be a better prediction factor of in-hospital death than single admission BG level alone. Third, our results revealed that mean BG had a higher AUC value in the ROC analysis and had a better prognostic performance for in-hospital mortality than admission BG, which had been largely ignored in previous studies (Figure 4). Moreover, multivariate regressive analyses

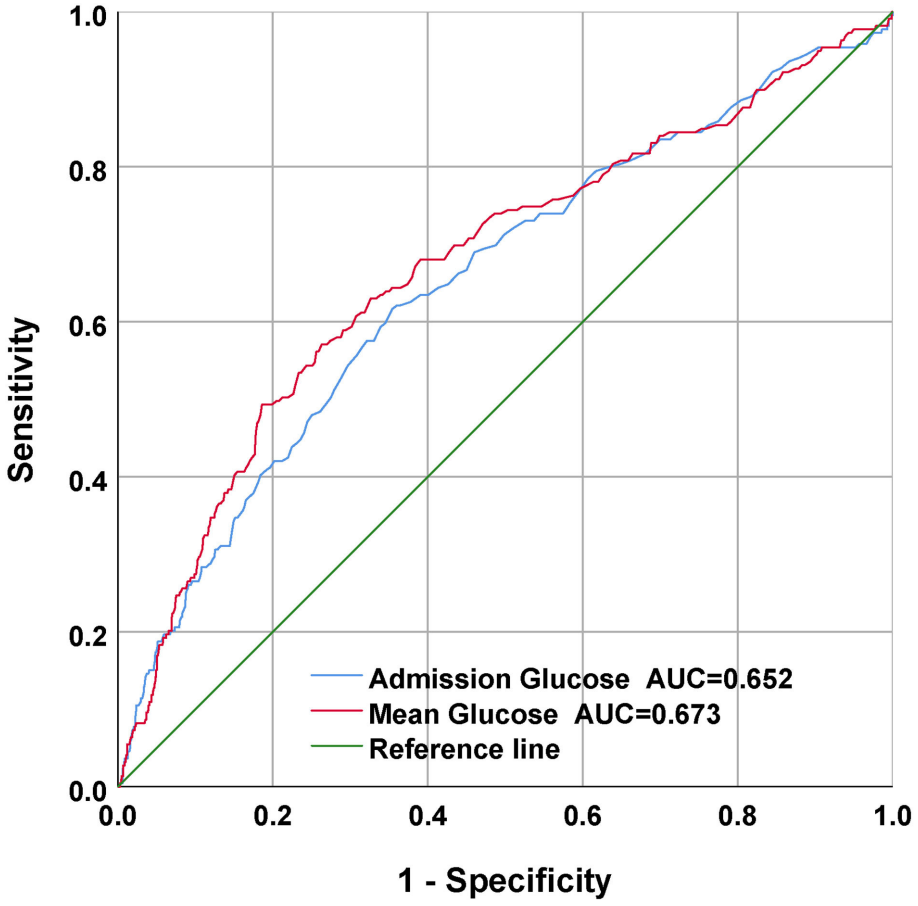

FIGURE 4
The receiver operating characteristic (ROC) curves of the predictive value of mean BG and admission BG for in-hospital mortality.

showed that mean BG was still related to in-hospital mortality after we adjusted potential risk factors ($p < 0.05$) (Table 2). Nevertheless, admission BG was not related to in-hospital mortality after we adjusted these factors ($p > 0.05$). Therefore, the average BG was utilized in this study.

A meta-analysis revealed that posterior to aneurysmal SAH, high BG contents on admission were common and that high BG on admission was related to unsatisfactory prognoses (24). There are several potential explanations for the relationship between high BG and unsatisfactory prognoses posterior to SAH. First, high BG on admission and high mean BG contents could derive from the stress response; therefore, they could merely denote the magnitude of the initial insult. Normally, it is suggested that the two main causal links related to stress high BG in acutely ill sufferers are reinforced liver GLU generation and elevated insulin resistance (25, 26). Second, high BG indicates aberrant GLU metabolic activity in diabetic illnesses or preexistent but undiscovered diabetic illnesses. A previous study revealed that diabetes mellitus increased the risk of poor outcomes following aneurysmal subarachnoid hemorrhage (27). Third, in this study, patients with high mean BG were older and had more comorbidities, all of which could be latent confounders eliciting elevated in-hospital ACM in SAH sufferers.

There were certain strengths in our research. The sample size herein was sufficient to determine a remarkable relationship between mean BG and in-hospital mortality in SAH sufferers. In addition, the in-depth analyses of covariable data enabled us to modify latent confounders which might influence the relationship between mean BG and mortality. Our study also explored the non-linear association between mean BG and poor outcomes, which indicated that aberrant mean BG might be related to elevated in-hospital mortality.

Despite the values of these findings, there remained certain deficiencies. First, this single-center research was finished retrospectively; therefore, multicenter prospective studies are required to substantiate our discoveries. Second, the data regarding the mean BG of certain sufferers were absent or not suitable for analysis. Therefore, these patients were excluded from this research, which might

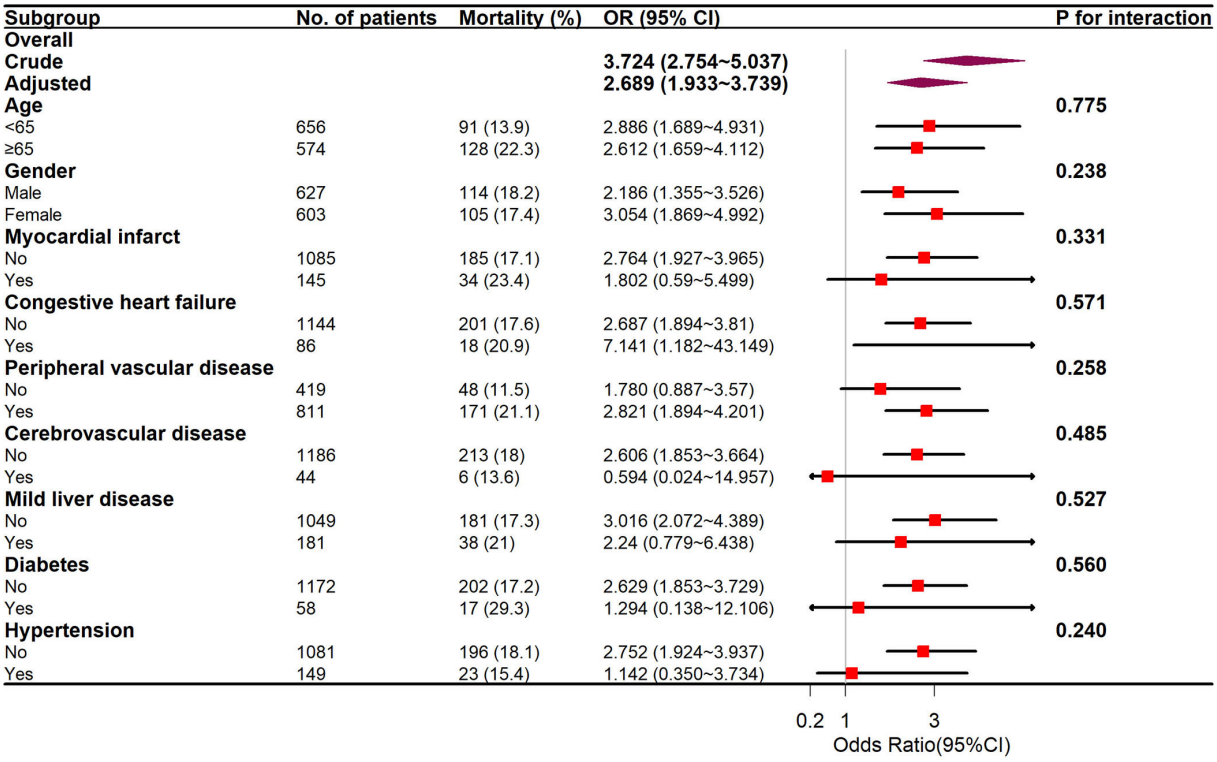

FIGURE 5
The relationship between mean BG and in-hospital mortality in subgroup analysis.

elicit selection bias, and this was one of the causes why the PSM method was utilized herein. Third, the mean BG could be influenced by a variety of factors such as diabetes, inflammation, and insulin, which might have biased the results.

Conclusion

Our findings demonstrated that the mean of 24-h venous BG was associated with in-hospital mortality among patients with SAH. According to our results, compared with patients with mean BG < 152.25 mg/dl, patients with mean BG ≥ 152.25 mg/dl were at higher risk of prolonged ICU stay, in-hospital mortality, 3-month mortality, and 6-month mortality.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: PhysioNet, https://physionet.org/, doi: 10.13026/s6n6-xd98.

Ethics statement

The studies involving human participants were reviewed and approved by the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. Written informed consent to participate in this study was provided by the participant's legal guardian/next of kin.

Author contributions

J-SY and J-HW designed this study, analyzed the data, and wrote the manuscript. H-KY, HL, and R-DC reviewed, interpreted, and checked clinical data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.904293/full#supplementary-material

References

1. Chang TR, Kowalski RG, Carhuapoma JR, Tamargo RJ, Naval NS. Impact of case volume on aneurysmal subarachnoid hemorrhage outcomes. *J Crit Care.* (2015) 30:469–72. doi: 10.1016/j.jcrc.2015.01.007

2. Udy AA, Vladic C, Saxby ER, Cohen J, Delaney A, Flower O, et al. subarachnoid hemorrhage patients admitted to intensive care in Australia and New Zealand: a multicenter cohort analysis of in-hospital mortality over 15 years. *Crit Care Med.* (2017) 45:e138–e45. doi: 10.1097/CCM.0000000000002059

3. Fang F, Wang P, Yao W, Wang X, Zhang Y, Chong W, et al. Association between D-dimer levels and long-term mortality in patients with aneurysmal subarachnoid hemorrhage. *Neurosurg Focus.* (2022) 52:E8. doi: 10.3171/2021.12.FOCUS21512

4. Wang P, Zhang Y, Wang X, Peng L, Jia L, Li T, et al. Association between serum albumin and hospital-acquired infections after aneurysmal subarachnoid hemorrhage. *Neurocrit Care.* (2021). doi: 10.1007/s12028-021-01421-y

5. Langer T, Zadek F, Carbonara M, Caccioppola A, Brusatori S, Zoerle T, et al. Cerebrospinal fluid and arterial acid-base equilibrium of spontaneously breathing patients with aneurismal subarachnoid hemorrhage. *Neurocrit Care.* (2022) 37:102–10. doi: 10.1007/s12028-022-01450-1

6. Wang J, Feng Q, Zhang Y, Qiu W, Gao H. Elevated glucose-potassium ratio predicts preoperative rebleeding in patients with aneurysmal subarachnoid hemorrhage. *Front Neurol.* (2021) 12:795376. doi: 10.3389/fneur.2021.795376

7. McGirt MJ, Woodworth GF, Ali M, Than KD, Tamargo RJ, Clatterbuck RE. Persistent perioperative hyperglycemia as an independent predictor of poor outcome after aneurysmal subarachnoid hemorrhage. *J Neurosurg.* (2007) 107:1080–5. doi: 10.3171/JNS-07/12/1080

8. Frontera JA, Fernandez A, Claassen J, Schmidt M, Schumacher HC, Wartenberg K, et al. Hyperglycemia after SAH: predictors, associated complications, and impact on outcome. *Stroke.* (2006) 37:199–203. doi: 10.1161/01.STR.0000194960.73883.0f

9. Latorre JG, Chou SH, Nogueira RG, Singhal AB, Carter BS, Ogilvy CS, et al. Effective glycemic control with aggressive hyperglycemia management is associated with improved outcome in aneurysmal subarachnoid hemorrhage. *Stroke.* (2009) 40:1644–52. doi: 10.1161/STROKEAHA.108.535534

10. Naidech AM, Levasseur K, Liebling S, Garg RK, Shapiro M, Ault ML, et al. Moderate hypoglycemia is associated with vasospasm, cerebral infarction, and 3-month disability after subarachnoid hemorrhage. *Neurocrit Care.* (2010) 12:181–7. doi: 10.1007/s12028-009-9311-z

11. Sadan O, Feng C, Vidakovic B, Mei Y, Martin K, Samuels O, et al. Glucose variability as measured by inter-measurement percentage change is predictive of in-patient mortality in aneurysmal subarachnoid hemorrhage. *Neurocrit Care.* (2020) 33:458–67. doi: 10.1007/s12028-019-00906-1

12. Liu D, Tang Y, Zhang Q. Admission hyperglycemia predicts long-term mortality in critically Ill patients with subarachnoid hemorrhage: a retrospective analysis of the MIMIC-III database. *Front Neurol.* (2021) 12:678998. doi: 10.3389/fneur.2021.678998

13. Eagles ME, Newton BD, Rosgen BK, Ayling OGS, Muram S, Tso MK, et al. Optimal glucose target after aneurysmal subarachnoid hemorrhage: a matched cohort study. *Neurosurgery.* (2021) 90:340–6. doi: 10.1227/NEU.0000000000001823

14. Okazaki T, Hifumi T, Kawakita K, Shishido H, Ogawa D, Okauchi M, et al. Blood glucose variability: a strong independent predictor of neurological outcomes in aneurysmal subarachnoid hemorrhage. *J Intensive Care Med.* (2018) 33:189–95. doi: 10.1177/0885066616669328

15. Johnson A, Bulgarelli L, Pollard T, Horng S, Celi LA, Mark R. (2021). MIMIC-IV (version 1.0). PhysioNet. https://doi.org/10.13026/s6n6-xd98.

16. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. *JAMA.* (2021) 326:1614–21. doi: 10.1001/jama.2021.18236

17. McIntyre MK, Halabi M, Li B, Long A, Van Hoof A, Afridi A, et al. Glycemic indices predict outcomes after aneurysmal subarachnoid hemorrhage: a retrospective single center comparative analysis. *Sci Rep.* (2021) 11:158. doi: 10.1038/s41598-020-80513-9

18. Zuurbier CCM, Bourcier R, Constant Dit Beaufils P, Redon R, Desal H, Investigators I, et al. Number of affected relatives, age, smoking, and hypertension prediction score for intracranial aneurysms in persons with a family history for subarachnoid hemorrhage. *Stroke.* (2022) 53:1645–50. doi: 10.1161/STROKEAHA.121.034612

19. Epstein D, Freund Y, Marcusohn E, Diab T, Klein E, Raz A, et al. Association between ionized calcium level and neurological outcome in endovascularly treated patients with spontaneous subarachnoid hemorrhage: a retrospective cohort study. *Neurocrit Care.* (2021) 35:723–37. doi: 10.1007/s12028-021-01214-3

20. Jung HM, Paik JH, Kim SY, Hong DY. Association of plasma glucose to potassium ratio and mortality after aneurysmal subarachnoid hemorrhage. *Front Neurol.* (2021) 12:661689. doi: 10.3389/fneur.2021.661689

21. Kruyt ND, Biessels GJ, DeVries JH, Luitse MJ, Vermeulen M, Rinkel GJ, et al. Hyperglycemia in aneurysmal subarachnoid hemorrhage: a potentially modifiable risk factor for poor outcome. *J Cereb Blood Flow Metab.* (2010) 30:1577–87. doi: 10.1038/jcbfm.2010.102

22. Kruyt ND, Roos YW, Dorhout Mees SM, van den Bergh WM, Algra A, Rinkel GJ, et al. High mean fasting glucose levels independently predict poor outcome and delayed cerebral ischaemia after aneurysmal subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry.* (2008) 79:1382–5. doi: 10.1136/jnnp.2007.142034

23. Pasternak JJ, McGregor DG, Schroeder DR, Lanier WL, Shi Q, Hindman BJ, et al. Hyperglycemia in patients undergoing cerebral aneurysm surgery: its association with long-term gross neurologic and neuropsychological function. *Mayo Clin Proc.* (2008) 83:406–17. doi: 10.4065/83.4.406

24. Kruyt ND, Biessels GJ, de Haan RJ, Vermeulen M, Rinkel GJ, Coert B, et al. Hyperglycemia and clinical outcome in aneurysmal subarachnoid hemorrhage: a meta-analysis. *Stroke.* (2009) 40:e424–30. doi: 10.1161/STROKEAHA.108.529974

25. Mizock BA. Alterations in fuel metabolism in critical illness: hyperglycaemia. *Best Pract Res Clin Endocrinol Metab.* (2001) 15:533–51. doi: 10.1053/beem.2001.0168

26. Dungan KM, Braithwaite SS, Preiser JC. Stress hyperglycaemia. *Lancet.* (2009) 373:1798–807. doi: 10.1016/S0140-6736(09)60553-5

27. Dumont T, Rughani A, Silver J, Tranmer BI. Diabetes mellitus increases risk of vasospasm following aneurysmal subarachnoid hemorrhage independent of glycemic control. *Neurocrit Care.* (2009) 11:183–9. doi: 10.1007/s12028-009-9232-x

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Neurofilament light chain and S100B serum levels are associated with disease severity and outcome in patients with aneurysmal subarachnoid hemorrhage

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Objectives: Serum neurofilament light chain (NfL) is a biomarker for neuroaxonal damage, and S100B is a blood marker for cerebral damage. In the present study, we investigated the relationship between serum NfL and S100B levels, severity, and outcomes in patients with aneurysmal subarachnoid hemorrhage (aSAH).

Methods: We prospectively recruited aSAH patients and healthy controls between January 2016 and January 2021. Clinical results included mortality and poor outcomes (modified Rankin scale score of 3-6) after 6 months. The ultrasensitive Simoa technique was used to evaluate NfL levels in the blood, and ELISA was used to detect S100B.

Results: A total of 91 patients and 25 healthy controls were included in the study, with a death rate of 15.4%. The group of aSAH patients had significantly higher serum levels of NfL and S100B ($P < 0.01$). Furthermore, the levels of NfL and S100B increased when the Hunt-Hess, World Federation of Neurological Surgeons (WFNS), and Fisher grades increased ($P < 0.01$). Serum NfL and S100B levels were linked to poor prognoses and low survival rates. The blood levels of NfL and S100B were found to be an independent predictor related to 6-month mortality in multivariable analysis. Additionally, the areas under the curves for NfL and S100B levels in serum were 0.959 and 0.912, respectively; the clinical diagnostic critical thresholds were 14.275 and 26.54 pg/ml, respectively; sensitivities were 0.947 and 0.921, and specificities were 0.849 and 0.811.

Conclusions: The NfL and S100B values for aSAH patients within 12 days of admission were considerably associated with Hunt-Hess grade, WFNS, and Fisher grade. The higher the grade, the higher the NfL and S100B value, and

the poorer the prognosis. Serum NfL and S100B values could be feasible biomarkers to predict the clinical prognosis of patients with aSAH.

KEYWORDS

aneurysmal subarachnoid hemorrhage, neurofilament protein light, S100B, biomarkers, prognosis

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is the deadliest form of a devastating disease, and poor-grade patients have poorly predicted outcomes. It has been reported that aSAH accounts for approximately 15% of cerebrovascular disease (1, 2). Without effective treatment, the mortality rate of patients with Fisher grade 3, and Hunt-Hess grading scales III or above is as high as 44% (3, 4). The prognosis of aSAH patients is even worse in most low- and middle-income countries owing to the lack of techniques and facilities for craniotomy and interventional embolization. Moreover, aSAH is usually followed by cerebral vasospasm (CVS) or even cerebral infarction in some extreme cases (5, 6). Thus, the early diagnosis and treatment of the disease are imperative, and it is also necessary to judge the prognosis of patients. Nearly 1 year after patients' admission to the hospital, brain damage and subsequent pathological changes occur, especially the long-term damage mechanism of hemoglobin and inflammatory molecules to the brain, fueling increased research interest (7, 8). The basic concept of early brain injury (EBI) is fundamental because it represents that the initial clinical presentation is the most important predictor of outcome (9). Therefore, the Hunt and Hess (H-H) grade, Fisher grades, and the World Federation of Neurological Surgeons (WFNS) scale are commonly used for predicting the prognosis of aSAH. Although it appears important for the outcome, the mechanisms behind brain injury are multifactorial and remain incompletely understood (10). In addition, the clinical predictive scoring system is somewhat subjective, and the patient may be in a state of sedation or coma when admitted to the hospital. In this condition, different doctors may give variable scores. Furthermore, WFNS only explained a minor proportion of variance in the outcome, and the contribution of the other predictors was substantially lower. To better understand the brain damage and the mechanism caused by aSAH, it is necessary to identify reliable biomarkers to promote further research and future clinical implementation.

Neurofilaments have three subunits; neurofilament light protein (NfL) is the smallest, neuron-specific protein abundant in myelinated axons and released into the extracellular compartment when neuronal damage occurs (11). Recent studies have proved cerebrospinal fluid (CSF) or plasma NfL as a useful neuroaxonal impairment biomarker in a diverse range of neurological diseases including degenerative conditions (11–13), as well as cerebral hemorrhage (14) and traumatic (15) brain injuries. A few studies have shown that the level of NfL in the plasma of patients with aSAH continues to increase, and the level of NfL is related to the severity of the patients' condition when admitted to the hospital as well as long-term outcomes (10, 16). However, most previous researchers focused on the late phase (>72 h) after bleeding, and the association among blood-borne neurofilament levels in the early brain injury phase, disease severity on admission to hospital, and the long-term consequences has not been previously explored.

S100B protein can effectively reflect brain tissue damage (17). In subsequent studies, many authors related pathologically increased serum levels of the S100B with head trauma (18). S100 B protein is recommended in current traumatic brain injury guidelines and is used in the clinical emergency routine for patients with head trauma. Moreover, S100B has been a well-studied marker for ischemic injury, stroke, coronary artery bypass graft surgery, and aSAH (19, 20). NfL and S100B release characteristics in patients with aSAH have not been reported.

In the present study, the levels of NfL and S100B in patients with aSAH were detected, and the correlation between them was also examined to explore the pathogenesis of aSAH to provide new insights into the clinical diagnosis of aSAH. The increase in serum NfL and S100B concentration following surgery or medical treatment may reflect disease severity and outcome in patients with aSAH.

Materials and methods

Patients and exclusion criteria

A total of 91 patients with aSAH who were diagnosed by computerized tomography (CT) and digital subtraction angiography (DSA) in the Department of Neurosurgery, Dujiangyan Medical Center, Chengdu, China, from January 2016 to January 2021 were enrolled as the observation group. These patients included 37 men and 54 women aged 41–75 years, with an average age of 56.06 ± 6.0 years. The CT scans and DSA images were evaluated by a neuroradiology consultant and scored according to the Fisher grade. Exclusion criteria were a preexisting hemorrhagic disease, treatment with antithrombotic drugs, active cancer or chemotherapy in the previous 3

months, and liver cirrhosis. In addition, patients who had experienced ischemic or hemorrhagic cerebral infarction within the previous 3 months, structural causes of SAH (arteriovenous malformation, tumor, or trauma), and autoimmune diseases were excluded. Twenty-five healthy people who matched the age and sex of physical examination in our hospital in the same period were selected as the health group. This study was discussed and approved by the hospital ethics committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Blood collection

All routine examinations were conducted immediately after admission to the observation group. Blood samples were collected on day 0, day 1, day 3, and day 10–12 after hemorrhage. Immediately, samples were centrifuged at 3,000 g for 10 min at 4°C and then stored at −80 °C until analysis. At the same time, serum NfL and S100B levels in the control group were examined.

Serum neurofilament light assay

The serum NfL levels were detected by a commercial nuclear factor kit (Quanterix, Lexington, MA, USA) of single molecular array immunoassay (SIMOA) on an HD-1 analyzer (Quanterix) (21). Briefly, samples were thawed at 25 °C, and vortexed, 10,000 RCF centrifugation was applied for 5 min. The samples were diluted with sample diluent at a ratio of 1:4 and bonded to paramagnetic magnetic beads on the instrument, which were coated with human NfL-specific antibodies. Then the biotinylated anti-NfL detection antibody was conjugated to streptavidin-β-galactosidase complex, and fluorescence detection was performed. Sample concentrations were calculated from a standard curve, fitted using a four-parameter logistic curve.

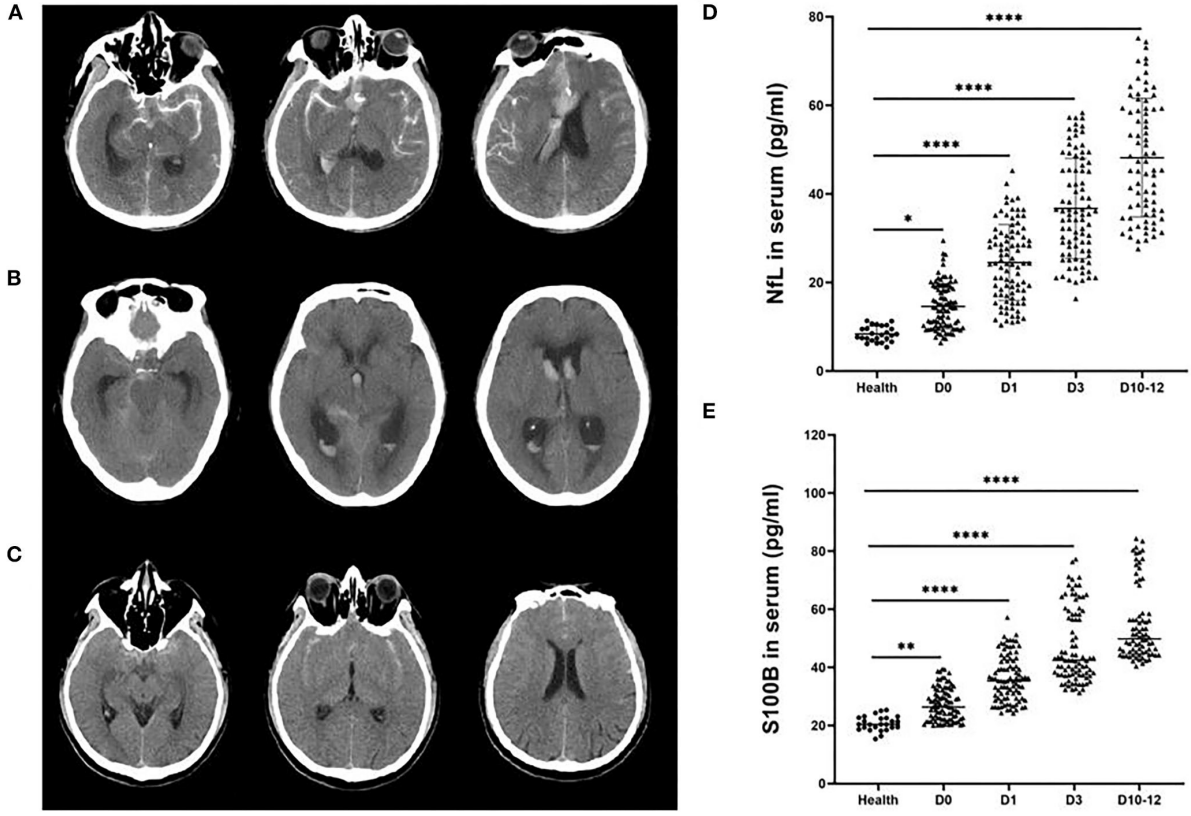

FIGURE 1
Representative head computed tomography (CT) scan of patients with aneurysmal subarachnoid hemorrhage (aSAH). **(A)** The head CT scans of a patient with a Hunt-Hess grade IV aSAH with global and focal edema. **(B)** The head CT scans of an aSAH patient with a Hunt-Hess grade III with hydrocephalus. **(C)** The head CT scans of an aSAH patient with a Hunt-Hess grade II without edema or hydrocephalus. NfL **(D)** and S100B **(E)** levels were significantly elevated in the serum of aSAH patients compared with control patients at each period. Ultrasensitive Simoa and ELISA results showed significant differences between the health group and patients with different Hunt-Hess grades. ***$P < 0.001$.

Serum S100B assay

An enzyme-linked immunosorbent assay kit detected the serum S100B level (purchased from Elabscience Company, Wuhan, China). Briefly, samples were thawed at 25 °C, vortexed, and 1750 RCF centrifugation was applied for 5 min. The samples were diluted with sample diluent at a ratio of 1:2 and added to microplate wells, which were coated with human S100B-specific antibodies. Then the biotinylated anti-S100B detection antibody was incubated with samples. Sample concentrations were calculated from a standard curve, fitted using a four-parameter logistic curve.

Clinical scales

CT images were used to grade the observation group. Fisher grading standard: grade 1: no blood was found in the subarachnoid space; grade 2: a thin layer of blood in the scanning layers such as longitudinal fissure and insular cistern, thickness $<$ 1 mm, or blood diffusely distributed in the subarachnoid space; grade 3: localized blood clot in the subarachnoid area, or blood clot thickness $\geq$ 1 mm in vertical layers grade 4: blood clot in the brain or ventricle, no or with diffuse subarachnoid hemorrhage (Figures 1A–C). The correlation between Fisher grade, serum NfL, and S100B levels after hemorrhage in the observation group was analyzed.

Patients were followed-up for 6 months after aSAH. The primary outcome was a functional state. Moreover, we assessed functional status using the modified Rankin scale (mRS) scores. Good outcome was defined as having the ability to perform activities of daily living (mRS score $\leq$ 2). Poor outcome was defined as an mRS score $\geq$ 3. According to the survival and mRS a half-year later, the patients in the observation group were divided into survival, non-survival, good prognosis, and poor prognosis subgroups. The serum NfL levels of different subgroups were compared, and the correlation between the prognosis and serum NfL level in the observation group was analyzed. The serum NfL level ROC curve predicting the prognosis of aneurysmal subarachnoid hemorrhage was evaluated.

TABLE 1 Baseline demographics and clinical findings of patients with aneurysmal subarachnoid hemorrhage.

Variables	Overall ($n = 91$)	Good outcome mRS (0–2) ($n = 53$)	Poor outcome mRS (3–6) ($n = 38$)	*P* value
Age (years)*		53.49 ± 4.26	59.65 ± 6.15	<0.01
Gender, *n* (%)				
Female/male	54/37	33/20	19/19	
Clinical findings, *n* (%)				
H-H grade				
I-II	47 (51.65%)	44	3	<0.01
III-V	44 (48.35%)	9	35	
WFNS score				
Good (I–III)	61 (67.03%)	53	8	<0.01
Poor (IV–V)	30 (32.97%)	0	30	
Fisher Grade, *n* (%)				
2	35 (38.46%)	33	2	<0.01
3	40 (43.96%)	20	20	
4	16 (17.58%)	0	16	<0.01
Brain edema, *n* (%)	31 (34.07%)	16	15	
Hydrocephalus, *n* (%)	29 (31.90%)	9	17	<0.01
CVS, n (%)	38(41.80%)	9	29	<0.01
Aneurysm site, *n* (%)	89			
ACoA	30 (32.97%)	22	8	
ICA	36 (39.56%)	30	6	
ACA	2 (2.20)	1	1	
MCA	15 (16.50)	9	6	
Vert.A	2 (2.20)	1	1	
PICA	1 (1.10)	1	0	
BA	2 (2.20)	1	1	
SCA	1 (1.10)	1	0	
PCA	2 (2.20)	1	1	

*Mean ± SD, aSAH, aneurysmal subarachnoid hemorrhage, ACA, anterior cerebral artery, ACoA, anterior communicating artery, ICA, internal carotid artery, mRS, modified Rankins Score, MCA, middle cerebral artery, PCoA, posterior communicating artery, PICA, posterior inferior cerebellar artery, SCA, superior cerebellar artery, Vert.A, vertebral artery, BA, basilar artery, PCA, posterior cerebral artery, H-H grade, Hunt-Hess grade, CVS, cerebral vasospasm, WFNS score, World Federation of Neurological Surgeons, WBC, white blood cells, APTT, activated partial thromboplastin time, INR, International Normalized Ratio.

Statistical analysis

SPSS 25.0 software was used to process the data. The measurement data were expressed as (mean ± SEM), and a one-way ANOVA was used to compare groups. The counting data were described by rate. Furthermore, the receiver operating curve (ROC) was used to study the value of serum NfL, S100B, in evaluating the poor prognosis of aSAH. Spearman correlation analysis was used to analyze the correlation among serum NfL, S100B and CT grade, Hunt-Hess grade, WFNS score, and Fisher grade. The logistic regression model was used to explore whether serum NfL and serum S100B were independent risk factors of aSAH.

Results

Ninety-one subjects were enrolled in this study, among whom 53 were in a good outcome group (mRS scores 0–2) and 38 were in a poor outcome group (mRS scores 3–6). The

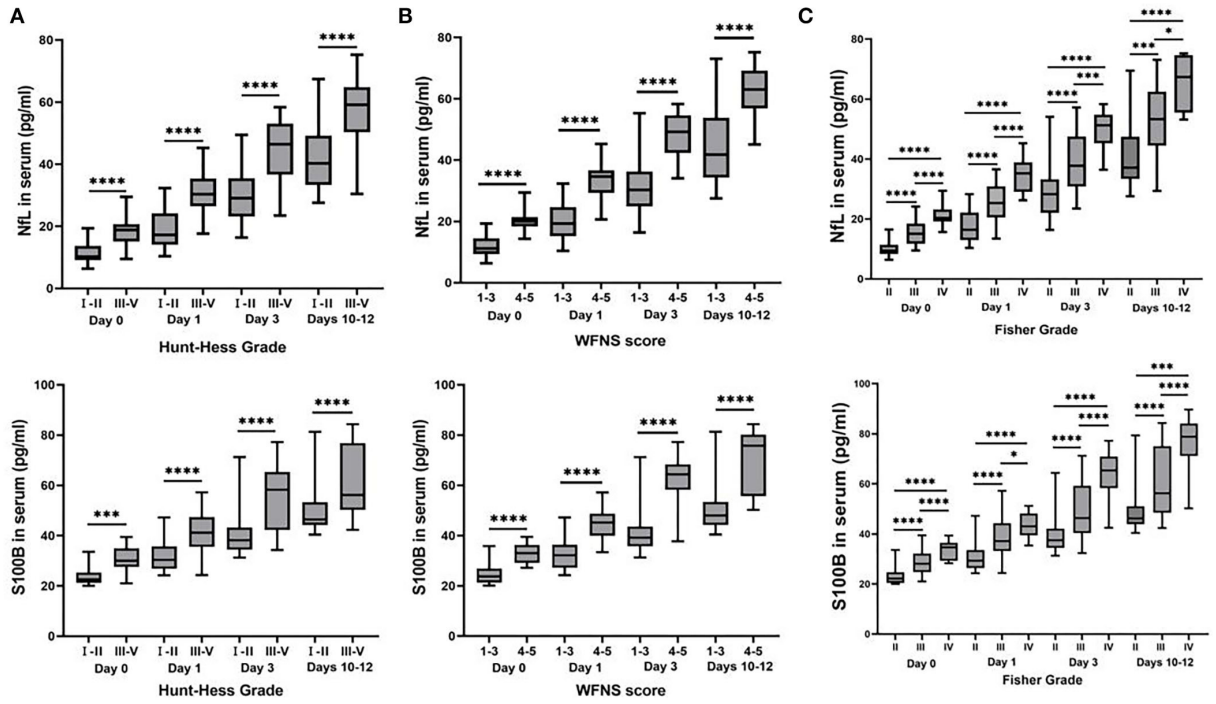

FIGURE 2
Box plots of concentrations of inflammatory cytokines classified by Hunt-Hess grade, World Federation of Neurological Surgeons (WFNS) grade, and Fisher grade. The serum NfL and S100B levels in patients with aSAH were considerably different from those various evaluations of neurological diseases. **(A)** The higher levels of serum NfL and S100B were detected in aSAH patients with H-H grades III and IV, compared with gradesland II at each time point ($P < 0.01$). **(B)** Serum NfL and S100B levels were much higher in aSAH patients with WFNS scores of 4 and 5 than those with WFNS scores of 1–3 at each time point ($P < 0.0001$). **(C)** The serum NfL and S100B concentrations in aSAH patients differed significantly in various Fisher grades at each time point ($P < 0.05$). All data were presented as mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

poor outcome group had more patients with an III-V Hunt-Hess grade than the good outcome group ($P < 0.01$). The same results were observed in the WFNS ($P < 0.01$) and Fisher ($P < 0.01$) grades and higher frequency of hydrocephalus ($P < 0.01$). The major aneurysm sites were the anterior communicating artery and internal carotid artery (32.97 and 39.56%, respectively) (Table 1).

The levels of NfL and S100B in serum of patients with aSAH were measured in different various periods (days 0, 1, 3, and 10–12, the day of aSAH patient admission was recorded as day 0). The levels of NfL (Figure 1D) and S100B (Figure 1E) were substantially increased in the serum of patients with aSAH in different periods compared with the health group ($P < 0.001$).

We found an increasing trend of NfL and S100B in aSAH patients with higher (H-H grades), World Federation of Neurological Surgeons grades (WFNS grades), and Fisher grades within days 0, 1, 3, and 10–12 (Figure 2). Compared with the lower H-H grades (grades I and II), the levels of NfL, and S100B in higher H-H grades (grades III to IV; $P < 0.0001$) were significantly higher (Figure 2A). The levels of NfL and S100B were considerably higher in aSAH patients with high WFNS grades (grades IV and V; $P < 0.0001$) (Figure 2B). Additionally, the same results were found in aSAH patients with different Fisher grades, The higher the serum NfL and S100B levels, the higher the corresponding Fisher grade, and there was a significant difference among grades II, III, and IV ($P < 0.001$). Meanwhile, the statistics results indicated that the correlation between NfL and Fisher Grade was stronger than that of S100B. The R squared value of S100B was all <0.5 on different days, but NfL was more than 0.5 except on days 10–12, the definition of R squared <0.5 was uncorrelated (Figure 2C).

For all subjects, we defined good and poor outcomes for patients with an mRS score of ≤2 and ≥3 at 6 months, respectively. We found that the levels at each time point were significantly correlated with poor outcomes when correlating NfL (Figures 3A–D) and S100B (Figures 3E–H) serum levels in aSAH patients with mRS scores (Figure 3). In addition, aSAH patients with a poor outcome had meaningfully higher serum NfL (Figure 4A) and S100B (Figure 4B) levels on days 0, 1, 3, and 10–12 than those with a good outcome.

Receiver operating curve analysis of serum NfL and S100B levels were applied to predict the prognosis of aSAH. On the day of admission, the AUC of serum NfL and S100B for predicting aSAH was 0.959 and 0.912, respectively. The best cutoff value of serum NfL for predicting the severity of aSAH on the day of

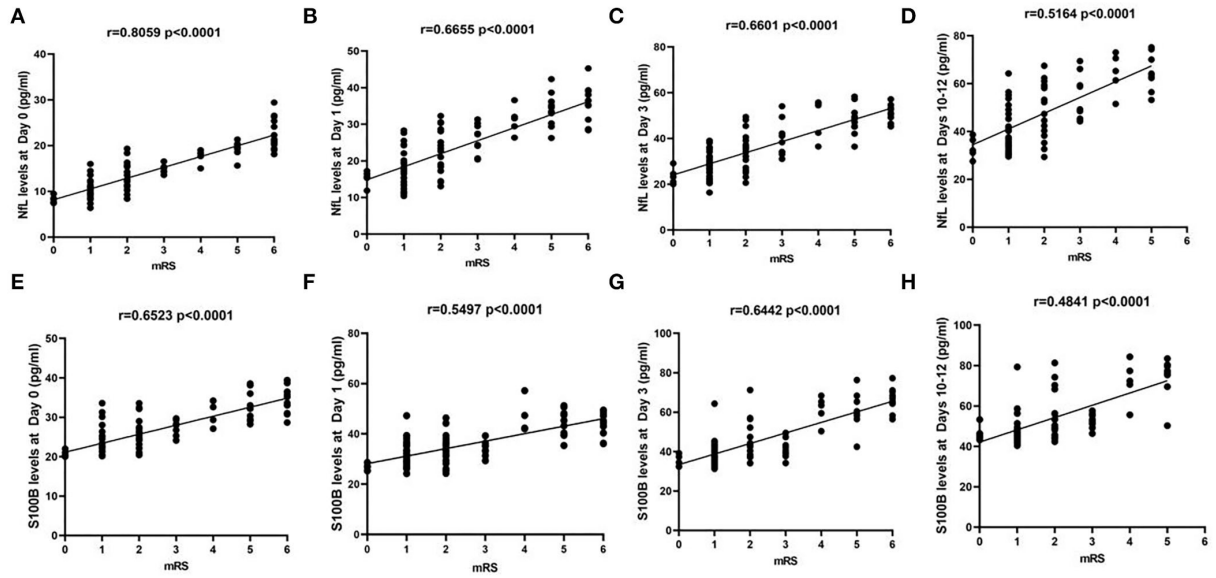

FIGURE 3
(A–D) The levels of aSAH patients serum NfL (Day 0, $r = 0.8059$, Day 1, $r = 0.6550$, Day 3, $r = 0.5960$, and Days 10–12, $r = 0.6468$, respectively; $P < 0.0001$), **(E–H)** S100B (Day 0, $r = 0.6530$, Day 1, $r = 0.5554$, Day 3, $r = 0.6200$, and Days 10–12, $r = 0.6345$, respectively; $P < 0.0001$) were significantly correlated with modified Rankin scale scores at each time points.

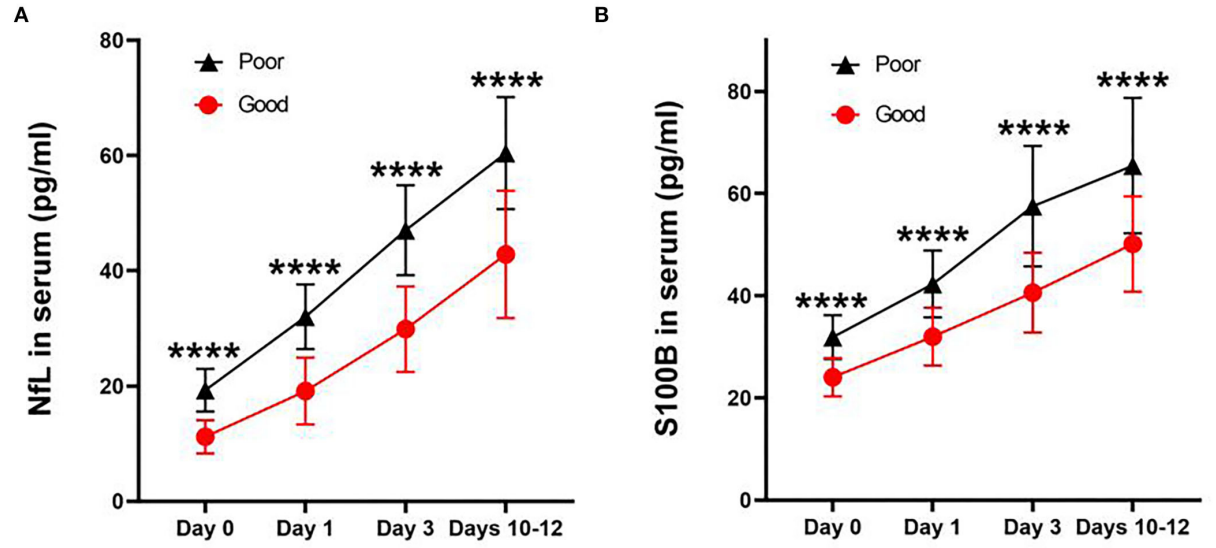

FIGURE 4
Line charts of concentration of NfL **(A)** and S100B **(B)** in serum after aneurysmal subarachnoid hemorrhage at each time point. Both concentrations were significantly higher in those with poor outcomes than in good outcomes ($P < 0.0001$). All data were presented as mean ± SEM. $^{****}P < 0.0001$.

admission was 14.275 pg/ml, and the sensitivity and specificity were 0.974 and 0.849, respectively (Figure 5A). The best cutoff value of serum S100B for predicting the survival of aSAH was 26.54 pg/ml, and the sensitivity and specificity were 0.921 and 0.811, respectively (Figure 5B).

Discussion

In recent years, many researchers have found a variety of biomarkers related to aSAH. These markers play a specific role in the development and prognosis of SAH, but the specificity and

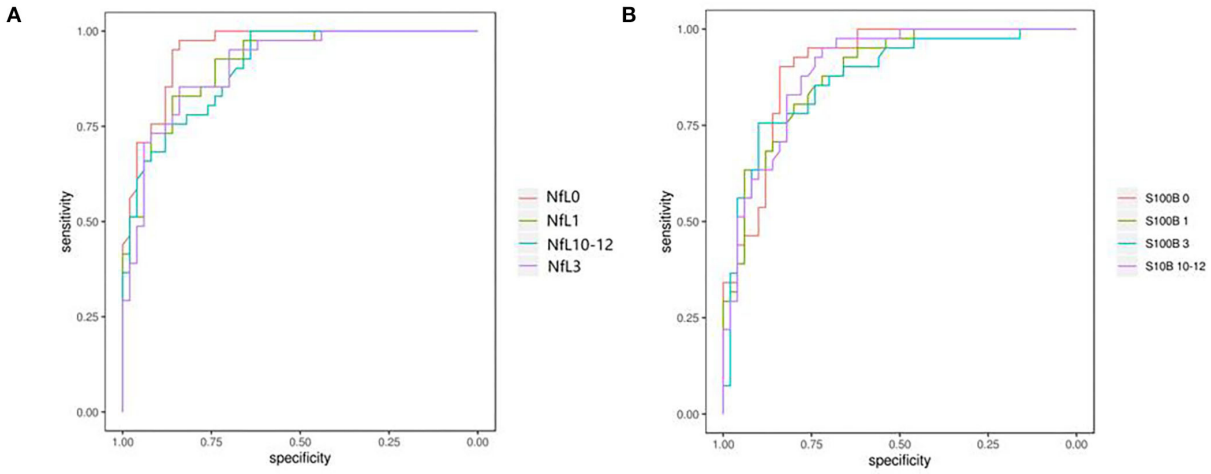

FIGURE 5
(A,B) Receiver operating characteristics revealed a sensitivity/specificity of NfL and S100B. The areas under curves for the levels of NfL and S100B in the serum were 0.959 and 0.912, respectively; the clinical diagnostic critical points were 14.275 and 26.54 pg/ml, respectively; sensibilities were 0.974 and 0.921, respectively, and specificities were 0.849 and 0.811, respectively.

sensitivity were not insufficient, and there were few prognostic markers for aSAH (16). Axonal white matter injury caused by hemorrhage was the main factor causing craniocerebral injury. Moreover, NfL was heavily expressed in axonal white matter, which was an essential part of the cytoskeleton and maintained the normal physiological function of axons, including the branching and growth of dendrites. Studies have shown that the level of NfL in CSF of patients with SAH was considerably higher, which can be used to evaluate the condition and prognosis of patients (22). However, because of the invasion of lumbar puncture, routine repeated lumbar puncture was not feasible in the clinic. So far, there is no study for serum NfL levels 3–12 days after admission. Therefore, we used the single molecular array immunoassay (SIMOA) on an HD-1 analyzer method to measure serum NfL levels in patients with aSAH to explore the relationship between NfL and prognosis. This was a highly sensitive immunoassay technology with dedicated hardware and software that quantified analyte concentrations by cingulated capture and reading of immunocomplexes on microbeads. The assay was at least 125 times more sensitive than conventional ELISA while maintaining high analytical performance (21).

Furthermore, the serum NfL levels of 91 patients with aneurysmal subarachnoid hemorrhage and 25 healthy subjects were analyzed. The results showed that the level of serum NfL in the observation group was significantly higher than in the health group. NfL should be detected in the serum of patients with aSAH as soon as admission. The concentration was considerably higher than that in the health group and increased rapidly from admission to days 10–12. This may be related to increased intracranial pressure and hypoxemia after aSAH. Further studies showed that the serum NfL concentration in patients in a poor prognosis subgroup was much higher than that in a good prognosis subgroup, indicating that the level of serum NfL in patients with aSAH was substantially correlated with the risk of severe injury. This was owing to the high expression of NfL in the axon. After being subjected to external forces, the neuron is damaged, and NfL enters the extracellular fluid and blood through the blood-brain barrier until the balance is reached. Therefore, the serum NfL concentration in patients with more severe aSAH was higher than in patients with mild aSAH.

S100B protein belongs to the S100 family and mainly exists in glial cells. Moreover, S100B is related to tumors, mental disorders, epilepsy, and brain injury. Under normal circumstances, S100B protein cannot pass through the blood-brain barrier, but aSAH leads to brain tissue damage, and the destruction of brain cells and the blood-brain barrier cause the blood S100B to increase rapidly, which has a specific value for the diagnosis and treatment of brain injury (23). The results of this study also demonstrate that the serum S100B protein increases quickly after an acute brain injury caused by aSAH, which is considerably higher than the health group, and the levels of serum S100B rise over time.

Further analysis showed less correlation between S100B protein and CT grade of aSAH, but serum NfL was positively correlated with CT grade of aSAH, the sensitivity of the detection methods may account for this difference. we used the SIMOA method to detect NfL while S100B was ELISA, the former is much more sensitive than the latter. In addition, S100B was mainly used to indicate brain injury, the volume, and area of local injury of brain injury were larger than that of aSAH. Compared with NfL, the distribution of S100B was

found to be higher in gray matter and lower in white matter in the cerebral cortex. The specific molecular mechanism needs to be further studied. For aSAH, local white matter damage may be more, resulting in a greater correlation between NfL and prognosis, which may be a better predictor compared with S100B. Demonstrating the degree of aSAH, NfL had some advantages compared with S100B protein.

In this study, by drawing the ROC curve, we found that the AUC of serum NfL and S100B on the day of admission to predict aSAH was 0.959 and 0.912, respectively. The sensitivity and specificity of predicting the risk in the severity of patients were 0.974, 0.921, 0.849, and 0.811, respectively. The sensitivity and specificity were high, and the results were similar to those of other studies. A recent study on cerebral spinal cord nerve sheath injury showed that NfL was a sign of axonal injury, and the concentration of NfL was related to the degree of axonal injury in an MRI (15). However, this study's authors did not evaluate the relationship between NfL and S100B concentrations and axonal injury, which had some limitations. Authors of follow-up studies maybe evaluate the degree of axonal injury and serum NfL and S100B concentrations by MRI, such as diffusion tensor imaging.

Overall, the level of serum NfL and S100B were positively correlated with the clinical prognosis of patients with aSAH. The detection of serum NfL and S100B levels helped in sensitively evaluating the severity of neuronal injury after aSAH. Serum NfL and S100B can be ideal biological markers for predicting aSAH. It was supportive in sensitively assessing the prognosis of patients, monitoring the development and curative effect of the disease, and improving the treatment methods in time, which is worthy of clinical attention.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Dujiangyan Medical Center Ethics Committee, and written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

ZZ and QL conceived, designed the study, and wrote the report. YZha collected and compiled data. SY, YZha, YZho, and XY performed the statistical analysis and interpreted the data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Lidington D, Wan H, Bolz SS. Cerebral autoregulation in subarachnoid hemorrhage. *Front Neurol.* (2021) 12:688362. doi: 10.3389/fneur.2021.688362

2. Qin B, Xiang Y, Zheng J, Xu R, Guo Z, Cheng C, et al. Increase in brain volume after aneurysmal subarachnoid hemorrhage leads to unfavorable outcome: a retrospective study quantified by CT scan. *Front Neurol.* (2021) 12:654419. doi: 10.3389/fneur.2021.654419

3. Chen XY, Chen Y, Lin N, Chen JY, Ding CY, Kang DZ, et al. A nomogram for predicting the need of postoperative tracheostomy in patients with aneurysmal subarachnoid hemorrhage. *Front Neurol.* (2021) 12:711468. doi: 10.3389/fneur.2021.711468

4. Jung HM, Paik JH, Kim SY, Hong DY. Association of plasma glucose to potassium ratio and mortality after aneurysmal subarachnoid hemorrhage. *Front Neurol.* (2021) 12:661689. doi: 10.3389/fneur.2021.661689

5. Smetana KS, Buschur PL, Owusu-Guha J, May CC. Pharmacologic management of cerebral vasospasm in aneurysmal subarachnoid hemorrhage. *Crit Care Nurs Q.* (2020) 43:138–56. doi: 10.1097/CNQ.0000000000000299

6. Zhang Z, Fang Y, Lenahan C, Chen S. The role of immune inflammation in aneurysmal subarachnoid hemorrhage. *Exp Neurol.* (2021) 336:113535. doi: 10.1016/j.expneurol.2020.113535

7. Etminan N, Vergouwen MD, Ilodigwe D, Macdonald RL. Effect of pharmaceutical treatment on vasospasm, delayed cerebral ischemia, and clinical

outcome in patients with aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis. *Cereb Blood Flow Metab.* (2011) 31:1443–51. doi: 10.1038/jcbfm.2011.7

8. Macdonald RL, Higashida RT, Keller E, Mayer SA, Molyneux A, Raabe A, et al. Randomized trial of clazosentan in patients with aneurysmal subarachnoid hemorrhage undergoing endovascular coiling. *Stroke.* (2012) 43:1463–9. doi: 10.1161/STROKEAHA.111.648980

9. Virta JJ, Satopää J, Luostarinen T, Raj R. One-year outcome after aneurysmal subarachnoid hemorrhage in elderly patients. *World Neurosurg.* (2020) 143:e334–43. doi: 10.1016/j.wneu.2020.07.127

10. Bodker Hviid CV, Lauridsen SV, Gyldenholm T, Sunde N, Parkner T, Hvas A-M. Plasma neurofilament light chain is associated with poor functional outcome and mortality rate after spontaneous subarachnoid hemorrhage. *Transl Stroke Res.* (2020) 11:671–7. doi: 10.1007/s12975-019-00761-4

11. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol.* (2018) 14:577–89. doi: 10.1038/s41582-018-0058-z

12. Hansson O, Janelidze S, Hall S, Magdalinou N, Lees AJ, Andreasson U, et al. Blood-based NfL: a biomarker for differential diagnosis of parkinsonian disorder. *Neurology.* (2017) 88:930–7. doi: 10.1212/WNL.0000000000003680

13. Mattsson N, Andreasson U, Zetterberg H, Blennow K. Alzheimer's disease neuroimaging initiative. Association of plasma neurofilament light with neurodegeneration in patients with alzheimer disease. *JAMA Neurol.* (2017) 74:557–66. doi: 10.1001/jamaneurol.2016.6117

14. Gendron TF, Badi MK, Heckman MG, Jansen-West KR, Vilanilam GK, Johnson PW, et al. Plasma neurofilament light predicts mortality in patients with stroke. *Sci Transl Med.* (2020) 12:eaay1913. doi: 10.1126/scitranslmed.aay1913

15. Shahim P, Politis A, van der Merwe A, Moore B, Chou YY, Pham DL, et al. Neurofilament light as a biomarker in traumatic brain injury. *Neurology.* (2020) 95:e610–22. doi: 10.1212/WNL.0000000000009983

16. Garland P, Morton M, Zolnourian A, Durnford A, Gaastra B, Toombs J, et al. Neurofilament light predicts neurological outcome after subarachnoid haemorrhage. *Brain.* (2021) 144:761–8. doi: 10.1093/brain/awaa451

17. Michetti F, D'Ambrosi N, Toesca A, Puglisi MA, Serrano A, Marchese E, et al. The S100B story: from biomarker to active factor in neural injury. *J Neurochem.* (2019) 148:168–87. doi: 10.1111/jnc.14574

18. Koh SX, Lee JK. S100B as a marker for brain damage and blood-brain barrier disruption following exercise. *Sports Med.* (2014) 44:369–85. doi: 10.1007/s40279-013-0119-9

19. Silva FP, Schmidt AP, Valentin LS, Pinto KO, Zeferino SP, Oses JP, et al. S100B protein and neuron-specific enolase as predictors of cognitive dysfunction after coronary artery bypass graft surgery: a prospective observational study. *Eur J Anaesthesiol.* (2016) 33:681–9. doi: 10.1097/EJA.0000000000000450

20. Kedziora J, Burzynska M, Gozdzik W, Kübler A, Kobylinska K, Adamik B. Biomarkers of neurological outcome after aneurysmal subarachnoid hemorrhage as early predictors at discharge from an intensive care unit. *Neurocrit Care.* (2021) 34:856–66. doi: 10.1007/s12028-020-01110-2

21. Hendricks R, Baker D, Brumm J, Davancaze T, Harp C, Herman A, et al. Establishment of neurofilament light chain simoa assay in cerebrospinal fluid and blood. *Bioanalysis.* (2019) 11:1405–18. doi: 10.4155/bio-2019-0163

22. Halawa I, Vlachogiannis P, Amandusson Å, Elf K, Engström ER, Zetterberg H, et al. Seizures, CSF neurofilament light and tau in patients with subarachnoid haemorrhage. *Acta Neurol Scand.* (2018) 137:199–203. doi: 10.1111/ane.12873

23. Mondal A, Saha P, Bose D, Chatterjee S, Seth RK, Xiao S, et al. Environmental microcystin exposure in underlying NAFLD-induced exacerbation of neuroinflammation, blood-brain barrier dysfunction, and neurodegeneration are NLRP3 and S100B dependent. *Toxicology.* (2021) 461:152901. doi: 10.1016/j.tox.2021.152901

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Day 1 neutrophil-to-lymphocyte ratio (NLR) predicts stroke outcome after intravenous thrombolysis and mechanical thrombectomy

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Background: The neutrophil-to-lymphocyte ratio (NLR) is a biomarker reflecting the balance between inflammation (as indicated by the neutrophil count) and adaptive immunity (as indicated by the lymphocyte count). We aimed to estimate ability of NLR at admission and at day 1 for predicting stroke outcome after two reperfusion therapies: intravenous thrombolysis (IVT) and mechanical thrombectomy (MT).

Methods: A retrospective analysis was performed on patients who received recombinant human tissue plasminogen activator (IVT) and/or underwent MT for acute ischemic stroke (AIS) at the First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China) from January 2018 to December 2020. Blood samples were taken on admission to hospital and on day 1 after stroke onset. Binary logistic regression models were applied to investigate potential associations between NLR at admission or day 1 and the following outcomes: symptomatic intracerebral hemorrhage (sICH), dependence, and mortality at 90 days. The ability of NLR to predict AIS outcome was analyzed using receiver operating characteristic (ROC) curves.

Results: Data for 927 patients (576 IVT and 351 MT) were reviewed. High admission NLR was associated with dependence in IVT treatment [adjusted odds ratio (OR) 1.21, 95% confidence interval (CI) 1.14–1.23] and 90-day mortality in MT patients (OR 1.09, 95% CI 1.04–1.13). In IVT patients, high NLR at day 1 predicted dependence (OR 1.09, 95% CI 1.02–1.11), sICH (OR = 1.07, 95% CI 1.01–1.12), and 90-day mortality (OR 1.06, 95% CI 1.01–1.15). In MT patients, high NLR at day 1 also predicted dependence (OR 1.08, 95% CI 1.02–1.11) and sICH (OR 1.03, 95% CI 1.01–1.09). ROC analysis confirmed that NLR at day 1 could predict dependence (cut-off 4.2; sensitivity 68.7%; specificity 79.6%), sICH (cut-off 5.1; sensitivity 57.9%, specificity 73.5%), and death (cut-off 5.4; sensitivity 78.8%; specificity 76.4%) in IVT patients. Z values of area under the curves were compared between admissioin and day 1 NLR in IVT patients and showed day 1 NLR can better predict dependence ($Z = 2.8$, $p = 0.004$) and 90-day death ($Z = 2.8$, $p = 0.005$).

Conclusions: NLR is a readily available biomarker that can predict AIS outcome after reperfusion treatment and day 1 NLR is even better than admission NLR.

KEYWORDS

neutrophil, lymphocyte, acute ischemic stroke, thrombolysis (tPA), thrombectomy, outcome

Introduction

Reperfusion therapies, namely, intravenous thrombolysis (IVT) and mechanical thrombectomy (MT), are the most effective treatment for patients with acute ischemic stroke (AIS), but they are associated with symptomatic intracerebral hemorrhage (sICH) and ischemic-reperfusion injury. Emerging evidence indicates that post-stroke immune responses can affect the neurovascular interface, leading to reperfusion injury and sICH. Neutrophils are among the first cells in the blood to respond after ischemic stroke, and they contribute to the disruption of the blood–brain barrier (BBB), cerebral edema, and brain injury (1). After ischemic stroke, the number of circulating neutrophils rises while the number of lymphocytes falls, resulting in an increased neutrophil-to-lymphocyte ratio (NLR) (2, 3). Some studies investigated the relationship between admission NLR and prognosis of patients with ischemic stroke treated with IVT or MT (4–7) and found that high pretreatment NLR was associated with sICH or worse 3-month functional outcomes. This may be due to neuroinflammation induced by increased NLR (5, 8). In patients with ischemic stroke, the number of circulating neutrophils begins to rise within 6 h, but neutrophil infiltration into the brain peaks at 24–48 h after stroke onset (9, 10). In addition, the levels of proinflammatory cytokines produced by neutrophils such as tumor necrosis factor-alpha increase within 24–48 h after stroke onset, then fall slightly by 72–144 h (11). The time gap between the early increase in circulating neutrophils and the peak of neuroinflammation begs the question of whether the NLR increase causes subsequent inflammation-induced outcomes.

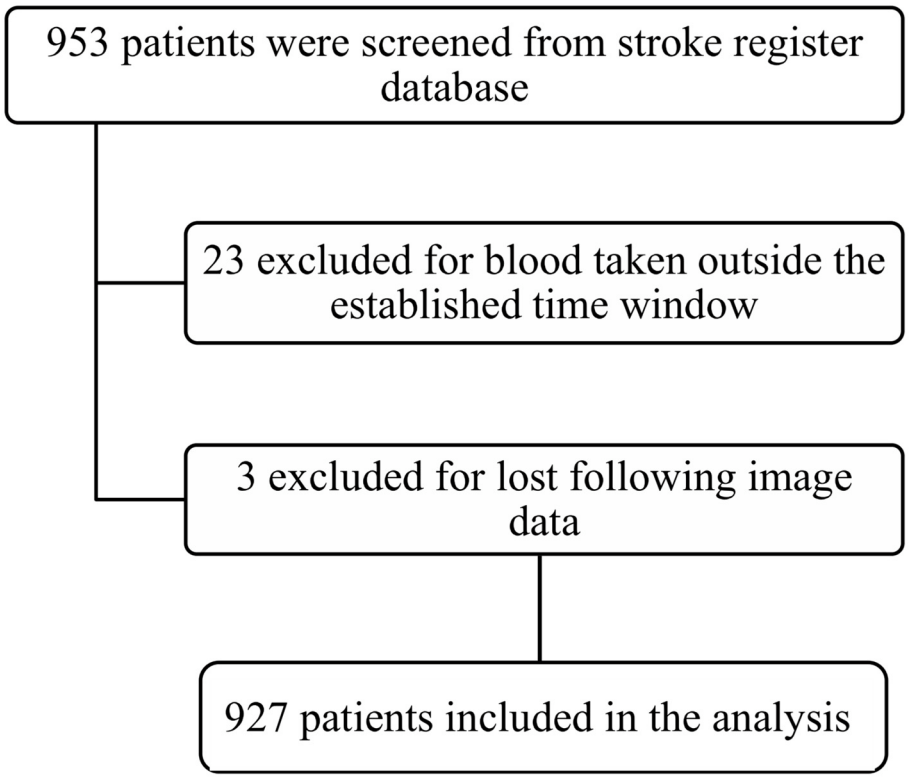

FIGURE 1
Patient flow-chart of the cohort.

Thus, it seems plausible to speculate that the NLR increase at 24 h after stroke onset may predict stroke outcome better, but the evidence is lacking. If NLR on day 1 has a prognostic value, then it may extend the time window for effective stroke treatment. Thus, in this study, we assessed whether NLR at admission and on day 1 after stroke onset is associated with stroke outcomes after IVT or MT. We then estimated cut-off values of NLR to predict outcomes in patients undergoing IVT or MT.

Materials and methods

Study population

We conducted a retrospective, observational study at the First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China) among patients consecutively included in the hospital registry from January 2018 to December 2020. Patients were included in the present study if they were diagnosed with ischemic stroke and were treated with IVT, MT, or both.

Patients were included whether they had available laboratory values with complete blood count from blood collected at admission (up to 4.5 h before IVT and up to 6 h before MT) and at day 1, corresponding to 12–36 h from stroke onset (referring to time from last known well in case of unwitnessed onset). The collected blood was mixed well and inserted into Sysmex Automated Analyzer by Mindray BC 6800, Shenzhen, China. Total white blood cell counts (WBCs) $\times 10^9$/L and their differentials neutrophils and lymphocytes were measured. Out of 953 patients with AIS treated with reperfusion, 927 total patients were included in the study, after excluding 23 patients whose blood samples were taken outside the established time window and 3 patients for missing follow-up imaging data (Figure 1). Blood collection was available at admission in 793 (85.5%) patients after a median time of 3.4 (0.7–5.9) h from stroke onset, whereas on day 1 in 924 (99.7%) patients after 22.9 (16.2–29.7) h from stroke onset. Seven hundred ninety patients (85.2%) had both admission and day 1 complete blood counts available.

For each patient, we recorded demographic data, prestroke functional status using a modified Rankin Scale (mRS), and vascular risk factors, namely, hypertension, diabetes mellitus, hyperlipidemia, stroke history, current smoker, and atrial fibrillation. Whether the patient had a history of atrial fibrillation and stroke was determined based on the patient's or the relatives' statement and confirmed by a review of the patient's past medical history. Hypertension and diabetes were assessed according to the 2010 Chinese guidelines for the management of hypertension (12) and the 2018 American Diabetes Association Standards of Medical Care in Diabetes (13), respectively. Hyperlipidemia was diagnosed when one of the following criteria was met: total cholesterol level $\geq$6.2 mmol/L, low-density lipoprotein cholesterol level $\geq$4.1 mmol/L, triglyceride level $\geq$2.3 mmol/L, or high-density lipoprotein chole $<$1.0 mmol/L. Current smoking is defined as regular smoking of at least 1 cigarette per day at the time of presentation. We recorded stroke severity assessed with the National Institutes of Health Stroke Scale (NIHSS) on admission and on day 1; administration of systemic thrombolysis; the site of the large vessel occlusion on baseline CT angiography; and stroke cause according to TOAST classification (14). Because of the small number for each type of small artery occlusion, the stroke of other determined etiology and undetermined etiology, these three types of stroke are referred to as "other" type together. NLR was calculated as the ratio of the number of neutrophils to the number of lymphocytes. The baseline NIHSS score was determined at admission by a staff neurologist. Treatment was classified as having received IVT [by recombinant human tissue plasminogen activator (rtPA) at the dose of 0.9 mg/kg] or mechanical thrombectomy (MT) whether with or without pretreatment of IVT. The outcome was measured by mRS at 90 days during clinical follow-up by trained staff. Additional demographic information was identified from the medical record.

Study outcomes

The occurrence of hemorrhagic transformation and sICH, after treatment, was assessed in one or more of the imaging techniques available during 24-h follow-up: non-contrast CT scan in 677 (73%) patients and brain magnetic resonance imaging in 436 (47%) patients. Symptomatic intracerebral hemorrhage was defined according to ECASS-II (European–Australian Acute Stroke Study II) definition (15): any HT and worsening by $\geq$4 points on the NIHSS. Functional outcomes at 3 months were assessed with the modified Rankin Scale (mRS) in-person or through a telephone interview by a certified evaluator. Dependence or poor outcome were defined as mRS $>$ 3 points. Death within 90 days and sICH were recorded as an index of safety outcomes.

The local Clinical Research Ethics Committee approved the study protocol under the requirements of national legislation in the field of biomedical research, the regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and the standards of Good Clinical Practice, and also with the Helsinki Declaration of 1975/1983. Patient consent was not required because of the retrospective nature of the study design and the lack of patient interaction.

Statistical analyses

Descriptive and frequency analyses were conducted for all demographic and clinical data. The normal distribution of data was tested by skewness and kurtosis analyses. The continuous

TABLE 1 Baseline characteristics of the study population and study outcomes.

Demographic characteristics	Total ($n = 927$)	IVT ($n = 576$)	MT ($n = 351$)	p
Age (years, median, IQR)	68 (59–76)	68 (59–76)	69 (60–76)	0.88
Sex, male *n* (%)	628 (68)	379 (65)	249 (71)	0.08
Hypertension, *n* (%)	664 (72)	417 (72)	247 (70)	0.59
Diabetes mellitus, *n* (%)	247 (30)	173 (30)	101 (29)	0.72
Hyperlipidemia, *n* (%)	86 (9)	47 (7)	39 (11)	0.2
Atrial fibrillation, *n* (%)	320 (35)	164 (28)	156 (44)	<0.001*
Current smoker, *n* (%)	152 (16)	145 (12)	7 (6)	0.067
Stroke history, *n* (%)	93 (10)	38 (7)	55 (16)	<0.001*
Admission NIHSS score, median (IQR)	9 (4–14)	5 (3–10)	14 (11–19)	<0.001*
mRS 0–1 before stroke, *n* (%)	859 (93)	549 (95)	310 (88)	<0.001*
Stroke onset to treat time, min, median (IQR)	249 (196–307)	173 (126–221)	318 (278–352)	<0.001*
Stroke etiology (TOAST) *n* (%)				
Large-artery atherosclerosis	557 (60)	373 (65)	184 (52)	<0.001*
Cardioembolism	284 (31)	128 (22)	156 (44)	<0.001*
Other	88 (9)	77 (13)	11 (3)	<0.001*
Anterior circulation, *n* (%)	794 (86)	497 (86)	297 (85)	0.56
Day 1 NIHSS score, median (IQR)	6 (2–13)	3 (1–8)	11 (7–21)	<0.001*
Hemorrhagic transformation, *n* (%)	172 (19)	67 (12)	105 (30)	<0.001*
sICH, *n* (%)	82 (9)	43 (8)	39 (11)	0.05
Functional outcome dependence (mRS>3), *n* (%)	376 (410)	143 (25)	233 (66)	<0.001*
mRS score at 90 day, median (IQR)	2 (0–4)	1 (0–2)	4 (1–5)	<0.001*
Mortality, *n* (%)	116 (13)	36 (6)	80 (23)	<0.001*
Admission WBC, median (IQR)	7.6 (6.0–9.7)	7.2 (5.8–9.1)	8.3 (6.4–10.7)	<0.001*
Admission neutrophils, median (IQR)	5.0 (3.6–7.1)	4.6 (3.5–6.4)	5.9 (4.2–8.6)	<0.001*
Admission lymphocytes, median (IQR)	1.6 (1.2–2.1)	1.7 (1.3–2.2)	1.5 (1.1–1.9)	<0.001*
Admission NLR, median (IQR)	3.7 (1.7–6.8)	2.6 (1.7–4.3)	4.3 (2.5–6.8)	<0.001*
Day 1 WBC, median (IQR)	8.3 (6.4–11.7)	7.6 (6.4–9.6)	9.6 (7.6–11.7)	<0.001*
Day 1 neutrophils, median (IQR)	6.1 (4.8–8.1)	5.4 (4.8–6.1)	7.7 (7.1–8.1)	<0.001*
Day 1 lymphocytes, median (IQR)	1.4 (1.0–2.1)	1.5 (1.1–2.1)	1.3 (1.0–1.8)	<0.001*
Day 1 NLR, median (IQR)	4.3 (2.7–8.6)	3.5 (2.7–5.6)	5.9 (3.9–8.6)	<0.001*

IVT, intravenous thrombolysis; MT, mechanical thrombectomy; NIHSS, National Institutes of Health Stroke Scale; IQR, interquartile range; TOAST, trial of Org 10 172 in acute stroke treatment; WBC, while blood cell; sICH, symptomatic intracerebral hemorrhagic; NLR, neutrophil over lymphocytes ratio. *Statistically significant.

variables in our study are all non-normal distributions. Changes in neutrophils, lymphocytes, and NLR before and after reperfusion procedures were compared by Wilcoxon signed ranks test. Univariate tests (χ^2-test for categorical variables, Mann–Whitney U-test for continuous variables) were first used to compare clinical, neuroradiological features, and NLR in patients for dependence, death, and sICH. Multivariable logistic regression models were used to test the independent effect of NLR on outcome measures including age and sex and other variables with a $P < 0.1$ in univariate analysis. Receiver operating characteristic (ROC) curves were used to determine the predictive values of the area under the curve and 95% CI. We considered an area under the curve value of 0.70 or higher as indicating acceptable discrimination. To compare the ability of NLR to predict stroke prognosis, we calculated the Z-value of the areas under the curve by the formula $Z =$ (S1-S2)/(SE1*SE1+SE2*SE2)^0.5 and compared the area by the Z-test. All statistical analyses were performed using IBM SPSS Statistics for Mac, version 23.0 (IBM Corp, Armonk, NY, USA) and the figures are performed using GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

Baseline characteristics and study outcomes

Patients' clinical and demographic baseline characteristics and stroke outcomes are displayed in Table 1. Across all patients, the median baseline NIHSS score was 9 [interquartile range

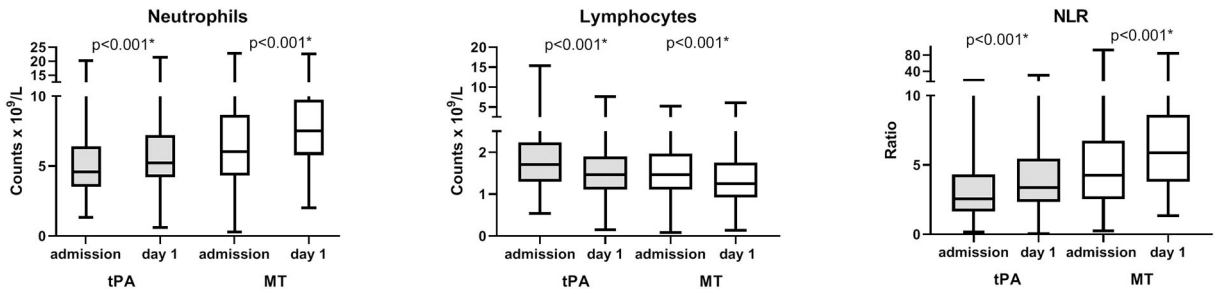

FIGURE 2
Longitudinal changes of neutrophil, lymphocyte counts, and neutrophil-to-lymphocyte ratio (NLR) over time. Analysis of repeated measures of neutrophil, lymphocyte counts, and NLR at admission and at day 1 in patients with both admission and day 1 complete blood counts available ($n = 495$ in tPA and $n = 295$ in MT). *P*-value refer to comparisons of repeated measures. Boxes, 25–75% interquartile range; central horizontal bars, median; outer horizontal bars, minimum and maximum values. Significant ($P < 0.001$) differences between admission and day 1 values were also obtained in comparisons of means (Mann–Whitney *U*-test) on the entire population (data not shown). IVT, intravenous thrombolysis; MT, mechanical thrombectomy.

(IQR) (4–14)] and the median 90-day mRS was 2 (IQR 0–4). Among the 351 patients treated with MT, 88 patients (25.1%) received IVT before MT. The median baseline NIHSS score was 14 (IQR 11–19) and the median 90-day mRS was 4 (IQR 1–5). Anterior circulation strokes comprised 85.7% of the population.

On admission, the median neutrophil percentage was 68% (IQR 57–78%) of the total WBC and the median lymphocyte percentage was 23% (IQR 14–31%), corresponding to a median NLR of 3.7 (IQR 1.7–6.8). On day 1, the median neutrophil percentage was 75% (IQR 66–83%) and the median lymphocyte percentage was 17% (IQR 12–25%), corresponding to a median NLR of 4.3 (IQR 2.7–8.6).

Across the entire cohort, 82 (8.8%) patients presented sICH and 116 (12.5%) died. A bad outcome was presented in 376 (39.6%) presented bad outcomes on day 90.

Patients with MT had significant more previous stroke history (15.7 vs. 6.6%), previous disability (11.7 vs. 4.7%), sICH after reperfusion therapy (11.1 vs. 7.5%), and more functional dependence at 90 days (66.4 vs. 24.8%) compared to the IVT group. Recanalization, which is demonstrated by a myocardial infarction (TIMI) score of 2 or 3, was achieved in 260 (74.1%) patients who underwent MT.

Changes in neutrophils, lymphocytes, and NLR before and after reperfusion procedures

In 790 patients with both admission and day 1, complete blood counts were available ($n = 495$ in IVT and $n = 295$ in MT), neutrophils increase in 516 (65.3%), lymphocytes decrease in 517 (65.4%), and NLR increased in 520 (65.8%) patients at day 1 compared to admission. In both patients with IVT and MT, neutrophils and NLR increase and lymphocytes decrease on day 1 (all $p < 0.001$, Figure 2).

Compare baseline characteristics and NLR in patients presented with dependence or not

In both patients with IVT and MT, high admission NIHSS is related to poor outcomes (both $p < 0.001$, Table 2) and old age is another factor leading to dependence ($p < 0.001$ and $p = 0.002$, respectively, Table 2). Female, atrial fibrillation, high admission, longer stroke onset to puncture time, and day 1 NLR in patients with IVT predict worse functional outcomes ($p = 0.04$, $p < 0.0001$, $p < 0.0001$, $p = 0.03$, and $p < 0.0001$, respectively, Table 2). Stroke classification types are different in the IVT group (Table 2). High day 1 NLR is associated the poor outcome in patients with MT ($p = 0.01$, Table 2).

Compare baseline characteristics and NLR in patients presented with sICH or not

In IVT, high admission NIHSS and old age are related to sICH ($p < 0.001$ and $p = 0.001$, Table 3). Female, disability before the stroke and atrial fibrillation are associated with sICH ($p = 0.03$, $p < 0.001$, and $p = 0.002$, respectively, Table 3). Admission and day 1 NLR is significantly higher in patients with sICH than in no sICH ones ($p = 0.001$ and $p < 0.001$, Table 3). In patients with MT, DM predicts sICH ($p = 0.03$, Table 3). No differences in either admission or day 1 NLR are found between patients with sICH and no sICH in MT.

TABLE 2 Comparison of clinical characteristics and NLR in patients presented with dependence or not at 90 day.

	IVT				MT			
	Yes ($n = 108$)	No ($n = 468$)	Statistic value	p	Yes ($n = 187$)	No ($n = 164$)	Statistic value	p
Admission NIHSS, median (IQR)	12 (9–15)	4 (2–8)	−12.8	<0.001*	16 (12–23)	12 (10–16)	−6.4	<0.001*
Age, median (IQR)	76 (68–83)	67 (58–74)	−7.2	<0.001*	71 (61–78)	66 (59–74)	−3.1	0.002*
Male, *n* (%)	62 (57)	317 (67)	4.2	0.04*	125 (66)	124 (75)	3.3	0.07
Hypertension, *n* (%)	79 (73)	338 (72)	0.04	0.85	134 (71)	113 (68)	0.32	0.57
Diabetes mellitus, *n* (%)	33 (30)	140 (30)	0.02	0.9	60 (32)	41 (25)	2.1	0.14
Hyperlipidemia, *n* (%)	5 (4)	42 (9)	2.2	0.14	16 (8)	23 (14)	2.6	0.1
Current smoker, *n* (%)	36 (33)	109 (23)	4.7	0.03	2 (1)	5 (3)	1.8	0.2
Stroke history, *n* (%)	11 (10)	27 (6)	2.8	0.09	34 (18)	21 (13)	1.9	0.2
mRS 0–1 before stroke, *n* (%)	101 (94)	448 (96)	0.9	0.3	169 (90)	141 (86)	1.6	0.2
Atrial fibrillation, *n* (%)	46 (42)	118 (22)	13.0	<0.001*	92 (49)	64 (39)	3.7	0.06
Admission NLR, median (IQR)	4.1 (2.5–6.1)	2.4 (1.6–4.0)	−4.8	<0.001*	4.5 (2.7–6.8)	3.9 (2.4–6.8)	−1.5	0.13
Day 1 NLR, median (IQR)	6.2 (4.4–10)	3.1 (2.2–4.9)	−8.9	<0.001*	6.4 (4.1–9.3)	5.2 (3.6–7.9)	−2.5	0.01*
Day 1 NHISS, median (IQR)	13 (10–20)	3 (1–5)	−13.9	<0.001*	12 (7–22)	7 (3–10)	−14.1	<0.001*
Stroke onset to treat time, min, median (IQR)	166 (127–188)	163 (122–185)	−1.2	0.16	314 (277–353)	321 (271–358)	−1.3	0.18
Stroke etiology (TOAST), *n* (%)								
Large-artery atherosclerosis	63 (58)	310 (66)	2.4	0.1	98 (52)	86 (52)	0	0.99
Cardioembolism	16 (15)	112 (24)	4.2	0.04*	80 (43)	76 (46)	0.5	0.5
Other	29 (27)	48 (10)	20.1	<0.001*	9 (5)	2 (2)	3.7	0.05
Anterior circulation, *n* (%)	93 (86)	404 (86)	0.3	0.6	157 (84)	140 (85)	0.1	0.7

IVT, intravenous thrombolysis; MT, mechanical thrombectomy; NIHSS, National Institutes of Health Stroke Scale; IQR, interquartile range; NLR, neutrophil over lymphocytes ratio.
*Statistically significant.

Compare baseline characteristics and NLR in patients presented with death or not

In patients with IVT, high admission NIHSS, female, and old age are related to 90-day death ($p < 0.001$, $p < 0.001$, and $p = 0.02$, respectively, Table 4). Atrial fibrillation and high day 1 NLR were associated with death ($p = 0.01$ and $p < 0.001$, Table 4). In patients with MT, high admission NIHSS and old age are related to 90-day death ($p = 0.007$ and $p < 0.001$, Table 4). DM and high day 1 NLR are found to relate to 90-day death (both $p = 0.03$, Table 4).

Predictive values of NLR for three end points

The ROC curves and the area underneath them for using NLR to predict outcomes are shown in Figure 3. For predicting poor outcomes in patients with IVT generally, NLR on day 1 showed a sensitivity of 68.7%, specificity of 79.6%, a positive likelihood ratio 2.33, and an area under the curve of 0.79 (95% CI 0.73–0.82, $p < 0.001$). For predicting death in patients with IVT, NLR on day 1 showed a sensitivity of 78.8%, specificity of 76.4%, a positive likelihood ratio of 1.03, and an area under the curve of 0.81 (95% CI 0.74–0.89, $p < 0.001$). For predicting sICH in patients with IVT, NLR on day 1 showed a sensitivity of 71.4%, specificity of 68.9%; a positive likelihood ratio of 2.11, and an area under the curve of 0.74 (95% CI 0.67–0.82, $p < 0.001$). The cut-off NLR of dependence, sICH, and death were 4.2, 5.1, and 4.7, respectively.

While for the NLR at admission for patients with IVT and that of patients with MT at admission and on day 1, the areas of ROC are under the discrimination value (Figure 3).

To further assess the ability of NLR to predict prognosis, Z values of the areas under ROC curves were compared between NLR at admission and day 1 in patients with IVT. We found that high NLR on day 1 predicted poor outcome and death at 90 days better than NLR at admission, while the two NLRs were similar in their ability to predict sICH (Table 5).

Association of NLR to three end points before and after adjustment

Before adjustment, in patients with IVT, both admission and day 1 NLR related to patients with poor outcomes at 3

TABLE 3 Comparison of clinical characteristics and NLR in patients presented with sICH or not.

	IVT				MT			
	Yes ($n = 43$)	No ($n = 533$)	Statistic value	p	Yes ($n = 39$)	No ($n = 312$)	Statistic value	p
Admission NIHSS, median (IQR)	13 (8–17)	5 (2–10)	−6.5	<0.001*	16 (11–22)	14 (11–19)	−1.51	0.13
Age, median (IQR)	75 (67–83)	68 (58–76)	−3.4	0.001*	70 (59–78)	69 (60–76)	−0.39	0.69
Male, *n* (%)	22 (51)	361 (68)	4.9	0.03*	28 (72)	203 (74)	0.69	0.4
Hypertension, *n* (%)	31 (72)	376 (71)	0.05	0.83	29 (74)	218 (70)	0.34	0.56
Diabetes mellitus, *n* (%)	16 (37)	157 (29)	1.14	0.3	17 (44)	84 (27)	4.6	0.03*
Hyperlipidemia, *n* (%)	1 (2)	46 (9)	2.1	0.15	8 (21)	31 (10)	3.9	0.05
Current smoker, *n* (%)	9 (20)	136 (26)	0.4	0.5	1 (3)	6 (2)	0	1
Stroke history, *n* (%)	2 (5)	36 (7)	0.3	0.6	6 (15)	49 (16)	0.003	0.96
mRS 0–1 before stroke, *n* (%)	33 (77)	507 (95)	22.9	<0.001*	35 (91)	275 (88)	0.001	0.98
Atrial fibrillation, *n* (%)	21 (49)	143 (27)	9.6	0.002*	20 (51)	136 (44)	0.83	0.36
Admission NLR, median (IQR)	4.2 (2.2–5.9)	2.5 (1.6–4.2)	−3.2	0.001*	3.6 (2.2–6.0)	4.3 (2.5–6.8)	−0.97	0.33
Day 1 NLR, median (IQR)	7.1 (3.9–10.6)	3.3 (2.3–5.3)	−5.3	<0.001*	6.1 (4.1–9.4)	5.8 (3.8–8.6)	−0.73	0.47
Day 1 NHISS, median (IQR)	16 (9–26)	3 (1–7)	−8.1	<0.001*	21 (12–29)	11 (6–18)	−4.1	<0.001*
Stroke onset to treat time, min, median (IQR)	164 (122–182)	167 (123–185)	−1.1	0.2	315 (277–352)	318 (270–356)	−1.3	0.2
Stroke etiology (TOAST), *n* (%)								
Large-artery atherosclerosis	29 (67)	343 (64)	1.1	0.3	20 (51)	164 (53)	0.02	0.9
Cardioembolism	11 (26)	117 (22)	0.3	0.6	17 (43)	139 (45)	0.01	0.9
Other	4 (9)	73 (14)	0.07	0.4	2 (5)	9 (3)	0.07	0.8
Anterior circulation, *n* (%)	35 (82)	462 (87)	0.94	0.3	38 (97)	259 (83)	5.5	0.01

IVT, intravenous thrombolysis; MT, mechanical thrombectomy; NIHSS, National Institutes of Health Stroke Scale; IQR, interquartile range; NLR, neutrophil over lymphocytes ratio.
*Statistically significant.

months ($p < 0.001$, Table 6) while only high day 1 NLR is associated with sICH and death within 3 months (both $p < 0.001$, Table 6). In patients with MT, admission NLR predicts death at 90 days ($p = 0.03$, Table 6) and on day 1 NLR predicts worse outcome and sICH ($p = 0.03$ and $p = 0.02$, Table 6) before adjustment.

After adjustment, admission NLR is still related to the poor outcome at 90 days ($p < 0.001$, Table 7) and on day 1 NLR is associated with poor outcome, sICH, and mortality ($p = 0.002$, $p = 0.04$, and $p = 0.003$, Table 7) in patients with IVT. For patients with MT, after adjustment, admission NLR is still related to 90-day mortality ($p = 0.04$, Table 7) and day 1 NLR is associated with poor outcome and sICH (both $p = 0.04$, Table 7).

Discussion

Here we assessed the clinical value of NLR for predicting stroke outcomes after reperfusion therapies. We observed that NLR at admission was associated with dependence and mortality, while NLR on day 1 predicted sICH, dependence, and mortality in patients with IVT at day 90. In patients with MT, admission NLR was associated with mortality, while NLR on day 1 predicted sICH and poor outcomes at day 90. Also, we found that on day 1 was a better prognostic indicator than NLR at admission in patients with AIS who received reperfusion treatment.

Here we confirmed an increase in NLR with time after stroke, which is in consistent with previous studies in patients who undergo IVT or MT (4, 6, 16). After ischemic stroke, complex networks connecting the brain and the immune system are triggered. Within several minutes after stroke, peripheral immune cells are activated and recruited to ischemic tissue, where they exert either beneficial or detrimental effects, depending on the stroke phase and the subtype of leukocytes involved (2, 17, 18). The rise in neutrophils after stroke reflects their enhanced production and release from the bone marrow and spleen, and potentially also reduced apoptosis in neutrophils (19).

In our work, high NLR was associated with an increased risk of sICH after IVT treatment and the findings are consistent with prior studies (6, 16). Circulating neutrophils are recruited to the site of cerebral injury shortly after

TABLE 4 Comparison of clinical characteristics and NLR in patients presented 90 death or not.

	IVT				MT			
	Yes ($n = 34$)	No ($n = 542$)	Statistic value	p	Yes ($n = 80$)	No ($n = 271$)	Statistic value	p
Admission NIHSS, median (IQR)	15 (12–17)	5 (2–10)	−10.5	<0.001*	19 (12–24)	14 (10–17)	−2.7	0.007*
Age, median (IQR)	81 (72–88)	68 (58–76)	−5.5	<0.001*	72 (63–79)	68 (59–76)	−5.0	<0.001*
Male, *n* (%)	16 (47)	363 (67)	5.6	0.02*	59 (73)	190 (70)	0.4	0.5
Hypertension, *n* (%)	25 (73)	392 (72)	0.02	0.9	57 (71)	190 (70)	0.04	0.84
Diabetes mellitus, *n* (%)	11 (32)	162 (29)	0.1	0.8	31 (38)	70 (25)	5	0.03*
Hyperlipidemia, *n* (%)	6 (18)	41 (8)	0.6	0.4	12 (15)	27 (10)	2.3	0.1
Atrial fibrillation, *n* (%)	16 (47)	148 (27)	6.1	0.01*	31 (38)	125 (46)	1.4	0.4
Current smoker, *n* (%)	9 (26)	136 (25)	0.03	0.8	1 (1)	6 (2)	0.3	0.6
Stroke history, *n* (%)	2 (5)	36 (7)	0.03	0.8	11 (14)	44 (16)	0.3	0.6
mRS 0–1 before stroke, *n* (%)	31 (91)	518 (95)	1.3	0.2	72 (90)	238 (88)	0.3	0.6
Admission NLR, median (IQR)	3.8 (1.9–5.7)	2.5 (1.6–4.3)	−1.7	0.09	4.5 (2.7–8.9)	4.2 (2.5–6.4)	−1.7	0.09
Day 1 NLR, median (IQR)	8.6 (5.5–12.9)	3.3 (2.3–5.4)	−6	<0.001*	7.1 (4.1–10.1)	5.5 (3.8–8.4)	−2.2	0.03*
Day 1 NHISS, median (IQR)	26 (19–30)	3 (1–7)	−9.4	<0.001*	29 (27–32)	10 (5–14)	−13.2	<0.001*
Stroke onset to treat time, min, median (IQR)	167 (122–179)	162 (119–183)	−1.2	0.16	323 (267–364)	319 (271–347)	−1.1	0.21
Stroke etiology (TOAST), *n* (%)								
Large-artery atherosclerosis	24 (71)	349 (64)	0.5	0.5	37 (46)	147 (54)	0.2	0.7
Cardioembolism	7 (20)	121 (22)	0.05	0.8	35 (44)	121 (45)	0.1	0.6
Other	5 (15)	70 (13)	0.02	0.9	8 (10)	3 (1)	0.002	0.9
Anterior circulation, *n* (%)	27 (79)	470 (87)	1.4	0.2	71 (89)	226 (83)	1.4	0.2

IVT, intravenous thrombolysis; MT, mechanical thrombectomy; NIHSS, National Institutes of Health Stroke Scale; IQR, interquartile range; NLR, neutrophil over lymphocytes ratio.
*Statistically significant.

ischemia occurs, and they contribute to BBB disruption and tissue damage by releasing matrix metalloproteinases, elastase, cathepsin G, proteinase 3, and reactive oxygen species (ROS), while migrating across the cerebral endothelium (20–23). In patients with MT, we observed that NLR at day 1 was related to sICH and poor functional outcomes, also in line with a previous study (4). In other words, NLR on day 1 but not at admission was related to sICH in patients receiving reperfusion treatment. This result indicates a link between NLR at 24 h after stroke and the concurrent peak of neuroinflammation within the brain, which leads to BBB leakage.

Poor outcomes at day 90 were associated with high NLR on day 1 in both reperfusion groups, which may have multiple explanations. First, high NLR at day 1 is associated with sICH, which increases mortality and worsens neurological deficit. Second, except for sICH caused by multifaceted inflammatory response inside the brain parenchymal, cerebral edema, and brain injury which play an important role in functional deterioration, are mediated by factors released from neutrophils including ROS, proteases, cytokines, and chemokines (2). Third, it was reported that neutrophils are also involved in the major processes that cause ischemic stroke, thrombosis, and atherosclerosis (24–26). They promote clot formation through interactions with platelets and release of prothrombotic molecules which may weaken the reperfusion effect or even infarct expansion.

Stroke causes high mortality worldwide, and changes in NLR may affect the mortality rate after stroke. On one hand, For example, high NLR may reflect neutrophil-mediated neuroinflammation as a complication of reperfusion treatment or brain edema that often occurs after a stroke. At the same time, infections such as pneumonia can increase long-term mortality after stroke (27–29). Immunosuppression indicated by lymphocyte decrease following a stroke can increase the risk of such infection. One of the vital mechanisms underlying the post-stroke infection is the impairment of the brain's immune system after a stroke, leading to a stroke-related immunosuppressive syndrome (30, 31). Neutrophils de-differentiate and undergo stimulation by growth factors, while apoptosis reduces lymphocyte numbers, increasing susceptibility to infections (32, 33).

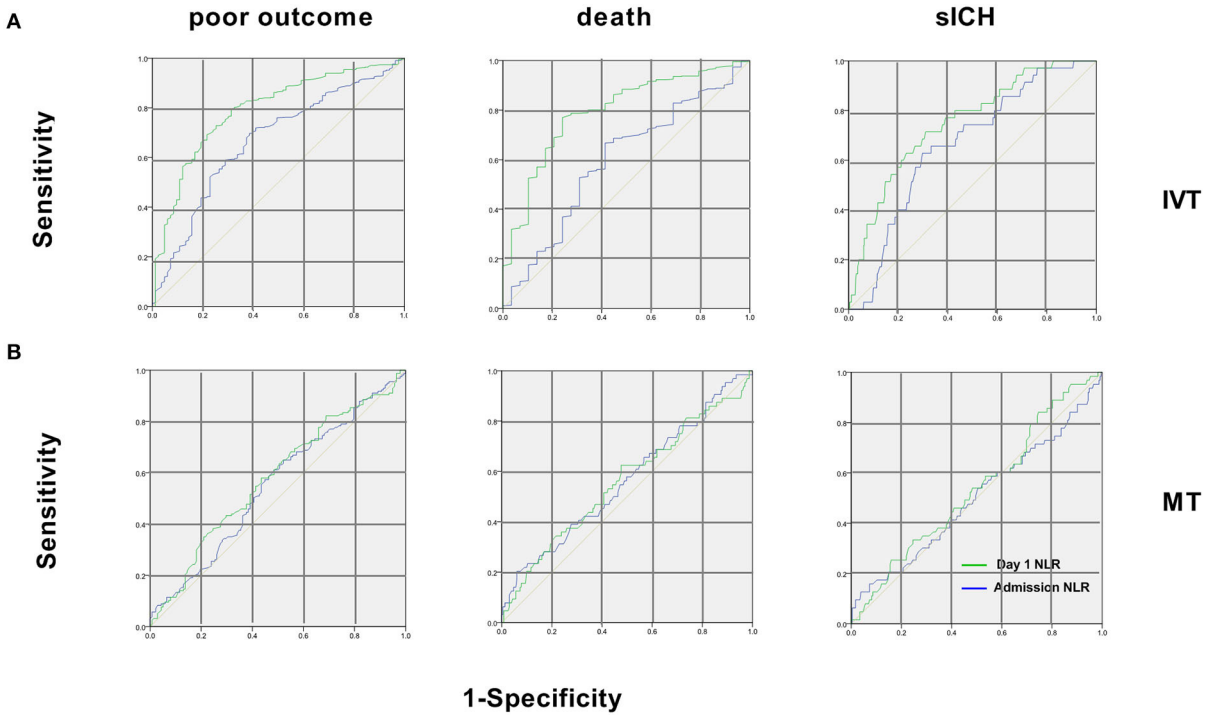

FIGURE 3
Receiver operating characteristic curves of NLR for the 3 endpoints in patients with IVT and MT. **(A)** In patients with IVT, for poor outcome, areas under the curve were 0.66 for admission NLR and 0.78 for day 1 NLR. For 90-day death, areas were 0.60 for admission NLR and 0.81 for day 1 NLR. For sICH, areas were 0.63 for admission NLR and 0.74 for day 1 NLR; **(B)** In patients with MT, areas under the curve were 0.55 (95% CI 0.48–0.6) for admission NLR and 0.57 (95% CI 0.48–0.66) for day 1 NLR for poor outcome. For 90-day death, areas were 0.57 (95% CI 0.44–0.63) for admission NLR and 0.58 (95% CI 0.45–0.67) of day 1 NLR. For sICH, areas were 0.51 (95% CI 0.42–0.65) for admission NLR and 0.56 (95% CI 0.43–0.6) for day 1 NLR. IVT, intravenous thrombolysis; MT, mechanical thrombectomy; sICH, symptomatic intracerebral hemorrhage.

In our study, NLR on day 1 was a better indicator of stroke outcomes in patients with AIS than NLR at admission, which may have several explanations. First, there are interactions between inflammatory agents and neutrophils. Circulating neutrophils recruited to the site of the cerebral facilitate releasing of inflammatory molecules inside the brain. A number of factors released after brain ischemia act on neutrophils including cytokines, chemokines, and damage-associated molecular patterns (DAMPs). These upregulated inflammatory molecules, in turn, further activate neutrophils and provoke additional recruitment of leukocytes from the peripheral blood. Levels of most inflammatory factors peak at 12–72 h after stroke onset, which means that neutrophils are continuously activated (34). Increased neutrophil counts provide a measure of the inflammation in the ischemic brain and they may also contribute directly to neuroinflammation. Second, reperfusion treatment may lead to ischemia-reperfusion injury after focal brain ischemia. Systemic inflammatory responses help circulating neutrophils gain access to the ischemic area and further activate ROS. However, except for increased susceptibility to infections, the mechanism of decreased lymphocyte numbers leading to the worse outcome of AIS remains unclear.

The importance of our work is that we found that posttreatment NLR was a better predictor of stroke outcomes than pre-reperfusion one, suggesting that the strategies to reduce NLR, especially reducing neutrophils after reperfusion is feasible because of extending the time window for treatment and clinically importance. The achievements in the treatment of stroke targeting neutrophils are fruitful in the lab but have challenges to translate to patients (2). The timing and duration of antineutrophil treatment are important determinants of success.

Our work presents several limitations. First, the modest sample size and retrospective analysis of prospectively collected data are important methodological shortcomings. Second chronic inflammatory conditions, prestroke infections, and cancer treatments may all affect a patient's NLR and will need to be taken into account. Intercurrent complications such as infection which is common in severe stroke leading to an increase in neutrophil and NLR cannot be ruled out. It was documented that increased NLR and lymphopenia with or without neutrophilia are linked to stroke-associated

TABLE 5 Comparison of the area under ROC curve between admission and day 1 NLR in patients with IVT.

Area under the curve	Dependence (95% CI)	Death (95% CI)	sICH (95% CI)
Admission	0.66 (0.60–0.73)	0.60 (0.49–0.70)	0.66 (0.58–0.74)
Day 1	0.78 (0.73–0.82)	0.80 (0.74–0.89)	0.75 (0.63–0.83)
Z-value	2.8	2.8	1.7
p	0.004*	0.005*	0.08

NLR, neutrophil over lymphocytes ratio; IVT, intravenous thrombolysis; sICH, symptomatic intracerebral hemorrhage. *Statistically significant.

TABLE 6 Unadjusted multivariate analysis for neutrophil–lymphocyte ratio (NLR) prediction models.

	IVT				MT			
	Admission		Day 1		Admission		Day 1	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Poor outcome	1.21 (1.14–1.23)	< 0.001*	1.21 (1.09–1.24)	< 0.001*	0.98 (0.96–1.12)	0.07	1.01 (1.01–1.07)	0.03*
sICH	1.07 (0.89–1.12)	0.95	1.16 (1.08–1.24)	< 0.001*	1.01 (0.98–1.05)	0.31	1.05 (1.01–1.10)	0.02*
Death	1.07 (0.84–1.32)	0.9	1.22 (1.07–1.24)	< 0.001*	0.88 (0.83–1.01)	0.03*	0.88 (0.86–1.01)	0.05

IVT, intravenous thrombolysis; MT, mechanical thrombectomy; sICH, symptomatic intracerebral hemorrhagic. *Statistically significant.

TABLE 7 Adjusted multivariate analysis for neutrophil–lymphocyte ratio (NLR) prediction models.

	IVT				MT			
	Admission		Day 1		Admission		Day 1	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Poor outcome	1.11 (1.07–1.16)	< 0.001*	1.09 (1.02–1.11)	0.002*	1.13 (0.99–1.11)	0.09	1.08 (1.02–1.11)	0.04*
sICH	1.03 (0.93–1.15)	0.5	1.06 (1.01–1.13)	0.04*	1.01 (0.98–1.04)	0.47	1.05 (1.01–1.10)	0.04*
Death	0.88 (0.64–1.12)	0.3	1.06 (1.01–1.15)	0.003*	1.09 (1.04–1.13)	0.04*	0.98 (0.96–1.08)	0.07

IVT, intravenous thrombolysis; MT, mechanical thrombectomy; sICH, symptomatic intracerebral hemorrhagic. *Statistically significant. The associations were adjusted for admission NIHSS score, age, sex, history of diabetes and atrial fibrillation, prestroke disability, day 1 NIHSS score, and stroke type.

pneumonia (35). Third, the skewed distribution of NLR may account for the discrepant findings between Mann–Whitney *U*-test and univariable logistic regression analyses evaluating the unadjusted association of NLR with 90-day three outcome mediators.

Conclusions

The NLR, particularly on day 1 is a readily available prognosis indicator for AIS outcome after reperfusion treatment.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Wenzhou Medical University Affiliated the First Hospital Clinical Research Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SYC, JHC, QY, ZSY, and YLZ searched, reviewed available studies, extracted and analyzed the data, and wrote the paper. YTL, GQH, FCC, MY, CLW, TTD, and XL made critical revisions to the paper. XL extracted the data and co-wrote the paper. ZZ reviewed and made critical revisions to the paper. All

authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Segel GB, Halterman MW, Lichtman MA. The paradox of the neutrophil's role in tissue injury. *J Leukocyte Biol.* (2011) 89:359–72. doi: 10.1189/jlb.0910538

2. Jickling GC, Liu D, Ander BP, Stamova B, Zhan X, Sharp FR. Targeting neutrophils in ischemic stroke: translational insights from experimental studies. *J Cereb Blood Flow Metab.* (2015) 35:888–901. doi: 10.1038/jcbfm.2015.45

3. Wang A, Quan K, Tian X, Zuo Y, Meng X, Chen P, et al. Leukocyte subtypes and adverse clinical outcomes in patients with acute ischemic cerebrovascular events. *Ann Trans Med.* 9:748. doi: 10.21037/atm-20-7931

4. Semerano A, Laredo C, Zhao Y, Rudilosso S, Renú A, Llull L, et al. Leukocytes, collateral circulation, and reperfusion in ischemic stroke patients treated with mechanical thrombectomy. *Stroke.* (2019) 50:3456–64. doi: 10.1161/STROKEAHA.119.026743

5. Brooks SD, Spears C, Cummings C, VanGilder RL, Stinehart KR, Gutmann L, et al. Admission neutrophil-lymphocyte ratio predicts 90 day outcome after endovascular stroke therapy. *J Neurointerventional Surg.* (2014) 6:578–83. doi: 10.1136/neurintsurg-2013-010780

6. Maestrini I, Strbian D, Gautier S, Haapaniemi E, Moulin S, Sairanen T, et al. Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes. *Neurology.* (2015) 85:1408–16. doi: 10.1212/WNL.0000000000002029

7. Zhu W, Guo Z, Yu S. Higher neutrophil counts before thrombolysis for cerebral ischemia predict worse outcomes. *Neurology.* (2016) 86:1077. doi: 10.1212/01.wnl.0000481976.41273.a1

8. Goyal N, Tsivgoulis G, Chang JJ, Malhotra K, Pandhi A, Ishfaq MF, et al. Admission neutrophil-to-lymphocyte ratio as a prognostic biomarker of outcomes in large vessel occlusion strokes. *Stroke.* (2018) 49:1985–7. doi: 10.1161/STROKEAHA.118.021477

9. Ross AM, Hurn P, Perrin N, Wood L, Carlini W, Potempa K. Evidence of the peripheral inflammatory response in patients with transient ischemic attack. *J Stroke Cerebrovasc Dis.* (2007) 16:203–7. doi: 10.1016/j.jstrokecerebrovasdis.2007.05.002

10. Tu XK, Yang WZ, Shi SS, Wang CH, Zhang GL, Ni TR, et al. Spatio-temporal distribution of inflammatory reaction and expression of TLR2/4 signaling pathway in rat brain following permanent focal cerebral ischemia. *Neurochem Res.* (2010) 35:1147–55. doi: 10.1007/s11064-010-0167-6

11. Nayak AR, Kashyap RS, Kabra D, Purohit HJ, Taori GM, Daginawala HF. Time course of inflammatory cytokines in acute ischemic stroke patients and their relation to inter-alfa trypsin inhibitor heavy chain 4 and outcome. *Ann Indian Acad Neurol.* (2012) 15:181–5. doi: 10.4103/0972-2327.99707

12. Liu LS. 2010 Chinese guidelines for the management of hypertension. *Zhonghua xin xue guan bing za zhi.* (2011) 39:579–615. doi: 10.1038/ajh.2011.248a

13. Chamberlain JJ, Johnson EL, Leal S, Rhinehart AS, Shubrook JH, Peterson L. Cardiovascular disease and risk management: review of the American Diabetes Association Standards of Medical Care in Diabetes 2018. *Ann Internal Med.* (2018) 168:640–50. doi: 10.7326/M18-0222

14. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke.* (1993) 24:35–41. doi: 10.1161/01.STR.24.1.35

15. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Second European-Australasian Acute Stroke Study Investigators. *Lancet.* (1998) 352:1245–51. doi: 10.1016/S0140-6736(98)08020-9

16. Guo Z, Yu S, Xiao L, Chen X, Ye R, Zheng P, et al. Dynamic change of neutrophil to lymphocyte ratio and hemorrhagic transformation after thrombolysis in stroke. *J Neuroinflamm.* (2016) 13:199. doi: 10.1186/s12974-016-0680-x

17. Planas AM. Role of immune cells migrating to the ischemic brain. *Stroke.* (2018) 49:2261–7. doi: 10.1161/STROKEAHA.118.021474

18. Salas-Perdomo A, Miró-Mur F, Urra X, Justicia C, Gallizioli M, Zhao Y, et al. T cells prevent hemorrhagic transformation in ischemic stroke by P-selectin binding. *Arterioscl Thromb Vasc Biol.* (2018) 38:1761–71. doi: 10.1161/ATVBAHA.118.311284

19. Tsai NW, Chang WN, Shaw CF, Jan CR, Lu CH. Leucocyte apoptosis in patients with acute ischaemic stroke. *Clin Exp Pharmacol Physiol.* (2010) 37:884–8. doi: 10.1111/j.1440-1681.2010.05398.x

20. Perez-de-Puig I, Miró-Mur F, Ferrer-Ferrer M, Gelpi E, Pedragosa J, Justicia C, et al. Neutrophil recruitment to the brain in mouse and human ischemic stroke. *Acta Neuropathol.* (2015) 129:239–57. doi: 10.1007/s00401-014-1381-0

21. Neumann J, Riek-Burchardt M, Herz J, Doeppner TR, König R, Hütten H, et al. Very-late-antigen-4 (VLA-4)-mediated brain invasion by neutrophils leads to interactions with microglia, increased ischemic injury and impaired behavior in experimental stroke. *Acta Neuropathol.* (2015) 129:259–77. doi: 10.1007/s00401-014-1355-2

22. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol.* (2013) 13:159–75. doi: 10.1038/nri3399

23. Enzmann G, Mysiorek C, Gorina R, Cheng YJ, Ghavampour S, Hannocks MJ, et al. The neurovascular unit as a selective barrier to polymorphonuclear granulocyte (PMN) infiltration into the brain after ischemic injury. *Acta Neuropathol.* (2013) 125:395–412. doi: 10.1007/s00401-012-1076-3

24. Ruf W, Ruggeri ZM. Neutrophils release brakes of coagulation. *Nat Med.* (2010) 16:851–2. doi: 10.1038/nm0810-851

25. Carbone F, Nencioni A, Mach F, Vuilleumier N, Montecucco F. Pathophysiological role of neutrophils in acute myocardial infarction. *Thromb Haemostasis.* (2013) 110:501–14. doi: 10.1160/TH13-03-0211

26. Mócsai A. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J Exp Med.* (2013) 210:1283–99. doi: 10.1084/jem.20122220

27. Saposnik G, Hill MD, O'Donnell M, Fang J, Hachinski V, Kapral MK. Variables associated with 7-day, 30-day, and 1-year fatality after ischemic stroke. *Stroke.* (2008) 39:2318–24. doi: 10.1161/STROKEAHA.107.510362

28. Vernino S, Brown RD Jr, Sejvar JJ, Sicks JD, Petty GW, O'Fallon WM. Cause-specific mortality after first cerebral infarction: a population-based study. *Stroke.* (2003) 34:1828–32. doi: 10.1161/01.STR.0000080534.98416.A0

29. Heuschmann PU, Kolominsky-Rabas PL, Misselwitz B, Hermanek P, Leffmann C, Janzen RW, et al. Predictors of in-hospital mortality and attributable risks of death after ischemic stroke: the German Stroke Registers Study Group. *Arch Internal Med.* (2004) 164:1761–8. doi: 10.1001/archinte.164.16.1761

30. Hannawi Y, Hannawi B, Rao CP, Suarez JI, Bershad EM. Stroke-associated pneumonia: major advances and obstacles. *Cereb Dis.* (2013) 35:430–43. doi: 10.1159/000350199

31. Li L, Zhang LH, Xu WP, Hu JM. Risk assessment of ischemic stroke associated pneumonia. *World J Emerg Med.* (2014) 5:209–13. doi: 10.5847/wjem.j.issn.1920-8642.2014.03.009

32. Shim R, Wong CH. Ischemia, immunosuppression and infection–tackling the predicaments of post-stroke complications. *Int J Mol Sci.* (2016) 17:64. doi: 10.3390/ijms17010064

33. Prass K, Meisel C, Höflich C, Braun J, Halle E, Wolf T, et al. Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. *J Exp Med.* (2003) 198:725–36. doi: 10.1084/jem.20021098

34. Nilupul Perera M, Ma HK, Arakawa S, Howells DW, Markus R, Rowe CC, et al. Inflammation following stroke. *J Clin Neurosci.* (2006) 13:1–8. doi: 10.1016/j.jocn.2005.07.005

35. Nam KW, Kim TJ, Lee JS, Kwon HM, Lee YS, Ko SB, et al. High neutrophil-to-lymphocyte ratio predicts stroke-associated pneumonia. *Stroke.* (2018) 49:1886–92. doi: 10.1161/STROKEAHA.118.021228

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Cerebral small vessel disease combined with cerebral collaterals to predict the prognosis of patients with acute large artery atherosclerotic stroke

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Background and purpose: Besides cerebral collaterals, few studies have examined other additional factors affecting the prognosis of patients with large artery atherosclerotic (LAA) stroke. Our study aims to explore the effect of the cerebral small vessel disease (SVD) and the effects of its interaction with cerebral collaterals on the prognosis of patients with acute LAA stroke.

Method: Patients aged 18 years or older with LAA stroke within 24 h after stroke onset were consecutively enrolled. The functional outcome was determined using the modified Rankin Scale (mRS) at 3 months after stroke onset. Logistic multivariate analyses were used to identify the risk factors for stroke prognosis. Receiver operating characteristic (ROC) curves were constructed to compare the effects of cerebral collaterals and SVD on predicting the prognosis.

Results: Of the 274 enrolled patients, 174 (63.50%) were identified as having a favorable prognosis, and 100 (36.50%) were identified as having an unfavorable prognosis. After adjusting for covariates, the logistic regression analysis identified that unfavorable prognosis was related to the total SVD score (Model 1, adjusted odds ratio = 1.73, 95% CI: 1.15–2.61, $P < 0.01$; Model 2, adjusted odds ratio = 1.85, 95% CI: 1.23–2.79, $P < 0.01$) and Tan score (Model 1, adjusted odds ratio = 0.38, 95% CI: 0.23–0.64, $P < 0.01$; Model 2, adjusted odds ratio = 0.52, 95% CI: 0.33–0.82, $P < 0.01$). Compared with cerebral collaterals (AUC = 0.59; 95% CI: 0.52–0.67; $P < 0.01$) or SVD (AUC = 0.62; 95% CI: 0.56–0.69; $P < 0.01$) alone, the combination of collaterals and SVD (AUC = 0.66; 95% CI: 0.59–0.73; $P < 0.01$) had higher diagnostic value for an unfavorable prognosis, and the optimal sensitivity and specificity were 77.01 and 53.00%, respectively.

Conclusions: The total SVD burden was related to the prognosis of patients with LAA stroke. Compared with cerebral collaterals or SVD alone, cerebral collaterals combined with total SVD burden are better at predicting the prognosis of patients with acute LAA stroke.

KEYWORDS

collaterals, cerebral small vessel disease, prognosis, large artery, stroke

Introduction

Large artery atherosclerosis is responsible for ∼17% of all cases of ischemic stroke (1) and is considered a systemic disease that may lead to both cardiovascular and cerebrovascular diseases (2). It is an important cause of global disability and death in patients with large artery atherosclerotic (LAA) stroke, despite considerable progress in the treatment of acute stroke with intravenous thrombolysis (IVT) and mechanical thrombectomy (MT) (3, 4). Several risk factors related to the prognosis of LAA stroke, including traditional risk factors (such as age, hypertension, diabetes, hyperlipidemia, smoking, etc.), and the role of cerebral collaterals has been the focus of research recently (5). A retrospective study from two comprehensive stroke centers indicated that collaterals predict patient outcomes, regardless of the time last known to be normal in patients with LAA stroke who were treated with MT (6). Moreover, in patients with ischemic stroke caused by occlusion of a proximal intracranial artery who were treated with EVT, higher collateral scores are associated with a better functional outcome (7).

Recently, with the development of neuroimaging, new neuroimaging markers of cerebral small vessel disease (SVD) related to predicting the prognosis of LAA stroke have attracted increasing attention. Markers of SVD on magnetic resonance imaging (MRI) include white matter hyperintensities (WMH), lacunes, cerebral microbleeds (CMBs) and enlarged perivascular spaces (EPVS). Some studies have indicated that cerebral SVD is potentially related to ischemic stroke (8–12); However, researchers have not determined whether cerebral SVD increases the risk of a poor prognosis for LAA stroke and few studies have explored the combined effect of cerebral collaterals and SVD on the prognosis. Therefore, our study aims to explore the effect of the SVD burden and its combined effects with cerebral collaterals on the prognosis of acute LAA stroke to provide more guidance for clinical decisions.

Abbreviations: LAA, Large artery atherosclerotic; TOAST, Trial of Org 10,172 in Acute Stroke Treatment; OR, odds ratio; CI, confidence interval; ROC, receiver operating characteristic; AUC, areas under ROC curves; mRS, median modified Rankin Scale; IVT, intravenous thrombolysis; EVT, endovascular treatment; MT, mechanical thrombectomy; MRI, magnetic resonance imaging; CMBs, cerebral microbleeds; WMH, white matter hyperintensities; EPVS: enlarged perivascular spaces; CTA, CT angiography; NIHSS, National Institute of Health Stroke Scale; SVD, small vascular disease; sICH, spontaneous intracerebral hemorrhage; SD, standard deviation; HR, heart rate; LDL-C indicates low-density lipoprotein cholesterol; HDL-C indicates high-density lipoprotein cholesterol; Lp(a), lipoprotein(a); TG, triglycerides; TC, total cholesterol; Lp-PLA2, lipoprotein associated phospholipase *A2*.

Methods

Data source

Patients were screened at the Affiliated Jiangning Hospital with Nanjing Medical University. The study was approved by the hospital ethics committee. Patients aged 18 years or older with acute ischemic stroke within 24 h after stroke onset were consecutively enrolled from 9 January 2019, to 21 December 2021. The inpatient medical record system contains data on patient demographics, clinical and imaging features and treatment details. Data on patient demographics, clinical history, clinical presentation, laboratory results, treatment, follow-up examinations and outcome were collected.

Study design and population

This observational, prospective, short-term follow-up and single-center study was conducted on adults with acute LAA stroke. Participants were included if they met all of the following criteria: (1) aged 18 years or older at baseline; (2) MRI and CT angiography (CTA) were performed within 12 h of admission; (3) patients were diagnosed with LAA stroke according to the Trial of Org 10,172 in Acute Stroke Treatment (TOAST) criteria (13): (a) clinical findings include cerebral cortical impairment or brain stem or cerebellar dysfunction; (b) cortical or cerebellar lesions and brainstem or subcortical hemispheric infarcts > 1.5 cm in diameter on CT or MRI; and (c) supportive evidence by duplex imaging of a stenosis of >50% of an appropriate intracranial or extracranial artery; and (4) higher image quality was available for a subsequent neuroimaging evaluation. Patients with poor functional outcomes in the preadmission

state (mRS scores of 3–6), acute intracranial hemorrhage, acute cardiovascular diseases, pulmonary insufficiency and intracranial tumors were excluded. A total of 925 patients aged 18 years or older with ischemic stroke were enrolled in the study. All patients provided informed consent and were enrolled if all inclusion criteria and none of the exclusion criteria were met. At the end of the study, only 274 eligible patients were analyzed, and a detailed study flowchart is shown in Figure 1.

Prognostic assessment

The primary outcome was the distribution of the mRS scores at 3 months. An mRS score < 3 indicated a favorable outcome, while an mRS score >2 indicated an unfavorable outcome (14). Patients were then grouped as having a favorable prognosis (mRS score of 0–2) and an unfavorable prognosis (mRS score of 3–6) at 3 months after stroke onset. Patients with recurrent stroke, death, or symptomatic cerebral hemorrhage were eligible for inclusion and classified as having an unfavorable prognosis. Neurological deficits on admission and discharge were assessed using the National Institute of Health Stroke Scale (NIHSS) score. Neurological improvement was defined as improvement of 4 or more points on the NIHSS or an NIHSS score of 0 at discharge (15). Patients were further grouped into NIHSS (favorable) with neurological improvement and NIHSS (unfavorable) without neurological improvement.

Neuroimaging evaluation

Enrolled patients underwent a brain MRI examination with a 3.0 T scanner (Philips Medical Systems, the Netherlands) with an 8-channel receiver array head coil. Standardized parameters of the MRI sequences, including T1-weighted, T2-weighted and fluid-attenuated inversion recovery images, were obtained. The burden of SVD was graded as 0–4 based on imaging markers (WMH, lacunes, EPVS and CMBs) on MRI using established criteria (16–18). Briefly, one point represents each of the following phenomena: more than 10 EPVS in basal ganglia, presence of lacuna, periventricular WMH with a Fazekas score of 3 or deep WMH with a Fazekas score of 2 or 3, and the presence of deep CMBs. The total SVD score was calculated by summing the scores for the SVD markers listed above.

CTA examinations of the carotid and intracranial arteries were performed with a 64-slice helical CT scanner (Philips Brilliance 64, Philips Healthcare, Amsterdam, Netherlands). Cerebral collaterals were assessed on CTA by consensus by 2 neuroradiologists using the Tan scale (19): 0, absence of collaterals; (1), collaterals filling $\leq 50\%$ of the occluded territory; (2), collaterals filling $> 50\%$ but $< 100\%$ of the occluded territory; and 3, collaterals filling 100% of the occluded territory.

Statistics

Continuous data are summarized as the mean values with SDs for data with a normal distribution or the median values with interquartile ranges for data with a skewed distribution. Categorical data are presented as frequencies with proportions. A two-sample t test was used to compare continuous data. Categorical data were analyzed using the chi-square test. Logistic multivariate analyses were performed to identify the risk factors for the stroke prognosis. Receiver operating characteristic (ROC) curves were constructed to compare the effects of cerebral collaterals and SVD on predicting the prognosis of LAA stroke, and areas under ROC curves (AUCs) were calculated. All statistical analyses were performed using SPSS 25.0 software (**SPSS, Chicago, IL**).

Results

Of the 274 enrolled patients, 174 (63.50%) were identified as having a favorable prognosis, and 100 (36.50%) were identified as having an unfavorable prognosis. Patients with a favorable prognosis were younger than those with an unfavorable prognosis (66.53 ± 11.34 vs. 71.87 ± 9.90, y, $P < 0.01$). Patients with a favorable prognosis presented lower homocysteine levels (16.59 ± 7.91 vs. 20.20 ± 10.13 μmol/L, $P < 0.01$), total SVD score (2.17 ± 1.04 vs. 2.64 ± 0.95, $P < 0.01$), mRS (2.41 ± 1.39 vs. 3.57 ± 1.09, $P < 0.01$) score and NIHSS score (4.15 ± 3.90 vs. 7.73 ± 5.39, $P < 0.01$) than patients with an unfavorable prognosis at baseline. Patients with a favorable prognosis had a higher Tan score (1.57 ± 0.70 vs. 1.13 ± 0.80, $P < 0.01$) than control subjects. The details are presented in Table 1.

We subsequently compared the differences in EPVS, lacunes, WMH and CMBs between patients with and without a favorable prognosis. Patients with a favorable prognosis presented lower ratios of WMH (52.87% vs. 76.00%, $P < 0.01$) and CMBs (14.94% vs. 31.00%, $P < 0.01$) than patients with an unfavorable prognosis, but the ratios of EPVS (71.84% vs. 72.00%, $P > 0.05$) and lacunes (77.59% vs. 85%, $P > 0.05$) were not significantly different between the two groups. This result suggests that the difference in the total SVD burden is mainly derived from WMH and CMBs (Figure 2).

After adjusting for covariates, the logistic regression analysis indicated that an unfavorable prognosis was related to the total SVD score (Model 1, OR = 1.73, 95% CI: 1.15–2.61, $P < 0.01$; Model 2, OR = 1.85, 95% CI: 1.23–2.79, $P < 0.01$) and Tan score (Model 1, OR = 0.38, 95% CI: 0.23–0.64, $P < 0.01$; Model 2, OR = 0.52, 95% CI: 0.33–0.82, $P < 0.01$). Homocysteine levels (Model 1, OR = 1.07, 95% CI: 1.02–1.13, $P = 0.01$; Model 2, OR = 1.06, 95% CI: 1.01–1.12, $P = 0.02$), the mRS score (Model 1, OR = 2.20, 95% CI: 1.60–3.04, $P < 0.01$) and NIHSS score (Model 2, OR = 1.23, 95% CI: 1.12–1.34, $P < 0.01$) were also associated with an unfavorable prognosis (Tables 2, 3).

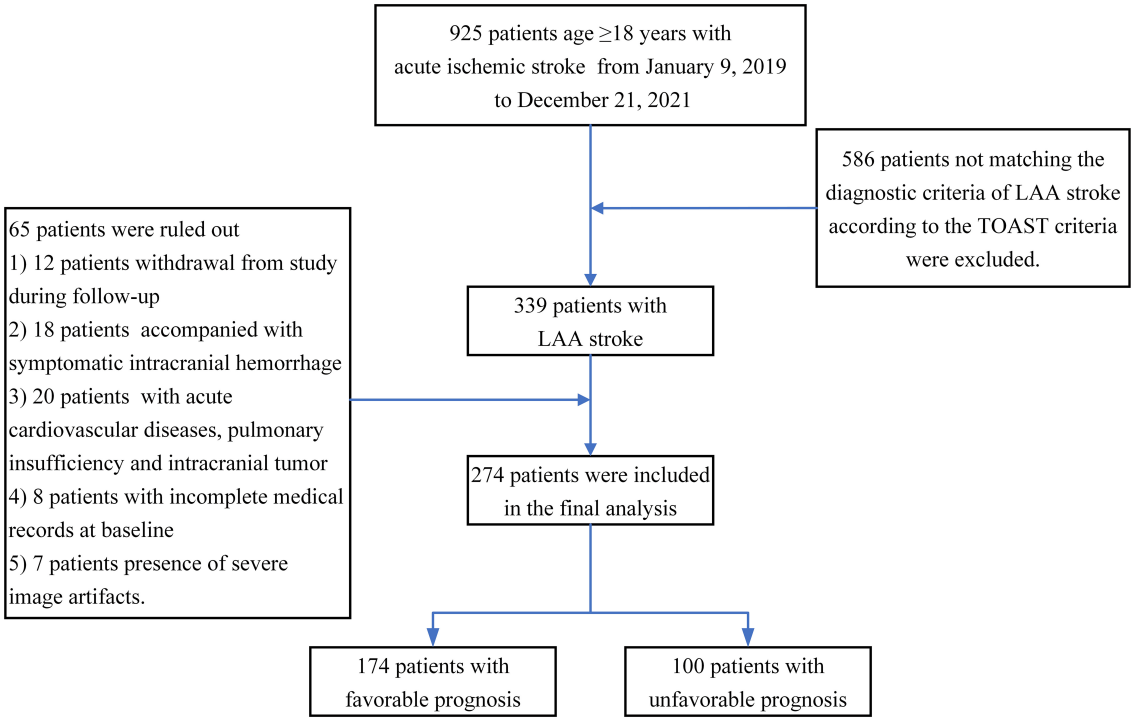

FIGURE 1
Schematic of the screening process.

Patients were stratified according to NIHSS (favorable) and NIHSS (unfavorable) scores. The Tan score and SVD score were compared between the two groups. The results showed that patients in the NIHSS (favorable) group presented significantly higher Tan scores (1.60 ± 0.79 vs. 1.32 ± 0.78, $P < 0.01$) than controls, but the comparison revealed no significant difference in SVD scores (2.23 ± 0.99 vs. 2.39 ± 1.05, $P = 0.21$) between the two groups (Figure 3).

ROC curves were created and AUCs were calculated to further evaluate the predictive values of cerebral collateral circulation and SVD in patients with an unfavorable prognosis (Figure 4). Compared with single cerebral collateral circulation (AUC = 0.59; 95% CI: 0.52–0.67; $P < 0.01$) or SVD (AUC = 0.62; 95% CI: 0.56–0.69; $P < 0.01$), the combination of collateral circulation and SVD (AUC = 0.66; 95% CI: 0.59–0.73; $P < 0.01$) has a higher diagnostic value for an unfavorable prognosis, and the optimal sensitivity and specificity were 77.01 and 53.00%, respectively.

Discussion

In the present study, (1) the total SVD burden was related to the prognosis of patients with LAA stroke, which might primarily arise from the discrepancy of WMH and CMBs. (2) Compared with cerebral collaterals or SVD alone, cerebral collaterals combined with total SVD burden are better at predicting the prognosis of patients with acute LAA stroke. (3) Moreover, cerebral collaterals, not SVD, are associated with poor short-term functional outcomes in patients with LAA stroke.

In general, occlusions of large arteries result from occlusion of the basilar artery, carotid artery and the proximal middle cerebral artery, leading to more serious outcomes, and poorly developed collaterals are often associated with a worse functional prognosis (20). Well-developed collaterals may compensate for acute cerebral hypoperfusion and prolong the time window of intravascular interventional therapy, which is important for extending the therapeutic time window in patients with acute ischemic stroke (21). Good collaterals might reduce the rate of hemorrhagic transformation after thrombolytic or endovascular therapies and the incidence of adverse events (22). Moreover, retrograde collateral flow may help to expose more portions of the thrombus to thrombolytic drugs and promote thrombus dissolution, which is very important in the therapy of acute ischemic stroke (23). Therefore, a good collateral status results in a higher recanalization rate, smaller infarct volume, and better neurological outcome.

Apart from collaterals, a more recent retrospective observational study with similar objective indicated that

TABLE 1 Clinical characteristics of patients with and without a favorable prognosis at baseline ($n = 274$).

Variables	Patients with a favorable prognosis ($n = 174$)	Patients with an unfavorable prognosis ($n = 100$)	*P* value
Age, y, mean ± SD	66.53 ± 11.34	71.87 ± 9.90	<0.01
Male, *n* (%)	113 (64.94)	59 (59.00)	0.33
HR, bpm, mean ± SD	75.25 ± 14.24	76.69 ± 14.71	0.43
Pulse pressure, mmHg, mean ± SD	67.60 ± 18.20	68.54 ± 18.33	0.83
Medical history, *n* (%)			
Hypertension	128 (73.56)	76 (76.00)	0.66
Diabetes	65 (37.36)	31 (31.00)	0.29
Coronary artery disease	15 (8.62)	13 (13.00)	0.25
Previous ischemic stroke	56 (32.18)	43 (43.00)	0.19
Atrial fibrillation	12 (6.90)	14 (14.00)	0.05
Current smoker	45 (25.86)	21 (21.00)	0.37
Current alcohol user	33 (18.97)	13 (13.00)	0.20
Laboratory findings, mean ± SD			
Troponin-I, ng/mL	0.02 ± 0.01	0.02 ± 0.02	0.37
Lp-PLA2, ng/mL	235.18 ± 131.80	273.43 ± 143.07	0.05
TC, mmol/L	4.13 ± 0.98	4.45 ± 4.97	0.41
LDL-C, mmol/L	2.58 ± 0.90	2.59 ± 1.07	0.97
HDL-C, mmol/L	1.02 ± 0.24	1.09 ± 0.85	0.31
TG, mmol/L	1.57 ± 0.96	1.46 ± 0.92	0.38
Lipoprotein (a), mg/L	261.02 ± 241.15	292.84 ± 289.89	0.34
Homocysteine, μmol/L	16.59 ± 7.91	20.20 ± 10.13	<0.01
Creatinine, μmol/L	70.46 ± 32.08	72.82 ± 26.48	0.54
Uric acid, μmol/L	313.78 ± 97.17	327.73 ± 105.60	0.27
Therapy, *n* (%)			
Statin therapy	71 (40.80)	41 (41.00)	0.98
antiplatelet therapy	97 (55.75)	55 (55.00)	0.86
IVT or EVT treatment	17 (9.77)	8 (8.00)	0.65
Related scales, mean ± SD			
Total SVD score	2.17 ± 1.04	2.64 ± 0.95	<0.01
Tan score	1.57 ± 0.70	1.13 ± 0.80	<0.01
mRS score	2.41 ± 1.39	3.57 ± 1.09	<0.01
NIHSS score	4.15 ± 3.90	7.73 ± 5.39	<0.01

Continuous variables are shown as the mean ± standard deviation (SD), categorical variables are shown as numbers combined with percentage (%).Pulse pressure means the difference between the systolic and diastolic pressures; HR, heart rate; IVT, intravenous thrombolysis; EVT, endovascular treatment; LDL-C indicates low-density lipoprotein cholesterol; HDL-C indicates high-density lipoprotein cholesterol; Lp(a), lipoprotein(a); TG, triglycerides; TC, total cholesterol; Lp-PLA2, lipoprotein associated phospholipase A2; mRS, median modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale.

atrophy and lacune were essential in evaluating stroke patients and could additionally improve the stroke outcome prediction (24). Dissimilarly, our findings suggest that the total burden of SVD was associated with the functional neurological prognosis of patients with LAA stroke, and the difference is mainly attributed to the influence of WMH and CMBs. However, result of the short-term functional outcome based on the changes of NIHSS score at admission and discharge indicated that there was no significant difference in SVD scores between the two groups (Figure 3), though the difference was not statistically significant, patients in the NIHSS (favorable) group exhibited a tendency of lower SVD scores. This is probably due to the relatively short observation time. WMH destroy white matter fiber tracts and the network architecture of the brain, and these changes in white matter tissue microstructure may lead to severe deficits related to impaired brain plasticity (25), potentially resulting in a poor outcome and stroke recurrence (26). Besides, a recent study showed that WMH burden had a dose-dependent relationship with poor collaterals and further led to poor prognosis (27). A randomized trial of large populations showed

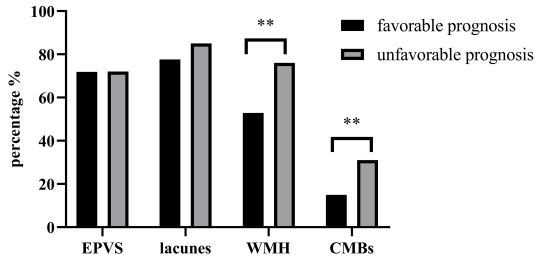

FIGURE 2
Comparison of EPVS, lacunes, WMH and CMBs between patients with and without a favorable prognosis. ** $P < 0.01$.

TABLE 2 The multivariate logistic regression analysis of Model 1.

Variables	β	Wals	OR (95% CI)	*P* value
Age	0.010	0.247	1.01 (0.97–1.05)	0.62
Atrial fibrillation	0.379	0.347	1.46 (0.42–5.14)	0.56
Lp-PLA2 level	0.001	0.006	1.00 (0.99–1.00)	0.94
Homocysteine level	0.066	6.490	1.07 (1.02–1.13)	0.01
Total SVD score	0.549	6.884	1.73 (1.15–2.61)	<0.01
Tan score	−0.959	13.458	0.38 (0.23–0.64)	<0.01
mRS score	0.790	22.995	2.20 (1.60–3.04)	<0.01

OR, odds ratio; CI, confidence interval. Model 1 adjust for Age, Atrial fibrillation, Lp-PLA2, Homocysteine and mRS score at baseline.

TABLE 3 The multivariate logistic regression analysis of Model 2.

Variables	β	Wals	OR (95% CI)	*P* value
Age	0.020	0.968	1.02 (0.98–1.06)	0.33
Atrial fibrillation	0.567	0.692	1.76 (0.46–6.72)	0.41
Lp-PLA2 level	0.001	0.004	1.00 (0.99–1.00)	0.95
Homocysteine level	0.060	5.597	1.06 (1.01–1.12)	0.02
Total SVD score	0.616	8.714	1.85 (1.23–2.79)	<0.01
Tan score	−0.659	7.873	0.52 (0.33–0.82)	<0.01
NIHSS score	0.206	20.367	1.23 (1.12–1.34)	<0.01

OR, odds ratio; CI, confidence interval. Model 2 adjust for Age, Atrial fibrillation, Lp-PLA2, Homocysteine and NIHSS score at baseline.

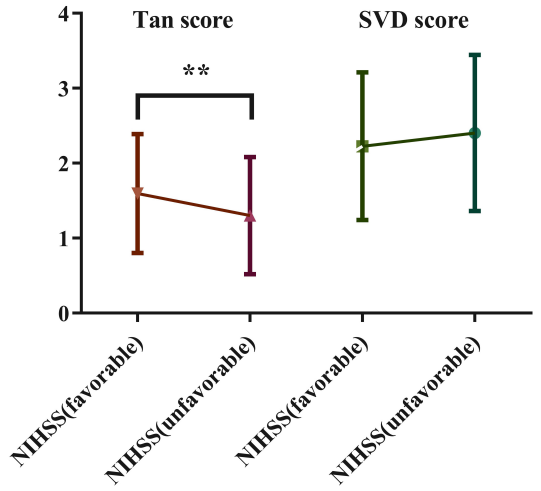

FIGURE 3
Comparison of the Tan score and SVD score between the two groups stratified based on the NIHSS score.

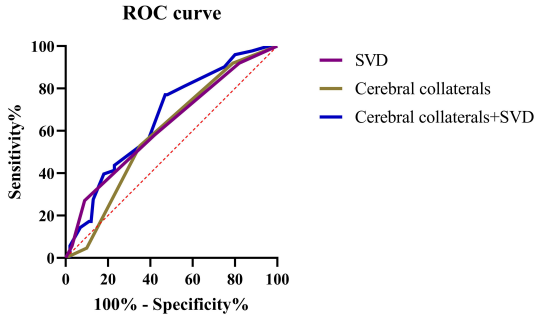

FIGURE 4
ROC curves for the cerebral collateral circulation and SVD in predicting the unfavorable prognosis of patients with LAA stroke.

that WMH was a risk factor for first-ever and recurrent stroke in the general population (28). A study of 307 patients illustrated that the large artery disease group had a higher prevalence of WMH than the other groups (9). Furthermore, several studies have shown that CMBs are associated with hemorrhagic stroke and significantly increase the risk of ischemic stroke, which also substantially affects the neurological function and prognosis of patients with LAA stroke (29, 30). The poor prognosis associated with CMBs may be related to hemorrhagic transformation after ischemic stroke. The results of a multicenter prospective cohort study indicated that patients with multiple CMBs have a six times higher risk of recurrent stroke than those without CMBs and exhibit an increased fatality rate of stroke (31). A population-based cohort study reported that an increase in the mean carotid intima-media thickness, a marker of LAA, was related to an increased risk of CMBs, especially in the deep and infratentorial brain regions (8). EPVS and lacunes may also be related to the prognosis of patients with LAA stroke, although our present study did not observe significant differences in these parameters. EPVS are strongly associated with age and may correlate with the functional outcome and prognosis of patients with LAA stroke. The pathological examination of EPVS revealed that the brain tissue surrounding the lesions was destroyed, accompanied by reactive gliosis, which may contribute to neurological deficits (32). Although lacunes are less severe and have better short-term

physical outcomes, patients with the condition are at increased risk of recurrence and neurological impairment over time (33), and lacunes and LAA stroke share common risk factors and influence each other (10). Therefore, more research is needed to prove the correlation between SVD and the prognosis of LAA stroke; nevertheless, research on the mechanism needs to continue.

The current study has several strengths and limitations. First, this study is one of the first to focus on the relationship between the total SVD burden and prognosis of patients with LAA stroke. Second, the study was performed with a short-term follow-up and dynamic observation of progression. Third, we explored the combined effects of SVD burden and cerebral collaterals on the prognosis of patients with acute LAA stroke. Our study is limited by a relatively small sample size and enrollment of patients at a single center, and the study is based on clinical research and does not explore the underlying mechanisms. Moreover, despite the widespread approval of intravenous thrombolysis treatment and endovascular treatment for patients with acute ischemic stroke, the narrow therapeutic windows limit their clinical application, and we did not discuss the effects of different treatments on the outcome. Therefore, further multicenter and in-depth mechanistic studies are needed to overcome the aforementioned limitations.

Taken together, our findings suggest that the total SVD burden was related to the prognosis of patients with LAA stroke, which might primarily arise from the differences in WMH and CMBs. Compared with cerebral collaterals or SVD alone, cerebral collaterals combined with total SVD burden have a better value to predict the prognosis of patients with acute LAA stroke. Moreover, cerebral collaterals, not SVD, are associated with poor short-term functional outcomes of patients with LAA stroke.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Affiliated Jiangning Hospital with Nanjing Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XC contributed to the study design. CW, TS, XT, and XY performed the data collection. XC, CW, TS, and YG were responsible for data analysis and imaging evaluation. CW wrote the manuscript. All authors approved the final manuscript for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Banerjee C, Turan TN. Large artery atherosclerosis: extracranial and intracranial. *Semin Neurol.* (2017) 37:307–15. doi: 10.1055/s-0037-1603588

2. Baldassarre D, De Jong A, Amato M, Werba JP, Castelnuovo S, Frigerio B, et al. Carotid intima-media thickness and markers of inflammation, endothelial damage and hemostasis. *Ann Med.* (2008) 40:21–44. doi: 10.1080/07853890701645399

3. Pico F, Lapergue B, Ferrigno M, Rosso C, Meseguer E, Chadenat ML, et al. Effect of in-hospital remote ischemic perconditioning on brain infarction growth and clinical outcomes in patients with acute ischemic stroke: the RESCUE BRAIN randomized clinical trial. *JAMA Neurol.* (2020) 77:725–34. doi: 10.1001/jamaneurol.2020.0326

4. Kim D, Park JM, Kang K, Cho YJ, Hong KS, Lee KB, et al. Dual vs. mono antiplatelet therapy in large atherosclerotic stroke. *Stroke.* (2019) 50:1184–92. doi: 10.1161/STROKEAHA.119.024786

5. Piedade GS, Schirmer CM, Goren O, Zhang H, Aghajanian A, Faber JE, et al. Cerebral collateral circulation: a review in the context of ischemic stroke and mechanical thrombectomy. *World Neurosurg.* (2019) 122:33–42. doi: 10.1016/j.wneu.2018.10.066

6. Sharma R, Llinas RH, Urrutia V, Marsh EB. Collaterals predict outcome regardless of time last known normal. *J Stroke Cerebrovasc Dis.* (2018) 27:971–7. doi: 10.1016/j.jstrokecerebrovasdis.2017.10.037

7. Berkhemer OA, Jansen IG, Beumer D, Fransen PS, van den Berg LA, Yoo AJ, et al. Collateral status on baseline computed tomographic angiography and intra-arterial treatment effect in patients with proximal anterior circulation stroke. *Stroke.* (2016) 47:768–76. doi: 10.1161/STROKEAHA.115.011788

8. Romero JR, Preis SR, Beiser A, DeCarli C, D'Agostino RB, Wolf PA, et al. Carotid atherosclerosis and cerebral microbleeds: the framingham heart study. *J Am Heart Assoc.* (2016) 5:e002377. doi: 10.1161/JAHA.115.002377

9. Lee SJ, Kim JS, Lee KS, An JY, Kim W, Kim YI, et al. The leukoaraiosis is more prevalent in the large artery atherosclerosis stroke subtype among Korean patients with ischemic stroke. *BMC Neurol.* (2008) 8:31. doi: 10.1186/1471-2377-8-31

10. Kloppenborg RP, Nederkoorn PJ, Grool AM, De Cocker LJ, Mali WP, van der Graaf Y, et al. Do lacunar infarcts have different aetiologies? Risk factor profiles of lacunar infarcts in deep white matter and basal ganglia: the second manifestations of arterial disease-magnetic resonance study. *Cerebrovasc Dis.* (2017) 43:161–8. doi: 10.1159/000454782

11. Zhu YC, Tzourio C, Soumaré A, Mazoyer B, Dufouil C, Chabriat H. Severity of dilated Virchow-Robin spaces is associated with age, blood pressure, and MRI markers of small vessel disease: a population-based study. *Stroke.* (2010) 41:2483–90. doi: 10.1161/STROKEAHA.110.591586

12. Yu X, Yu Y, Wei C, Wang L, Jiang J, Zhang R, et al. Association between small dense low-density lipoprotein cholesterol and neuroimaging markers of cerebral small vessel disease in middle-aged and elderly Chinese populations. *BMC Neurol.* (2021) 21:436. doi: 10.1186/s12883-021-02472-6

13. Adams HP Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke.* (1993) 24:35–41. doi: 10.1161/01.STR.24.1.35

14. Ebinger M, Siegerink B, Kunz A, Wendt M, Weber JE, Schwabauer E, et al. Association between dispatch of mobile stroke units and functional outcomes among patients with acute ischemic stroke in Berlin. *Jama.* (2021) 325:454–66. doi: 10.1001/jama.2020.26345

15. Nagaraja N, Warach S, Hsia AW, Adams HP Jr., Auh S, Latour LL, et al. Association between neurologic improvement with decline in blood pressure and recanalization in stroke. *JAMA Neurol.* (2014) 71:1555–8. doi: 10.1001/jamaneurol.2014.2036

16. Lau KK, Li L, Schulz U, Simoni M, Chan KH, Ho SL, et al. Total small vessel disease score and risk of recurrent stroke: validation in 2 large cohorts. *Neurology.* (2017) 88:2260–7. doi: 10.1212/WNL.0000000000004042

17. Du H, Wilson D, Ambler G, Banerjee G, Shakeshaft C, Cohen H, et al. Small vessel disease and ischemic stroke risk during anticoagulation for atrial fibrillation after cerebral ischemia. *Stroke.* (2021) 52:91–9. doi: 10.1161/STROKEAHA.120.029474

18. Xiao L, Lan W, Sun W, Dai Q, Xiong Y, Li L, et al. Chronic kidney disease in patients with lacunar stroke: association with enlarged perivascular spaces and total magnetic resonance imaging burden of cerebral small vessel disease. *Stroke.* (2015) 46:2081–6. doi: 10.1161/STROKEAHA.114.008155

19. Tan JC, Dillon WP, Liu S, Adler F, Smith WS, Wintermark M. Systematic comparison of perfusion-CT and CT-angiography in acute stroke patients. *Ann Neurol.* (2007) 61:533–43. doi: 10.1002/ana.21130

20. Roman LS, Menon BK, Blasco J, Hernandez-Perez M, Davalos A, Majoie C, et al. Imaging features and safety and efficacy of endovascular stroke treatment: a meta-analysis of individual patient-level data. *Lancet Neurol.* (2018) 17:895–904. doi: 10.1016/S1474-4422(18)30242-4

21. Zhang C, Ling C-L, Pang L, Wang Q, Liu J-X, Wang B-S, et al. Direct macromolecular drug delivery to cerebral ischemia area using neutrophil-mediated nanoparticles. *Theranostics.* (2017) 7:3260–75. doi: 10.7150/thno.19979

22. Bang OY, Saver JL, Kim SJ, Kim GM, Chung CS, Ovbiagele B, et al. Collateral flow averts hemorrhagic transformation after endovascular therapy for acute ischemic stroke. *Stroke.* (2011) 42:2235–9. doi: 10.1161/STROKEAHA.110.604603

23. Iwasawa E, Ichijo M, Ishibashi S, Yokota T. Acute development of collateral circulation and therapeutic prospects in ischemic stroke. *Neural Regen Res.* (2016) 11:368–71. doi: 10.4103/1673-5374.179033

24. Zhou JY, Shi YB, Xia C, Lu CQ, Tang TY, Lu T, et al. Beyond collaterals: brain frailty additionally improves prediction of clinical outcome in acute ischemic stroke. *Eur Radiol.* (2022). doi: 10.1007/s00330-022-08792-6

25. Fierini F, Poggesi A, Pantoni L. Leukoaraiosis as an outcome predictor in the acute and subacute phases of stroke. *Expert Rev Neurother.* (2017) 17:963–75. doi: 10.1080/14737175.2017.1371013

26. Schulz UG, Gruter BE, Briley D, Rothwell PM. Leukoaraiosis and increased cerebral susceptibility to ischemia: lack of confounding by carotid disease. *J Am Heart Assoc.* (2013) 2:e000261. doi: 10.1161/JAHA.113.000261

27. Lin MP, Brott TG, Liebeskind DS, Meschia JF, Sam K, Gottesman RF. Collateral recruitment is impaired by cerebral small vessel disease. *Stroke.* (2020) 51:1404–10. doi: 10.1161/STROKEAHA.119.027661

28. Ederle J, Davagnanam I, van der Worp HB, Venables GS, Lyrer PA, Featherstone RL, et al. Effect of white-matter lesions on the risk of periprocedural stroke after carotid artery stenting versus endarterectomy in the International Carotid Stenting Study (ICSS): a prespecified analysis of data from a randomised trial. *Lancet Neurol.* (2013) 12:866–72. doi: 10.1016/S1474-4422(13)70135-2

29. Charidimou A, Turc G, Oppenheim C, Yan S, Scheitz JF, Erdur H, et al. Microbleeds, cerebral hemorrhage, and functional outcome after stroke thrombolysis. *Stroke.* (2017) 48:2084–90. doi: 10.1161/STROKEAHA.116.012992

30. Wilson D, Charidimou A, Ambler G, Fox ZV, Gregoire S, Rayson P, et al. Recurrent stroke risk and cerebral microbleed burden in ischemic stroke and TIA: a meta-analysis. *Neurology.* (2016) 87:1501–10. doi: 10.1212/WNL.0000000000003183

31. Lim JS, Hong KS, Kim GM, Bang OY, Bae HJ, Kwon HM, et al. Cerebral microbleeds and early recurrent stroke after transient ischemic attack: results from the Korean transient ischemic attack expression registry. *JAMA Neurol.* (2015) 72:301–8. doi: 10.1001/jamaneurol.2014.3958

32. Loos CM, Klarenbeek P, van Oostenbrugge RJ, Staals J. Association between perivascular spaces and progression of white matter hyperintensities in lacunar stroke patients. *PLoS ONE.* (2015) 10:e0137323. doi: 10.1371/journal.pone.0137323

33. van Dijk AC, Fonville S, Zadi T, van Hattem AM, Saiedie G, Koudstaal PJ, et al. Association between arterial calcifications and nonlacunar and lacunar ischemic strokes. *Stroke.* (2014) 45:728–33. doi: 10.1161/STROKEAHA.113.003197

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High serum amyloid A predicts risk of cognitive impairment after lacunar infarction: Development and validation of a nomogram

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Background: Post-stroke cognitive impairment (PSCI) after lacunar infarction was worth attention in recent years. An easy-to-use score model to predict the risk of PSCI was rare. This study aimed to explore the association between serum amyloid A (SAA) and cognitive impairment, and it also developed a nomogram for predicting the risk of PSCI in lacunar infarction patients.

Methods: A total of 313 patients with lacunar infarction were enrolled in this retrospective study between January 2021 and December 2021. They were divided into a training set and a validation set at 70%:30% randomly. The Chinese version of the Mini-Mental State Examination (MMSE) was performed to identify cognitive impairment 3 months after discharge. Univariate and multivariate logistic regression analyses were used to determine the independent risk factors for PSCI in the training set. A nomogram was developed based on the five variables, and the calibration curve and the receiver operating characteristic (ROC) curve were drawn to assess the predictive ability of the nomogram between the training set and the validation set. The decision curve analysis (DCA) was also conducted in both sets.

Results: In total, 52/313 (16.61%) participants were identified with PSCI. The SAA levels in patients with PSCI were significantly higher than non-PSCI patients in the training set ($P < 0.001$). After multivariate analysis, age, diabetes mellitus, white blood count, cystatin C, and SAA were independent risk predictors of PSCI. The nomogram demonstrated a good discrimination performance between the training set (AUC = 0.860) and the validation set (AUC = 0.811). The DCA showed that the nomogram had a well clinical utility in the two sets.

Conclusion: The increased SAA is associated with PSCI in lacunar infarction patients, and the nomogram developed with SAA can increase prognostic information for the early detection of PSCI.

KEYWORDS

serum amyloid A, cognitive impairment, lacunar infarction, nomogram, prediction model

Introduction

Stroke is the second leading cause of death around the world, which endangers the life quality and safety of patients due to high morbidity and high disability (1). Especially, post-stroke cognitive impairment (PSCI) is the most common critical issue concerning population health and the burden on caregivers in an aging society (2, 3). The survivors of stroke have an increased risk of progressive cognitive impairment, even minor stroke (4). The presence of PSCI also affects the treatment of stroke patients in turn and nearly increases two-fold the risk of adverse outcomes (5). Lacunar infarction accounts for about 25% of stroke patients, and approximately half of the patients develop cognitive impairment in subsequent years (6). With the increased duration of ischemia and decreased mortality with minor stroke, the number of patients with PSCI will be increased (7). Therefore, it is crucial to realize the associations between PSCI and predictive factors, especially in high-risk patients.

The available evidence confirms that hypertension, diabetes, smoking, and other vascular risk factors are highly correlated with the increased risks of PSCI (8). Besides, frontal lobe dysfunction and brain gray matter atrophy were also associated with cognitive impairment in lacunar patients (9). Recently, the developed SIGNAL2 scale and CHANGE scale based on the clinical characteristics and neuroimaging variables were useful to identify PSCI after stroke (10, 11). However, the scale depends on the neurologist's appraising of the MRI and was difficult to promote in the community. In addition, the expression of biomarkers, such as interleukin 6 (IL-6), C-reactive protein (CRP), serum uric acid (UA), and malondialdehyde (MDA), was also independently associated with PSCI in increasing studies (12, 13). Therefore, it is necessary to explore reliable biomarkers to identify patients at higher risk of PSCI easily and conveniently.

Serum amyloid A (SAA) protein is a protein of only 104 amino acids and is mainly synthesized in the liver (14). As an acute phase protein (APP), SAA was significantly upregulated in acute and chronic inflammatory conditions (such as trauma, infection, and ischemia), which was in response to the elevator of the inflammatory cytokines IL-6 and tumor necrosis factor (TNF)-α during the acute-phase response (15). Schweizer et al.'s study (16) found that SAA was a novel blood biomarker, which was independent to predict post-stroke infection among ischemic stroke patients. A recent study confirmed that the increased secretion of SAA could activate the inflammatory response of microglia and stimulate NLRP3 activation in microglia after stroke, which induced neurological inflammation (17). Although studies have indicated that elevated SAA was associated with short-term cognitive impairment after ischemic stroke (18), the role of SAA has not yet been evaluated in the cognitive impairment after lacunar infarction.

Therefore, we aimed to develop and verify a nomogram to predict the risk of PSCI in lacunar infarction patients, which will be convenient for clinicians to identify cognitive disorders early and conveniently.

Methods

Study design and patients

This study retrospectively enrolled patients with lacunar infarction who were hospitalized at the Second Affiliated Hospital of Wannan Medical College between January 2021 and December 2021. All patients were admitted to the hospital within 7 days of symptom onset with a National Institute of Health Stroke Scale (NIHSS) score ≤3. This study was approved by the Institutional Review Board of the Second Affiliated Hospital of Wannan Medical College (No. WYEFYLS202205) and conducted by the guiding principles of the Declaration of Helsinki.

The inclusion criteria were as follows: (1) age >18 years; (2) patients who met the diagnostic criteria for lacunar infarction confirmed on cranial computed tomography (CT) scan or magnetic resonance imaging (MRI) examination; and (3) patients who were able to complete scale measurements. The patients were excluded if they had any of the following: (1) previous diagnosis with dementia or Alzheimer's disease; (2) cardioembolic source or large-vessel diseases (large artery stenosis >50%); (3) patients with incomplete clinical data; (4) had been treated with intervention and thrombolytic therapy; and (5) loss to follow-up.

Finally, a total of 313 patients were enrolled in this study and were randomly divided into a training set and a validation set at 70%:30% (Figure 1).

Baseline clinical characteristics collection

The baseline clinical characteristics were collected within 24 h of admission from the health information system (HIS). The first part was demographic characteristics, such as age, gender, education time, initial National Institutes of Health Stroke Scale (NIHSS) score, and vital signs (blood pressure, heart rate, temperature, breath rate). The second part was comorbidities (diabetes mellitus, atrial fibrillation, coronary heart disease, hypertension, tumor, and chronic obstructive pulmonary disease). The third part was laboratory examinations, which included red blood count, white blood count, hemoglobin, platelet, cystatin C, and total cholesterol.

Serum amyloid A

All blood samples were collected in the morning from all patients within 24 h of admission, and all patients were fasting for more than 8 h. The blood samples were collected with

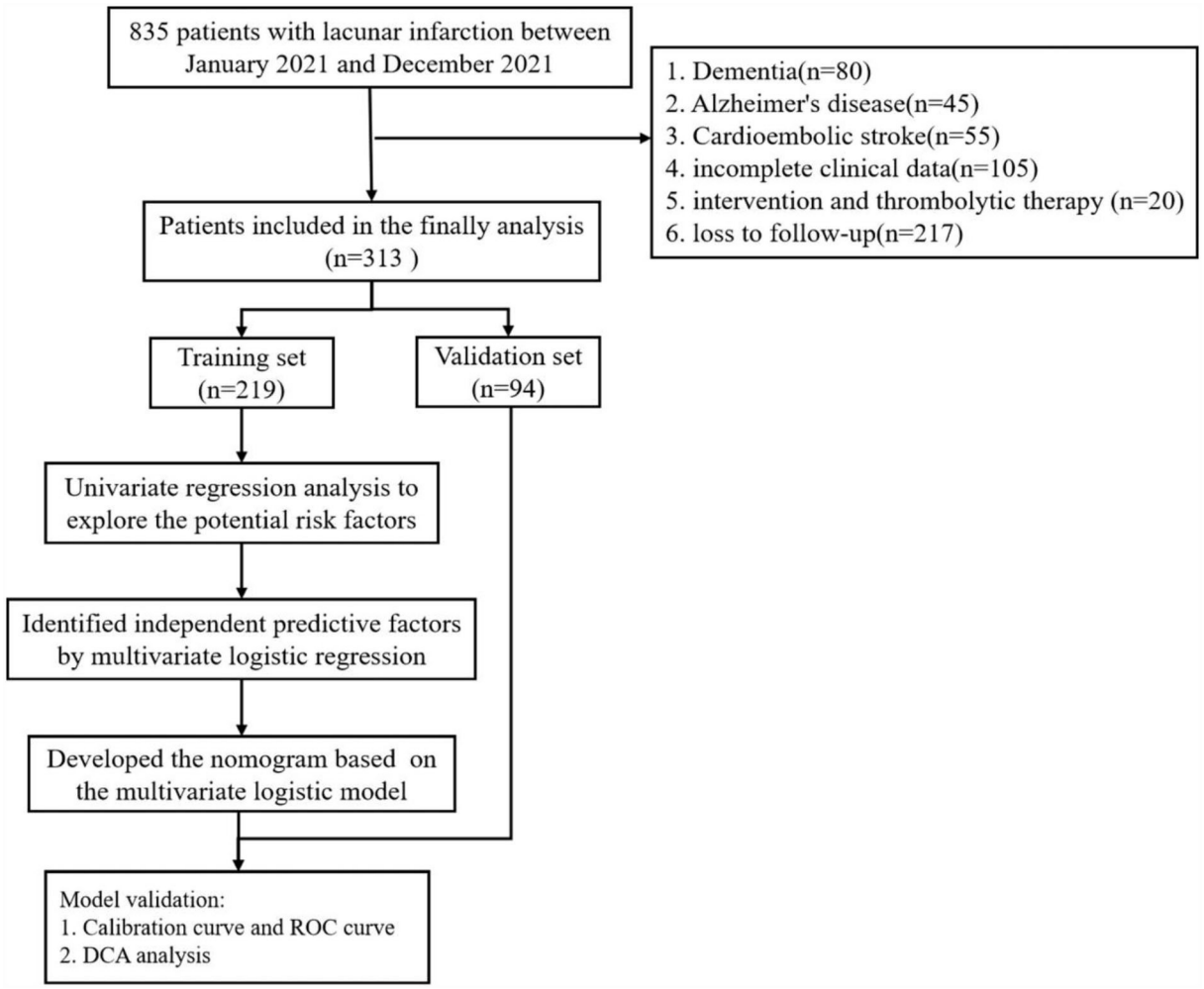

FIGURE 1
Schematic of patient's inclusion process and flowchart with the study.

heparin anticoagulant tubes and centrifuged at 1,000 *g* for 5 min to separate serum. The serum amyloid A level was measured by the latex-enhanced immunoturbidimetric method with the automatic biochemical analyzer (Hitachi-7600). The reference value of serum amyloid A ranged from 0 to 10.00 mg/L. All sample testing was performed by laboratory personnel blinded to the study.

Lacunar infarction definition

All patients routinely completed MRI examination or CT scanning. Lacunar infarction was defined as lacunar infarcts <20 mm in the subcortical or brain stem verified by CT or MRI (19, 20). Radiologic images were read by a radiologist and were reviewed by an experienced neurologist.

Assessment of cognitive function

Cognitive function was evaluated by experienced neurological physicians using the Chinese version of the Mini-Mental State Examination (MMSE) scale 3 months after lacunar infarction (21). The MMSE scale was widely used in cognitive function assessment in China (22, 23). The total score of MMSE was 30, and the lower scores indicated the worse cognitive function. According to the previous studies, a score of MMSE <24 was considered as a cognitive impairment in this study (24, 25).

Statistical analysis

The statistical analysis was performed by using SPSS 25.0 and R software (version 3.6.2). Continuous variables

with normal distribution were expressed as mean ± standard deviation, and the interquartile range were not normally distributed. Categorical variables were presented as frequency (percentage).

Univariate analysis was applied to screen the potential risk factors for PSCI. To determine independent risk factors for PSCI in lacunar infarction patients, the variables with $P < 0.05$ in the univariate analysis were included in the multivariate logistic regression model. Then, the predictive nomogram was developed based on the independent risk factors by using the "rms" package in R software. The receiver operating characteristic (ROC) curve was generated, and the area under the curve (AUC) of ROC was used to evaluate the discrimination of the nomogram. Meanwhile, the AUC of the nomogram was compared with all the independent risk variables. Finally, the net benefit of the decision curve analysis (DCA) was drawn to estimate the clinical value of the nomogram in the training set and validation set, respectively. Linear regression analysis was used to analyze the association between the serum SAA and the MMSE score. The statistical significance for all variables was set at $P < 0.05$ (two-sided tests), and the regression coefficients reported 95% confidence intervals (CI).

Results

Clinical characteristics of patients in the training and validation set

A total of 313 patients with lacunar infarction were included in this study, of which 219 patients were in the training set and 94 patients were in the validation set; 38 (17.40%) patients in the training set and 14 (14.90%) patients in the validation set were diagnosed with PSCI, respectively. The characteristics of patients in the training set and the validation set were no significant differences in Table 1 ($P > 0.05$).

Baseline characteristics of patients stratified by PSCI in the training set

Descriptive analysis revealed that significant differences between the two groups were confirmed for age ($P < 0.001$), years of education ($P = 0.001$), diabetes mellitus ($P = 0.006$), white blood count ($P < 0.001$), APTT ($P = 0.036$), cystatin C ($P < 0.001$), ApoA1 ($P = 0.005$), and serum amyloid A ($P < 0.001$) in Table 2.

Identifying the independent risk factors for PSCI

All the potential risk factors ($P < 0.05$) in the univariate regression analysis were included in the multivariate regression model. Multivariate logistic regression analysis revealed that age (OR = 1.099, 95%CI: 1.012–1.193, $P = 0.025$), diabetes mellitus (OR = 2.679, 95% CI: 1.029–6.976, $P = 0.044$), white blood count (OR = 1.271, 95% CI: 1.028–1.572, $P = 0.027$), cystatin C (OR = 3.118, 95% CI:1.053–9.228, $P = 0.040$), and serum amyloid A (OR = 1.031, 95% CI: 1.009–1.054, $P = 0.007$) were independent risk predictors of PSCI in patients with lacunar infarction (Table 3).

The predictive nomogram development

The nomogram was developed for predicting the risk of PSCI probability based on the results from the multivariate logistic model, which included five variables (Figure 2). A vertical line was drawn up to the "Point" axis to calculate the score of each variable, and the total score was summarized by the preliminary scores. The total score was located on the "Total Points" axis, and then, the predicted risk of PSCI probability could be located on the bottom axis.

The performance of the nomogram

The calibration curve of the nomogram for the probability of PSCI demonstrated a good agreement between prediction and observation for both sets (Figure 3). The Hosmer-Lemeshow H test indicated that the model did not depart from perfect fit, which had non-statistical significance in the training set ($P = 0.336$) and validation set ($P = 0.399$).

The AUC for the nomogram was 0.860 (95% CI: 0.794–0.925) in the training set (Figure 4A) and was confirmed to be 0.811 (95% CI: 0.686–0.936) through internal validation in the validation set (Figure 4B), which demonstrated that the nomogram had a greater discriminatory performance. In addition, the discrimination ability of the nomogram calculated by the AUC was superior to the other risk factors in the training set: age (0.734, 95%CI: 0.643–0.824, $P < 0.001$), diabetes mellitus (0.599, 95% CI: 0.493–0.704, $P = 0.056$), white blood count (0.691, 95% CI: 0.592–0.791, $P < 0.001$), cystatin C (0.711, 95% CI: 0.625–0.797, $P < 0.001$), and serum amyloid A (0.760, 95% CI: 0.674–0.846, $P < 0.001$; Figure 4A).

Clinical use

Moreover, the DCA was used to assess the clinical validity of the nomogram, which indicated the predictive nomogram to be clinically useful (Figure 5).

TABLE 1 Baseline and clinical characteristics of lacunar infarction patients in the training set and validation set.

Variables	Total ($n = 313$)	Training set ($n = 219$)	Validation set ($n = 94$)	$t/z/\chi^2$	*P*-value
Demographic characteristics					
Age, years, Mean ± SD	69.57 ± 10.83	69.62 ± 10.81	69.45 ± 10.95	0.127	0.899
Male, *n* (%)	153 (48.90)	102 (46.60)	51 (54.30)	1.552	0.213
Education, years, median (IQR)	5.00 (3.00, 6.00)	5.00 (3.00, 6.00)	5.00 (3.00, 8.00)	−0.955	0.340
Comorbidity					
Diabetes mellitus, *n* (%)	65 (20.80)	45 (20.50)	20 (21.30)	0.021	0.884
Atrial fibrillation, *n* (%)	43 (13.70)	25 (11.40)	18 (19.10)	3.319	0.068
Coronary heart disease, *n* (%)	53 (16.90)	41 (18.70)	12 (12.80)	1.658	0.198
Hypertension, *n* (%)	153 (48.90)	113 (51.60)	40 (42.60)	2.153	0.142
COPD, *n* (%)	42 (13.40)	33 (15.10)	9 (9.60)	1.709	0.191
Tumor, *n* (%)	33 (10.50)	25 (11.40)	8 (8.50)	0.588	0.443
Laboratory examination					
WBC, $\times 10^9$/L, median (IQR)	5.47 (4.52, 6.80)	5.46 (4.54, 6.91)	5.49 (4.47, 6.46)	−1.155	0.248
RBC, $\times 10^{12}$/L, median (IQR)	4.20 (3.82, 4.55)	4.19 (3.81, 4.56)	4.23 (3.90, 4.54)	−0.306	0.760
HB, g/L, median (IQR)	127.00 (115.00, 139.00)	127.00 (114.00, 139.00)	127.00 (117.00, 140.00)	−0.539	0.590
PLT, $\times 10^{12}$/L, median (IQR)	164.00 (135.00, 212.00)	166.00 (140.00, 208.00)	159.50 (123.75, 216.50)	−1.292	0.196
PT, s, median (IQR)	11.00 (10.40, 12.05)	10.90 (10.40, 12.10)	11.10 (10.50, 11.93)	−0.399	0.690
APTT, s, median (IQR)	25.30 (22.80, 28.60)	25.00 (22.80, 28.40)	25.50 (22.80, 28.78)	−0.625	0.532
FIB, g/L, median (IQR)	2.40 (2.10, 2.96)	2.50 (2.10, 3.00)	2.30 (2.00, 2.90)	−1.315	0.188
CysC, mg/L, median (IQR)	1.03 (0.87, 1.23)	1.03 (0.87, 1.23)	1.02 (0.87,1.22)	−0.591	0.555
ApoA1, g/L, median (IQR)	1.32 (1.12, 1.59)	1.33 (1.12, 1.59)	1.30 (1.11,1.55)	−0.559	0.576
ApoB, g/L, median (IQR)	0.83 (0.66, 1.02)	0.81 (0.63, 1.03)	0.88 (0.71, 1.02)	−1.292	0.196
SAA, mg/L, median (IQR)	9.00 (4.50, 19.50)	9.30 (4.80, 21.10)	8.10 (3.75, 17.20)	−1.249	0.212
TC, mmol/L, median (IQR)	4.09 (3.40, 4.87)	4.09 (3.39, 4.84)	4.09 (3.39, 4.96)	−0.290	0.772
LDL, mmol/L, median (IQR)	2.15 (1.51, 22.74)	2.12 (1.49, 2.71)	2.19 (1.51, 2.76)	−0.458	0.647
TG, mmol/L, median (IQR)	1.16 (0.83, 1.64)	1.18 (0.84, 1.62)	1.14 (0.74, 1.67)	−0.940	0.347
ALT, μ/L, median (IQR)	14.00 (10.00, 21.00)	15.00 (10.00, 21.00)	14.00 (9.75, 20.25)	−0.472	0.637
AST, μ/L, median (IQR)	22.00 (18.00, 27.00)	22.00 (18.00, 27.00)	22.00 (17.00, 27.00)	−0.450	0.653
ALB, g/L, median (IQR)	40.30 (37.60, 43.20)	40.10 (37.30, 43.00)	40.70 (38.30, 43.33)	−1.145	0.252
GLO, g/L, median (IQR)	28.10 (25.10, 30.95)	28.10 (25.10, 30.80)	27.85 (24.73, 31.40)	−0.292	0.771
TBIL, μmol/L, median (IQR)	11.70 (9.20, 14.95)	11.70 (9.10, 14.80)	11.65 (9.75, 16.20)	−0.287	0.774
GLU, mmol/L, median (IQR)	5.52 (4.77, 7.22)	5.61 (4.79, 7.54)	5.40 (4.64, 6.88)	−1.563	0.118
CREA, μmol/L, median (IQR)	73.00 (62.00, 87.30)	72.00 (62.00, 88.00)	74.90 (63.00, 86.25)	−0.401	0.688
CK, mmol/L, median (IQR)	77.00 (55.50, 109.50)	81.00 (58.00, 117.00)	71.50 (52.75, 94.50)	−1.839	0.066
UA, mmol/L, median (IQR)	332.80 (275.00, 402.00)	330.00 (275.00, 392.30)	344.00 (275.25, 418.25)	−1.046	0.296
MMSE, score, median (IQR)	26.00 (25.00, 28.00)	26.00 (25.00, 28.00)	27.00 (25.00, 28.00)	−1.611	0.107
Subtypes of lacunar infarction				6.418	0.268
Pure motor hemiparesis, *n* (%)	47 (15.00)	36 (16.40)	11 (11.70)		
Pure sensory stroke, *n* (%)	83 (26.50)	64 (29.20)	19 (20.20)		
Sensorimotor syndrome, *n* (%)	55 (17.60)	38 (17.40)	17 (18.10)		
Ataxic hemiparesis, *n* (%)	40 (12.80)	27 (12.30)	13 (13.80)		
Dysarthria clumsy, *n* (%)	36 (11.50)	23 (10.50)	13 (13.80)		
Atypical lacunar syndromes, *n* (%)	52 (16.60)	31 (14.20)	21 (22.30)		

SD, standard deviation; IQR, interquartile range; COPD, chronic obstructive pulmonary disease; RBC, red blood count; WBC, white blood count; HB, hemoglobin; PLT, platelet; CysC, cystatin C; TC, total cholesterol; LDL, low-density lipoprotein; TG, triglyceride; ALB, albumin; TBIL, total bilirubin; CREA, creatinine; BUN, blood urea nitrogen; UA, uric acid; ALT, alanine transaminase; SAA, serum amyloid A; MMSE, Mini-Mental State Examination.

TABLE 2 Baseline and clinical characteristics of lacunar infarction patients in the training set.

Variables	Total ($n = 219$)	PSCI ($n = 38$)	Non-PSCI ($n = 181$)	$t/z/\chi^2$	*P*-value
Demographic characteristics					
Age, years, Mean ± SD	69.62 ± 10.81	76.47 ± 10.07	68.18 ± 10.42	4.486	<0.001
Male, *n* (%)	102 (46.60)	16 (42.10)	86 (47.50)	0.369	0.543
Education, years, median (IQR)	5.00 (3.00, 6.00)	3 (0.00, 5.00)	5.00 (3.00, 6.00)	−3.350	0.001
Comorbidity					
Diabetes mellitus, *n* (%)	45 (20.50)	14 (36.80)	31 (17.10)	7.477	0.006
Atrial fibrillation, *n* (%)	25 (11.40)	7 (18.40)	18 (9.90)	2.231	0.135
Coronary heart disease, *n* (%)	41 (18.70)	10 (26.30)	31 (17.10)	1.743	0.187
Hypertension, *n* (%)	113 (51.60)	25 (65.80)	88 (48.60)	3.708	0.054
COPD, *n* (%)	33 (15.10)	7 (18.40)	26 (14.40)	0.404	0.525
Tumor, *n* (%)	25 (11.40)	3 (7.90)	22 (12.20)	0.564	0.453
Laboratory examination					
WBC, $\times 10^9$/L, median (IQR)	5.46 (4.54, 6.91)	6.97 (5.43, 8.16)	5.29 (4.47, 6.63)	−3.705	<0.001
RBC, $\times 10^{12}$/L, median (IQR)	4.19 (3.81, 4.56)	4.12 (3.66, 4.43)	4.20 (3.82, 4.57)	−0.941	0.347
HB, g/L, median (IQR)	127.00 (114.00, 139.00)	118.00 (106.50, 137.50)	128.00 (116.50, 139.00)	−1.559	0.119
PLT, $\times 10^{12}$/L, median (IQR)	166.00 (140.00, 208.00)	191.50 (143.50, 249.25)	164.00 (138.50, 200.00)	−1.507	0.132
PT, s, median (IQR)	10.90 (10.40, 12.10)	11.40 (10.50, 12.98)	10.90 (10.40, 12.05)	−1.754	0.079
APTT, s, median (IQR)	25.00 (22.80, 28.40)	26.45 (23.85, 32.28)	24.70 (22.80, 28.05)	−2.098	0.036
FIB, g/L, median (IQR)	2.50 (2.10, 3.00)	2.70 (2.15, 3.03)	2.40 (2.08, 2.99)	−1.851	0.064
CysC, mg/L, median (IQR)	1.03 (0.87, 1.23)	1.24 (1.03, 1.58)	1.01 (0.86, 1.17)	−4.089	<0.001
ApoA1, g/L, median (IQR)	1.33 (1.12, 1.59)	1.18 (1.06, 1.42)	1.37 (1.14, 1.61)	−2.783	0.005
ApoB, g/L, median (IQR)	0.81 (0.63, 1.03)	0.79 (0.64, 1.00)	0.83 (0.63, 1.04)	−0.542	0.588
SAA, mg/L, median (IQR)	9.30 (4.80, 21.10)	29.55 (10.15, 48.18)	8.40 (4.00, 15.10)	−5.031	<0.001
TC, mmol/L, median (IQR)	4.09 (3.39, 4.84)	4.08 (3.56, 4.78)	4.09 (3.34, 4.85)	−0.420	0.675
LDL, mmol/L, median (IQR)	2.12 (1.49, 2.71)	2.35 (1.88, 2.87)	2.09 (1.46, 2.66)	−1.878	0.060
TG, mmol/L, median (IQR)	1.18 (0.84, 1.62)	1.12 (0.87, 1.35)	1.20 (0.83, 1.67)	−0.396	0.692
ALT, μ/L, median (IQR)	15.00 (10.00, 21.00)	14.00 (8.00, 21.50)	15.00 (10.00, 21.00)	−0.486	0.627
AST, μ/L, median (IQR)	22.00 (18.00, 27.00)	22.00 (18.75, 26.00)	22.00 (18.00, 27.50)	−0.059	0.953
ALB, g/L, median (IQR)	40.10 (37.30, 43.00)	39.25 (36.38, 41.78)	40.20 (37.45, 43.20)	−1.364	0.172
GLO, g/L, median (IQR)	28.10 (25.10, 30.80)	29.25 (25.10, 32.78)	27.90 (25.15, 30.45)	−1.266	0.206
TBIL, μmol/L, median (IQR)	11.70 (9.10, 14.80)	12.45 (9.08, 14.95)	11.60 (9.05, 14.80)	−0.504	0.614
GLU, mmol/L, median (IQR)	5.61 (4.79, 7.54)	6.32 (4.92, 9.19)	5.47 (4.77, 7.25)	−1.705	0.088
CREA, μmol/L, median (IQR)	72.00 (62.00, 88.00)	79.35 (66.60, 89.00)	70.00 (61.00, 86.00)	−1.960	0.050
CK, mmol/L, median (IQR)	81.00 (58.00, 117.00)	73.00 (54.75, 115.75)	81.00 (58.00, 117.50)	−0.752	0.452
UA, mmol/L, median (IQR)	330.00 (275.00, 392.30)	360.00 (286.75, 400.10)	322.00 (266.50, 390.85)	−1.677	0.094

PSCI, post-stroke cognitive impairment; SD, standard deviation; IQR, interquartile range; COPD, chronic obstructive pulmonary disease; RBC, red blood count; WBC, white blood count; HB, hemoglobin; PLT, platelet; CysC, cystatin C; TC, total cholesterol; LDL, low-density lipoprotein; TG, triglyceride; ALB, albumin; TBIL, total bilirubin; CREA, creatinine; BUN, blood urea nitrogen; UA, uric acid; ALT, alanine transaminase; SAA, serum amyloid A.

The association between the serum SAA and the probability of PSCI, the serum SAA, and the MMSE score

We found a positive correlation between the serum SAA and the probability of PSCI, in which the predicted probabilities for PSCI were more than 50% after 58 mg/L of serum amyloid A (Figure 6A). Besides, linear regression analysis showed that the level of serum SAA was negatively associated with the MMSE score (regression equation: $y = 54.75–1.54x$, $P < 0.001$; Figure 6B).

Discussion

Post-stroke cognitive impairment is a clinical syndrome of cognitive impairment that occurs after an ischemic

TABLE 3 Univariate and multivariate logistic regression analyses of risk factors for PSCI in the training set.

Variables	Univariate logistic regression			Multivariate logistic regression		
	β	Odds ratio (95% CI)	*P*-value	*β*	Odds ratio (95% CI)	*P*-value
Age, years	1.092	1.047–1.138	<0.001	1.099	1.012–1.193	0.025
Education, years	0.828	0.737–0.930	0.001	1.098	0.863–1.396	0.446
Diabetes mellitus						
No		Ref			Ref	
Yes	2.823	1.315–6.061	0.008	2.679	1.029–6.976	0.044
WBC	1.440	1.195–1.736	<0.001	1.271	1.028–1.572	0.027
APTT	1.047	1.003–1.092	0.036	1.031	0.985–1.080	0.194
CysC	4.569	1.857–11.245	0.001	3.118	1.053–9.228	0.040
ApoA1	0.183	0.056–0.599	0.005	0.593	0.140–2.518	0.479
SAA	1.044	1.025–1.064	<0.001	1.031	1.009–1.054	0.007

WBC, white blood count; CysC, cystatin C; SAA, serum amyloid A; APTT, activated partial thromboplastin time.

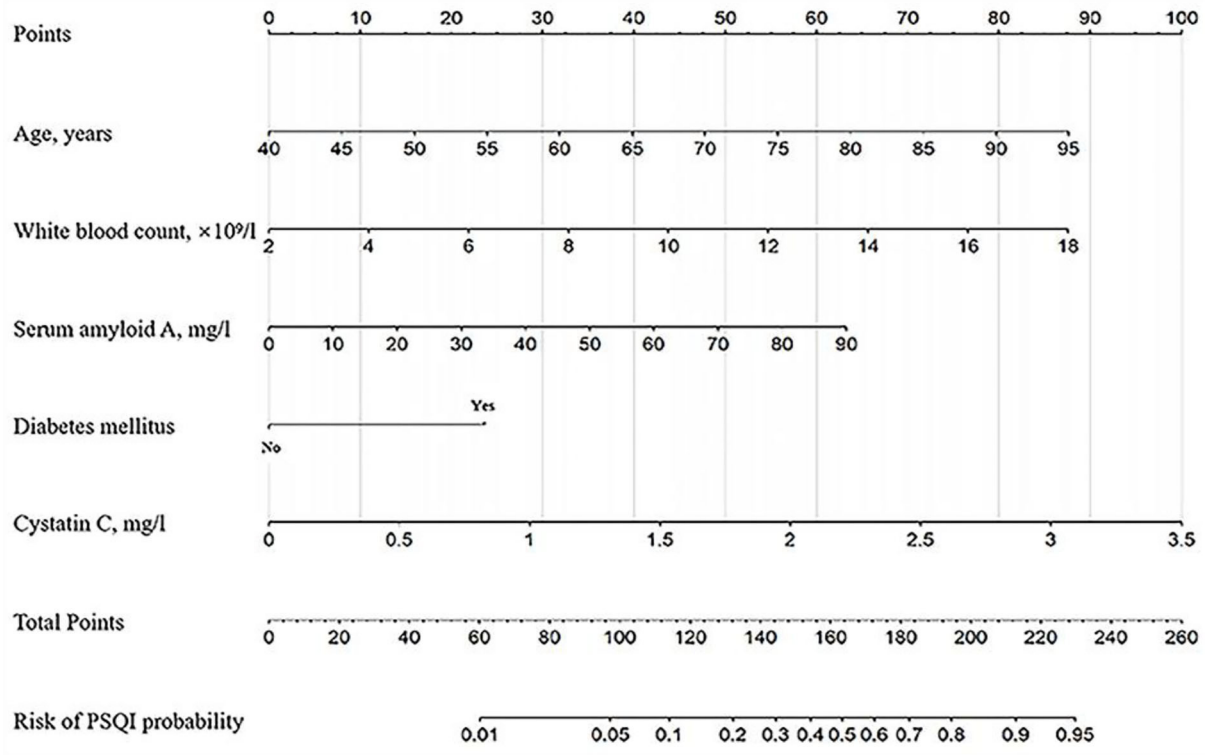

FIGURE 2
The nomogram for predicting the risk of PSCI probability based on the 5 independent prognostic factors. For example, a 65-year-old (40 points) patient with diabetes mellitus (25 points) with white blood counts of 12.0 × 10^9/L (55 points), serum amyloid A of 50 mg/L (35 points), and cystatin C of 1.5 mg/L (42 points) arrived at a total point of 197, with a probability of 80% to develop PSCI.

stroke (18). Approximately 37.3% of respondents developed PSCI in a retrospective study of 209 patients with mild ischemic stroke (11). Therefore, it is important to assess PSCI early and conveniently. In this study, we investigated the association of SAA concentrations with the risk of cognitive impairment after lacunar infarction. Several pivotal results were found in this analysis. First, the increased SAA levels were significantly associated with a higher risk of PSCI. Second, we proved that several traditional risk factors, such as age, diabetes mellitus, white blood count, and cystatin C, were independent risk predictors of PSCI in patients with lacunar infarction. Third, we

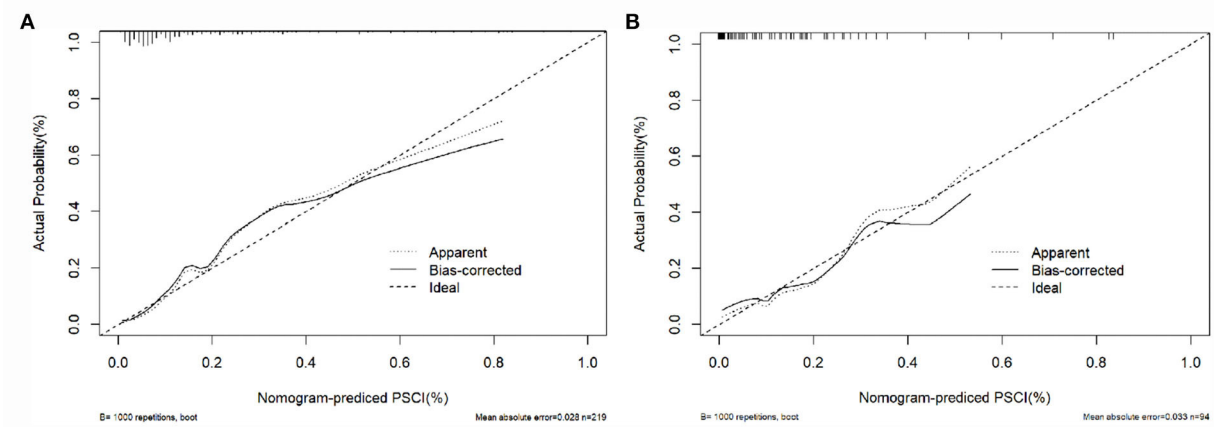

FIGURE 3
Calibration curves of the nomogram in the training set and the validation set. **(A)** The nomogram in the training set ($n = 219$); **(B)** the nomogram in the validation set ($n = 94$). The y-axis represents the observed rate of PSCI, and the x-axis represents the nomogram-predicted probability of PSCI. The dotted lines represented by the nomogram are closer to the diagonal gray lines representing a better prediction.

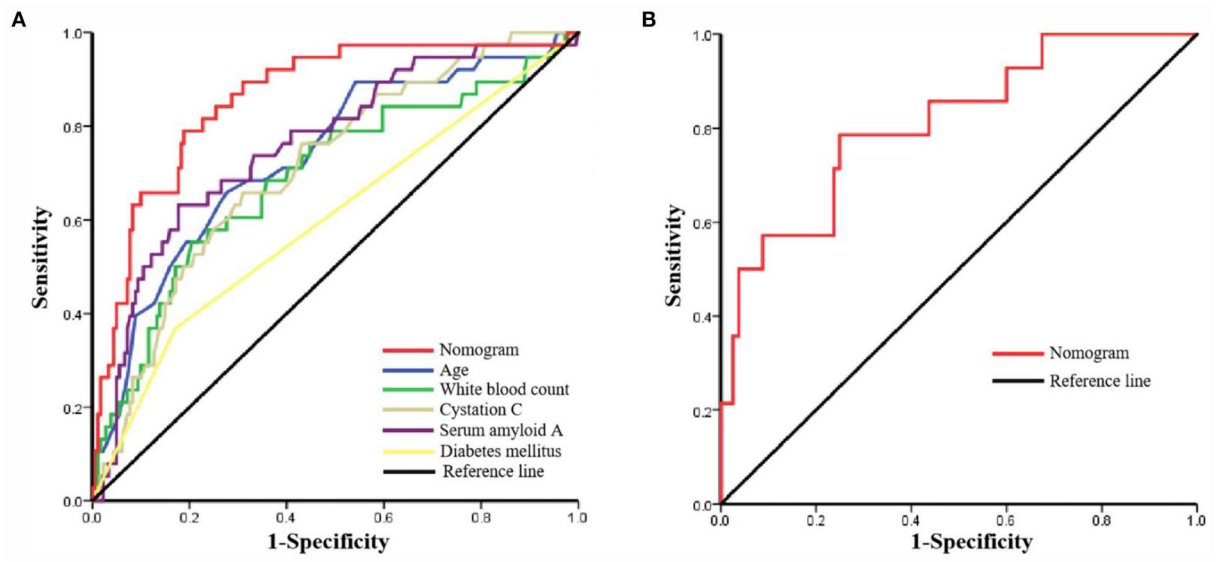

FIGURE 4
The receiver operating characteristic (ROC) curve of the nomogram in the training set and the validation set. **(A)** ROC in the training set; **(B)** ROC in the validation set.

constructed a nomogram model based on SSA that can predict PSCI effectively.

Each brain's morphology is unique, and aging changes brain morphology in both healthy and pathological conditions (26). Heart failure, atrial fibrillation, and renal insufficiency may contribute to acute stroke with increasing age, especially in patients over 85 years of age (27). Overton's research found that the older age groups had more possibility of having cognitive impairment (28). Morley's study also came to the data that nearly 40% of the persons older than 65 years had mild cognitive impairment in the United States (29). In our study, we found a positive association between age and the occurrence of cognitive impairment after lacunar infarction. The key point of brain aging is the cellular senescence of neurons and microglia (30). Evidence proved that since the age of 40 years, about 5% of neuron cells are destroyed every 10 years, which directly leads to a decrease in brain volume (29). In addition, as an important component of immunity for the central nervous system, microglia plays an indispensable role to maintain tissue homeostasis (31). Since microglia are

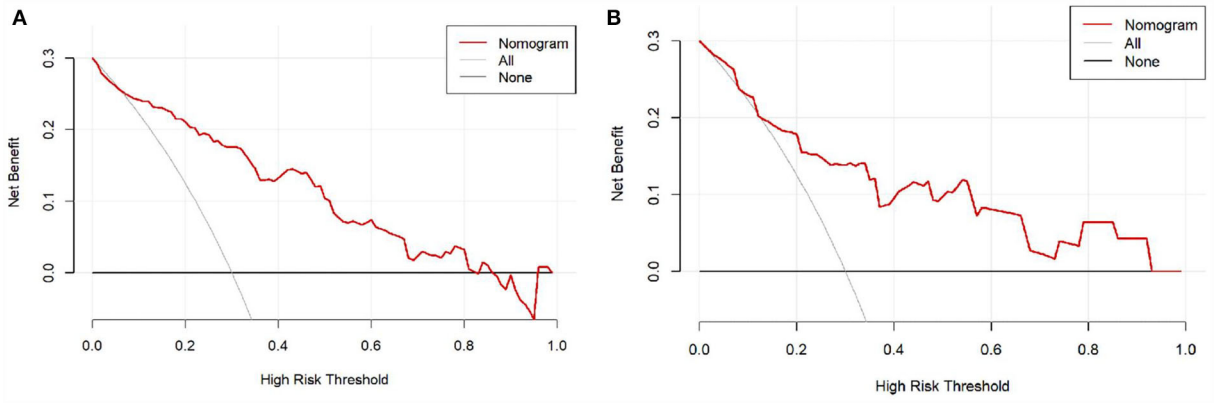

FIGURE 5
The decision curve analysis (DCA) of the nomogram in the training set and validation set. **(A)** DCA in the training set; **(B)** DCA in the validation set.

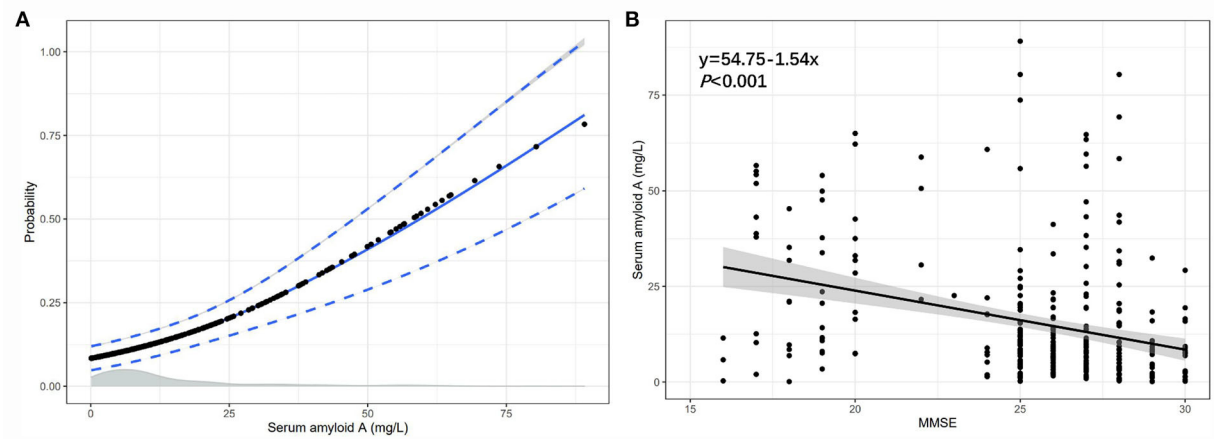

FIGURE 6
The association between the serum SAA and the probability of PSCI, the serum SAA, and the MMSE score. **(A)** The association of SAA levels with the probability of PSCI in patients with lacunar infarction; **(B)** The association between SAA levels and MMSE score.

found around lesions in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis, the release of inflammatory factors mediated by microglia is thought to be the key to the onset or progression of neurodegenerative diseases (32).

Diabetes mellitus is a kind of noninfectious and multiple organs affected chronic disease (33). Liccini's research concluded that 20% of patients aged between 50 and 65 years were diagnosed with diabetes mellitus who had cognitive impairment, and the situation may be more severe in diabetes mellitus who had metabolic syndrome (34–36). Van Sloten et al. revealed that diabetes-related microvascular dysfunction affected the exchange of gases, nutrients, proteins, and metabolites in the human body environment (37), which was a key factor in the occurrence of cognitive impairment (38). In addition, the health of people with diabetes could be negatively affected due to cognitive impairment. Therefore, we need to face up to the fact that stroke patients with diabetes have more prevalence of cognitive impairment (39), and clinicians should pay more attention to the cognitive abilities of diabetes patients.

Inflammatory responses are closely related to ischemic stroke; it could promote the development of ischemic injury and neuronal death after stroke (40, 41). The higher white cell counts within the normal range were associated with cognitive impairment in older adults (42, 43). Studies based on population have confirmed the relationship between inflammation and cognitive impairment, that is, the inflammatory states can negatively impact cognitive function (44, 45). Furthermore, the animal experiment revealed that white blood cells promoted the immune system to degenerate brain tissue in stroke animal models (46). In our study, we confirmed that white blood count played an important role in predicting cognitive impairment

after lacunar infarction. This suggested that neuronal inflammation prevention may reduce cognitive impairment and improve neurological outcomes in stroke patients.

Cystatin C is an endogenous cysteine proteinase inhibitor that exists nearly in all human cells and body fluids; it belongs to the type 2 cystatin superfamily (47, 48). Sarnak's research showed that higher levels of cystatin C were associated with cognitive impairment (49). Meanwhile, the higher serum cystatin C was an independent risk factor for PSCI in patients with acute mild ischemic stroke (50), which can provide early prediction of cognitive decline in the elderly (51). This is consistent with the conclusion of our study. Cognitive impairment could have a negative impact on the daily life of patients; therefore, reducing the level of serum cystatin C may provide a new treatment for the prevention of PSCI, and it is of great significance to timely predict the occurrence of cognitive impairment (52).

Serum amyloid A is a protein secreted by hepatocytes (53). The synthesis of SAA is associated with inflammatory cytokines, which can rise rapidly when infection and inflammation occur (18, 54). It is widely used as a follow-up marker for diagnosis, prognosis, or treatment of disease (55, 56). SAA has been recognized as being associated with cognitive impairment (57). Xu's research found the relationship between cognitive function and SAA levels in patients with vascular dementia and investigated the higher levels of SAA in patients with vascular dementia (58, 59). The elevation of SAA exacerbates neuroinflammation and changes the morphology of microglia to increase their activity, eventually leading to brain damage and memory loss (54, 60, 61). Therefore, for patients with lacunar infarction with elevated SAA, it is necessary for clinicians and healthcare organizations to take preventive actions against cognitive impairment that may occur in the future.

The nomogram based on the five variables would improve the predictive ability for PSCI in lacunar infarction patients. Compared with five independent risk factors, the nomogram exhibited good discrimination ability by the ROC analysis. In addition, DCA was applied in the training set and validation set, which confirmed the net benefit based on the threshold probability.

There were some limitations in this study. First, the study detected only the serum SAA levels within 24 h of admission, but did not examine the serum SAA levels before the stroke and within 3 months of discharge dynamically. Second, the patients did not perform the cognitive function assessment during admission, although patients with neurological disease and dementia were excluded. Third, the independent variables included in the study lack the relevant indicators of magnetic resonance imaging (cerebral atrophy and gray matter lesions), genetic risk factors, and environmental risk factors. Finally, there might be some bias in the selection of patients, because this study enrolled mild stroke patients with NHISS <3 only. Therefore, these issues need further exploration in the future prospective external studies.

Conclusion

This study revealed the association of SAA level with PSCI, which was an independent risk factor to predict cognitive impairment in lacunar infarction patients. In addition, this study constructed the nomogram to predict PSCI based on the five independent risk factors, which has proven clinical utility and is useful for PSCI risk decision-making in patients with lacunar infarction undergoing clinical assessment.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of the Second Affiliated Hospital of Wannan Medical College. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SY and LX designed this study and provided the funding support. SY and HP drafted the first manuscript and analyzed the data. JX, WL, and BW took part in the sample collection and acquired the data. LX and BW followed up with the patient. LX reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Tao X, Qiu C, Feng X, Wang L. Predictive analysis of serum NO, PGI2, and Ox-LDL levels on disease progression in patients with lacunar cerebral infarction. *Comput Math Methods Med.* (2022) 2022:1221810. doi: 10.1155/2022/1221810

2. Nakamori M, Matsushima H, Tachiyama K, Hayashi Y, Imamura E, Mizoue T, et al. Association of ankle-brachial index with cognitive decline in patients with lacunar infarction. *PLoS ONE.* (2022) 17:e0263525. doi: 10.1371/journal.pone.0263525

3. Teng Z, Dong Y, Zhang D, An J, Lv P. Cerebral small vessel disease and post-stroke cognitive impairment. *Int J Neurosci.* (2017) 127:824–30. doi: 10.1080/00207454.2016.1261291

4. Mijajlovic MD, Pavlovic A, Brainin M, Heiss WD, Quinn TJ, Ihle-Hansen HB, et al. Post-stroke dementia - a comprehensive review. *BMC Med.* (2017) 15:11. doi: 10.1186/s12916-017-0779-7

5. D'Souza CE, Greenway MRF, Graff-Radford J, Meschia JF. Cognitive impairment in patients with stroke. *Semin Neurol.* (2021) 41:75–84. doi: 10.1055/s-0040-1722217

6. Cukierman-Yaffe T, McClure LA, Risoli T, Bosch J, Sharma M, Gerstein HC, et al. The relationship between glucose control and cognitive function in people with diabetes after a lacunar stroke. *J Clin Endocrinol Metab.* (2021) 106:e1521–e8. doi: 10.1210/clinem/dgab022

7. Kwon HS, Lee D, Lee MH Yu S, Lim JS Yu KH, et al. Post-stroke cognitive impairment as an independent predictor of ischemic stroke recurrence: PICASSO sub-study. *J Neurol.* (2020) 267:688–93. doi: 10.1007/s00415-019-09630-4

8. Lo Coco D, Lopez G, Corrao S. Cognitive impairment and stroke in elderly patients. *Vasc Health Risk Manag.* (2016) 12:105–16. doi: 10.2147/VHRM.S75306

9. Grau-Olivares M, Arboix A, Junque C, Arenaza-Urquijo EM, Rovira M, Bartres-Faz D. Progressive gray matter atrophy in lacunar patients with vascular mild cognitive impairment. *Cerebrovasc Dis.* (2010) 30:157–66. doi: 10.1159/000316059

10. Chander RJ, Lam BYK, Lin X, Ng AYT, Wong APL, Mok VCT, et al. Development and validation of a risk score (CHANGE) for cognitive impairment after ischemic stroke. *Sci Rep.* (2017) 7:12441. doi: 10.1038/s41598-017-12755-z

11. Kandiah N, Chander RJ, Lin X, Ng A, Poh YY, Cheong CY, et al. Cognitive impairment after mild stroke: development and validation of the SIGNAL2 risk score. *J Alzheimers Dis.* (2016) 49:1169–77. doi: 10.3233/JAD-150736

12. Zhang X, Bi X. Post-stroke cognitive impairment: a review focusing on molecular biomarkers. *J Mol Neurosci.* (2020) 70:1244–54. doi: 10.1007/s12031-020-01533-8

13. Sun J, Lv X, Gao X, Chen Z, Wei D, Ling Y, et al. The association between serum uric acid level and the risk of cognitive impairment after ischemic stroke. *Neurosci Lett.* (2020) 734:135098. doi: 10.1016/j.neulet.2020.135098

14. Sack GH Jr. Serum amyloid A (SAA) proteins. *Subcell Biochem.* (2020) 94:421–36. doi: 10.1007/978-3-030-41769-7_17

15. Abouelasrar Salama S, Gouwy M, Van Damme J, Struyf S. The turning away of serum amyloid A biological activities and receptor usage. *Immunology.* (2021) 163:115–27. doi: 10.1111/imm.13295

16. Schweizer J, Bustamante A, Lapierre-Fetaud V, Faura J, Scherrer N, Azurmendi Gil L, et al. SAA (Serum Amyloid A): a novel predictor of stroke-associated infections. *Stroke.* (2020) 51:3523–30. doi: 10.1161/STROKEAHA.120.030064

17. Yu J, Zhu H, Taheri S, Mondy W, Bonilha L, Magwood GS, et al. Serum amyloid A-mediated inflammasome activation of microglial cells in cerebral ischemia. *J Neurosci.* (2019) 39:9465–76. doi: 10.1523/JNEUROSCI.0801-19.2019

18. Zhang Y, Feng Y, Zuo J, Shi J, Zhang S, Yang Y, et al. Elevated serum amyloid A is associated with cognitive impairment in ischemic stroke patients. *Front Neurol.* (2021) 12:789204. doi: 10.3389/fneur.2021.789204

19. Su JH, Meng LW, Dong D, Zhuo WY, Wang JM, Liu LB, et al. Noninvasive model for predicting future ischemic strokes in patients with silent lacunar infarction using radiomics. *BMC Med Imaging.* (2020) 20:77. doi: 10.1186/s12880-020-00470-7

20. Sun T, Xie T, Zhang A, Fan L, Xu Z, Chen X, et al. Relation between left atrial structure and lacunar infarction in patients with hypertension. *Aging.* (2020) 12:17295–304. doi: 10.18632/aging.103697

21. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* (1975) 12:189–98. doi: 10.1016/0022-3956(75)90026-6

22. Yao YY, Wei ZJ, Zhang YC Li X, Gong L, Zhou JW, et al. Functional disability after ischemic stroke: a community-based cross-sectional study in Shanghai, China. *Front Neurol.* (2021) 12:649088. doi: 10.3389/fneur.2021.649088

23. Zhu Y, Fang C, Zhang Q, Lu Y, Zhang R, Wang A, et al. Soluble ST2 and risk of cognitive impairment after acute ischemic stroke: a prospective observational study. *BMC Geriatr.* (2021) 21:330. doi: 10.1186/s12877-021-02288-6

24. Zhou S, Chen J, Cheng L, Fan K, Xu M, Ren W, et al. Age-dependent association between elevated homocysteine and cognitive impairment in a post-stroke population: a prospective study. *Front Nutr.* (2021) 8:691837. doi: 10.3389/fnut.2021.691837

25. Sitzia C, Sterlicchio M, Crapanzano C, Dozio E, Vianello E, Corsi Romanelli MM. Intra-erythrocytes magnesium deficiency could reflect cognitive impairment status due to vascular disease: a pilot study. *J Transl Med.* (2020) 18:458. doi: 10.1186/s12967-020-02645-w

26. Blinkouskaya Y, Cacoilo A, Gollamudi T, Jalalian S, Weickenmeier J. Brain aging mechanisms with mechanical manifestations. *Mech Ageing Dev.* (2021) 200:111575. doi: 10.1016/j.mad.2021.111575

27. Arboix A, Miguel M, Ciscar E, Garcia-Eroles L, Massons J, Balcells M. Cardiovascular risk factors in patients aged 85 or older with ischemic stroke. *Clin Neurol Neurosurg.* (2006) 108:638–43. doi: 10.1016/j.clineuro.2005.10.010

28. Overton M, Pihlsgard M, Elmstahl S. Prevalence and incidence of mild cognitive impairment across subtypes, age, and sex. *Dement Geriatr Cogn Disord.* (2019) 47:219–32. doi: 10.1159/000499763

29. Morley JE. An overview of cognitive impairment. *Clin Geriatr Med.* (2018) 34:505–13. doi: 10.1016/j.cger.2018.06.003

30. Raz N, Daugherty AM. Pathways to brain aging and their modifiers: free-radical-induced energetic and neural decline in senescence (FRIENDS) model - a mini-review. *Gerontology.* (2018) 64:49–57. doi: 10.1159/000479508

31. Subhramanyam CS, Wang C, Hu Q, Dheen ST. Microglia-mediated neuroinflammation in neurodegenerative diseases. *Semin Cell Dev Biol.* (2019) 94:112–20. doi: 10.1016/j.semcdb.2019.05.004

32. Xu L, He D, Bai Y. Microglia-mediated inflammation and neurodegenerative disease. *Mol Neurobiol.* (2016) 53:6709–15. doi: 10.1007/s12035-015-9593-4

33. Faselis C, Katsimardou A, Imprialos K, Deligkaris P, Kallistratos M, Dimitriadis K. Microvascular complications of type 2 diabetes mellitus. *Curr Vasc Pharmacol.* (2020) 18:117–24. doi: 10.2174/1570161117666190502103733

34. Liccini A, Malmstrom TK, Morley JE. Metformin use and cognitive dysfunction among patients with diabetes mellitus. *J Am Med Dir Assoc.* (2016) 17:1063–5. doi: 10.1016/j.jamda.2016.08.026

35. Zilliox LA, Chadrasekaran K, Kwan JY, Russell JW. Diabetes and cognitive impairment. *Curr Diab Rep.* (2016) 16:87. doi: 10.1007/s11892-016-0775-x

36. Lyu F, Wu D, Wei C, Wu A. Vascular cognitive impairment and dementia in type 2 diabetes mellitus: an overview. *Life Sci.* (2020) 254:117771. doi: 10.1016/j.lfs.2020.117771

37. Horton WB, Barrett EJ. Microvascular dysfunction in diabetes mellitus and cardiometabolic disease. *Endocr Rev.* (2021) 42:29–55. doi: 10.1210/endrev/bnaa025

38. van Sloten TT, Sedaghat S, Carnethon MR, Launer LJ, Stehouwer CDA. Cerebral microvascular complications of type 2 diabetes: stroke,

cognitive dysfunction, and depression. *Lancet Diabetes Endocrinol.* (2020) 8:325–36. doi: 10.1016/S2213-8587(19)30405-X

39. Ward R, Li W, Abdul Y, Jackson L, Dong G, Jamil S, et al. NLRP3 inflammasome inhibition with MCC950 improves diabetes-mediated cognitive impairment and vasoneuronal remodeling after ischemia. *Pharmacol Res.* (2019) 142:237–50. doi: 10.1016/j.phrs.2019.01.035

40. Quan K, Wang A, Zhang X, Wang Y. Leukocyte count and adverse clinical outcomes in acute ischemic stroke patients. *Front Neurol.* (2019) 10:1240. doi: 10.3389/fneur.2019.01240

41. Kim H, Seo JS, Lee SY, Ha KT, Choi BT, Shin YI, et al. AIM2 inflammasome contributes to brain injury and chronic post-stroke cognitive impairment in mice. *Brain Behav Immun.* (2020) 87:765–76. doi: 10.1016/j.bbi.2020.03.011

42. Kao TW, Chang YW, Chou CC, Hu J, Yu YH, Kuo HK. White blood cell count and psychomotor cognitive performance in the elderly. *Eur J Clin Invest.* (2011) 41:513–20. doi: 10.1111/j.1365-2362.2010.02438.x

43. Koren-Morag N, Tanne D, Goldbourt U. White blood cell count and the incidence of ischemic stroke in coronary heart disease patients. *Am J Med.* (2005) 118:1004–9. doi: 10.1016/j.amjmed.2005.03.010

44. Narasimhalu K, Lee J, Leong YL, Ma L, De Silva DA, Wong MC, et al. Inflammatory markers and their association with post stroke cognitive decline. *Int J Stroke.* (2015) 10:513–8. doi: 10.1111/ijs.12001

45. Halazun HJ, Mergeche JL, Mallon KA, Connolly ES, Heyer EJ. Neutrophil-lymphocyte ratio as a predictor of cognitive dysfunction in carotid endarterectomy patients. *J Vasc Surg.* (2014) 59:768–73. doi: 10.1016/j.jvs.2013.08.095

46. Ren X, Hu H, Farooqi I, Simpkins JW. Blood substitution therapy rescues the brain of mice from ischemic damage. *Nat Commun.* (2020) 11:4078. doi: 10.1038/s41467-020-17930-x

47. Mussap M, Plebani M. Biochemistry and clinical role of human cystatin C. *Crit Rev Clin Lab Sci.* (2004) 41:467–550. doi: 10.1080/10408360490504934

48. Cui Z, Cao G, Wang Y, Ma Q, Wang C, Xu Y, et al. Effects of cystatin C on cognitive impairment in older chinese adults. *Am J Alzheimers Dis Other Demen.* (2020) 35:1533317520965101. doi: 10.1177/1533317520965101

49. Sarnak MJ, Katz R, Fried LF, Siscovick D, Kestenbaum B, Seliger S, et al. Cystatin C and aging success. *Arch Intern Med.* (2008) 168:147–53. doi: 10.1001/archinternmed.2007.40

50. Yan X, Chen H, Shang XL. Association between serum cystatin C level and post-stroke cognitive impairment in patients with acute mild ischemic stroke. *Brain Behav.* (2022) 12:e2519. doi: 10.1002/brb3.2519

51. Wei Y, Wei YK, Zhu J. Early markers of kidney dysfunction and cognitive impairment among older adults. *J Neurol Sci.* (2017) 375:209–14. doi: 10.1016/j.jns.2017.01.071

52. Nair P, Misra S, Nath M, Vibha D, Srivastava AK, Prasad K, et al. Cystatin C and risk of mild cognitive impairment: A systematic review and meta-analysis. *Dement Geriatr Cogn Disord.* (2020) 49:471–82. doi: 10.1159/000510219

53. Matsumoto J, Dohgu S, Takata F, Iwao T, Kimura I, Tomohiro M, et al. Serum amyloid A-induced blood-brain barrier dysfunction associated with decreased claudin-5 expression in rat brain endothelial cells and its inhibition by high-density lipoprotein *in vitro*. *Neurosci. Lett.* (2020) 738:135352. doi: 10.1016/j.neulet.2020.135352

54. Malle E, Sodin-Semrl S, Kovacevic A. Serum amyloid A: an acute-phase protein involved in tumour pathogenesis. *Cell Mol Life Sci.* (2009) 66:9–26. doi: 10.1007/s00018-008-8321-x

55. De Buck M, Gouwy M, Wang JM, Van Snick J, Opdenakker G, Struyf S, et al. Structure and expression of different serum amyloid A (SAA) vari ants and their concentration-dependent functions during host insults. *Curr Med Chem.* (2016) 2016, 23, 1725-1755. doi: 10.2174/0929867323666160418114600

56. Sun L, Ye RD. Serum amyloid A1: structure, function and gene polymorphism. *Gene.* (2016) 583:48–57. doi: 10.1016/j.gene.2016.02.044

57. Salahudeen MS, Chyou TY, Nishtala PS. Serum anticholinergic activity and cognitive and functional adverse outcomes in older people: a systematic review and meta-analysis of the literature. *PLoS ONE.* (2016) 11:e0151084. doi: 10.1371/journal.pone.0151084

58. Xu M, He XY, Huang P. The Relationship between serum amyloid A level and cognitive dysfunction in patients with vascular dementia: preliminary findings. *Biomed Res Int.* (2021) 2021:6676144. doi: 10.1155/2021/6676144

59. Kisilevsky R, Manley PN. Acute-phase serum amyloid A: perspectives on its physiological and pathological roles. *Amyloid.* (2012) 19:5–14. doi: 10.3109/13506129.2011.654294

60. Zhang C, Liu Y, Gilthorpe J, van der Maarel JR. MRP14 (S100A9) protein interacts with Alzheimer beta-amyloid peptide and induces its fibrillization. *PLoS ONE.* (2012) 7:e32953. doi: 10.1371/journal.pone.0032953

61. Jang S, Jang WY, Choi M, Lee J, Kwon W, Yi J, et al. Serum amyloid A1 is involved in amyloid plaque aggregation and memory decline in amyloid beta abundant condition. *Transgenic Res.* (2019) 28:499–508. doi: 10.1007/s11248-019-00166-x

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Association of the stress hyperglycemia ratio and clinical outcomes in patients with stroke: A systematic review and meta-analysis

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Objective: Stress hyperglycemia (SH) is common in patients with acute diseases, such as stroke and myocardial infarction. Stress hyperglycemia ratio (SHR) is calculated by glucose/glycated hemoglobin and has been widely used for evaluating SH. But whether SHR is associated with clinical outcomes in stroke patients remains unclear so far. Although many studies have shown that higher SHR means poor outcomes, there is still no absolute evidence that SHR plays a critical role in stroke patients. Hence, we performed a systematic review and meta-analysis aiming to investigate the association between SHR and clinical outcomes in stroke patients.

Methods: We performed a comprehensive literature search of the PubMed, Embase, Cochrane Library databases, Clinicaltrials.gov, and WHO-ICTRP. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), we performed our study. The Newcastle-Ottawa Scale (NOS) tool was used to examine the potential bias of included studies. The endpoints including poor outcome, mortality, neurological deficit, hemorrhagic transformation (HT), and infectious complications were statistically analyzed.

Results: Sixteen retrospective studies met the eligibility criteria, and a number of 183,588 patients were included. Our meta-analysis demonstrated a significant increase in the incidence of poor outcome, according to assessment by the modified Rankin Scale (mRS) $\geq$ 3 points [odds ratio (OR) 2.53, 95% confidence interval (CI) 1.99–3.22, $P < 0.00001$, $I^2 = 68\%$], mortality (OR 1.96, 95% CI 1.58–2.44, $P < 0.00001$, $I^2 = 61\%$), neurological deficit (OR 1.99, 95% CI 1.47–2.70, $P < 0.00001$, $I^2 = 75\%$), hemorrhagic transformation (HT) (OR 3.70, 95% CI 2.69–5.08, $P < 0.00001$, $I^2 = 0\%$), and infectious complications [(Pneumonia) OR 2.06, 95% CI 1.57–2.72, $P < 0.00001$, $I^2 = 24\%$; (Urinary tract infection) OR 2.53, 95% CI 1.45–4.42, $P = 0.001$, $I^2 = 57\%$] in stroke patients with higher SHR. However, no significant influence was observed for recanalization rate (OR 0.86, 95% CI 0.54–1.38, $P = 0.53$, $I^2 = 0\%$).

Conclusion: With or without diabetes, no matter whether undergoing intravenous thrombolysis or mechanical thrombectomy, higher SHR

significantly increased the occurrence of poor outcomes, mortality, neurological deficit, HT, and infectious complications. The recanalization rate was not statistically significant between the two groups. More attention must be paid in clinical practice to SH. Future investigation should focus on the diagnostic value of SHR and the early control of hyperglycemia. Meanwhile, whether SHR could become a novel and promising target for early intervention is worthy of attention in further research. Besides, the influence of the dynamic change of glucose-to-HbA1c ratio, namely SHR, on intracerebral hemorrhage outcomes requires further investigation in future research. Although no randomized double-blind studies have been conducted, the available massive sample studies reflect the actual situation in the clinic and assist clinical decision makers.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier: CRD42022345587.

KEYWORDS

stroke, stress hyperglycemia, stress hyperglycemia ratio, ratio of glucose to glycated hemoglobin, clinical outcome, meta-analysis

Introduction

Stroke, including ischemic and hemorrhagic, is a pervasive type of acute cerebrovascular disease among which hemorrhagic stroke is the second most common stroke sub-type leading to the highest morbidity and mortality (1, 2). Even though treatment for stroke patients is timely and effective now, the earlier intervention of the risk factors for adverse results is still vital to optimize outcomes. In the past 40 years, the stroke burden in China has increased without a stop, and in the recent past 7 years (from 2013 to 2019), the prevalence of stroke in China has continued to increase (3). In 2017, stroke was the leading cause of death, years of life lost, and disability-adjusted life years at the national level in China (4). An investigation involving 480,687 adults aged $\geq$ 20 years showed that the age-standardized prevalence and incidence rate of stroke were 1,114.8/100,000/year and 246.8/100,000/year, respectively (5). Therefore, the prevention and treatment of stroke still have a long way to go.

Stress hyperglycemia (SH), known as transient hyperglycemia secondary to neurohormonal disorders and inflammation reaction (6), is a common manifestation found in patients with myocardial infarction, stroke, and other critical illnesses (6–9). Stress hyperglycemia ratio (SHR) was first applied for assessing SH by Roberts et al. (10). Because of the stability of glycosylated hemoglobin (HbA1c) in patients with diabetes over the previous 8–12 weeks, SHR was defined as the admission glucose concentration/estimated average glucose (eAG) concentration (10, 11). However, due to discrepancies between eAG and average blood glucose, some scholars pointed out that eAG should be carefully used for clinical practice. Another definition of SHR using the ratio of glucose to HbA1c was more practical and widely applied.

SH is associated with the severity of stroke (12, 13) and poor outcomes, especially in patients without diabetes mellitus (7). Nevertheless, the association between SH and the outcomes of patients with diabetes mellitus is controversial, not only for stroke patients but also for some other critical illnesses (12, 14, 15). A study concentrating on acute ischemic stroke patients with diabetes showed that SHR could be a better predictor for the severity and poor outcome of stroke (16). But owing to its characteristic of a single-center and small sample study, the limitation of the results was obvious. Because admission glucose could be influenced by the diabetic status and the food. Therefore, fasting blood glucose (FBG) rather than random or admission glucose could be a more reliable marker, as previously suggested (17).

Many studies evaluating the association between SHR and clinical outcomes in patients with stroke have been performed in recent years (18–33). But whether SHR is associated with clinical outcomes in stroke patients remains unclear. So far, no systematic reviews and meta-analyses have been reported concerning the SHR and clinical outcomes in patients with stroke and there is still no absolute evidence that SHR plays a critical role in stroke patients. Hence, we performed a systematic review and meta-analysis aiming to investigate the association between SHR and clinical outcomes in stroke patients. Herein, we performed the first meta-analysis based on the available studies to determine the followings: (1) the relationship between SHR and clinical outcomes during the follow-up in stroke patients; (2) the influence on recanalization rate in patients accepting mechanical thrombectomy or intravenous thrombolysis.

Methods

Aims and PICO statement

This study was performed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (34) and was registered with PROSPERO (CRD42022345587) (35). The detailed information is presented in Supplementary Table S1. And the PICO statements were as follows: (1) **Population**: Stroke patients with or without diabetes. (2) **Intervention**: Mechanical thrombectomy or intravenous thrombolysis or neither. (3) **Comparisons**: Relative low SHR *vs.* relative high SHR (based on different groupings, if there are three groups, we defined the first group as low SHR and the rest of two groups as high SHR. Similarly, if there are four groups, the first two groups are low SHR and the remaining two groups are high SHR. (4) **Outcomes**: We defined poor outcome as the mRS $\geq$ 3 points at follow-up. Symptomatic intracerebral hemorrhage (SICH) and intracerebral hemorrhage (ICH) were regarded as HT. Besides, mortality, neurological deficit, recanalization rate, and infectious complications were also extracted during the follow-up.

Literature search strategy

We performed a comprehensive literature search of the PubMed, Embase, and Cochrane Library databases. Two reviewers (Huang YW and Yin XS) systematically screened the electronic databases for the appropriate articles that were published from inception to the end of July 2022. Meanwhile, the clinical trials registry centers, including *clinicaltrials.gov* and *WHO-ICTRP*, were also screened for possible findings. The following search strategy was applied: ("stroke" [all fields]) AND ("stress hyperglycemia" [all fields]) for the above databases and the clinical trials registry centers. The detailed search strategy is presented in Supplementary Table S2.

Inclusion and exclusion criteria

All potential studies were appraised independently with regard to the inclusion and exclusion criteria by two reviewers (Huang YW and Yin XS). The investigators selected studies that met all the following criteria: (1) types of publication: articles published in peer-reviewed medical journals; (2) types of participants: stroke patients with complete data on FBG and HbA1c upon admission; (3) types of comparison: relative low SHR *vs.* relative high SHR; (4) types of outcome measure: poor outcome, according to assessment by the mRS $\geq$ 3 points; mortality; neurological deficit; HT; infectious complications (pneumonia and urinary tract infection) and recanalization rate.

Case reports, reviews, notes, meta-analyses, editorials, letters to the editor, commentaries, conference abstracts, and non-English studies were excluded.

Data extraction

Two reviewers independently extracted data using the same standardized tables. The following information was extracted from the included studies: (1) basic characteristics: study ID (year of publication + first author name), country, study design, and number of participants; (2) participant characteristics: rate of male, type of stroke, operation, primary endpoint, secondary endpoint, and clinical follow-up; (3) data on outcomes of interest, etc.

Risk of bias assessment

The Newcastle-Ottawa Scale (NOS) tool (36) was applied to appraise the potential risk of bias (RoB) in included studies. The approach based on NOS included three parts (each part three points): (1) the selection of studies; (2) the comparability of studies; (3) the assessment of exposure/outcome. Each study might be appraised on up to 9 points. More than 6 scores were considered to indicate the high quality of the study. The assessment was performed independently by three reviewers (Huang YW, Yin XS, and Li ZP). Any differences were resolved in a group investigator discussion if required.

Statistical analysis

We calculated odds ratios (ORs) and their corresponding 95% confidence interval (CIs) when comparing the different endpoints of high SHR and low SHR among stroke patients. Considering clinical heterogeneity, we used DerSimonian and Laird random-effects model to perform the meta-analyses (37). P-value < 0.05 was considered statistically significant. The heterogeneity between studies was appraised by the Cochrane Q test ($P < 0.1$ or $I^2 > 50\%$ was considered to represent significant heterogeneity) (38). Specific data of the high SHR and low SHR groups were extracted from the studies based on our definition of high SHR and low SHR. The possibility of publication bias was assessed by the analysis of the funnel plot. All statistical analyses were conducted with the Review Manager software (version 5.3.0; https://training.cochrane.org/online-learning/core-softwarecochrane-reviews/revman).

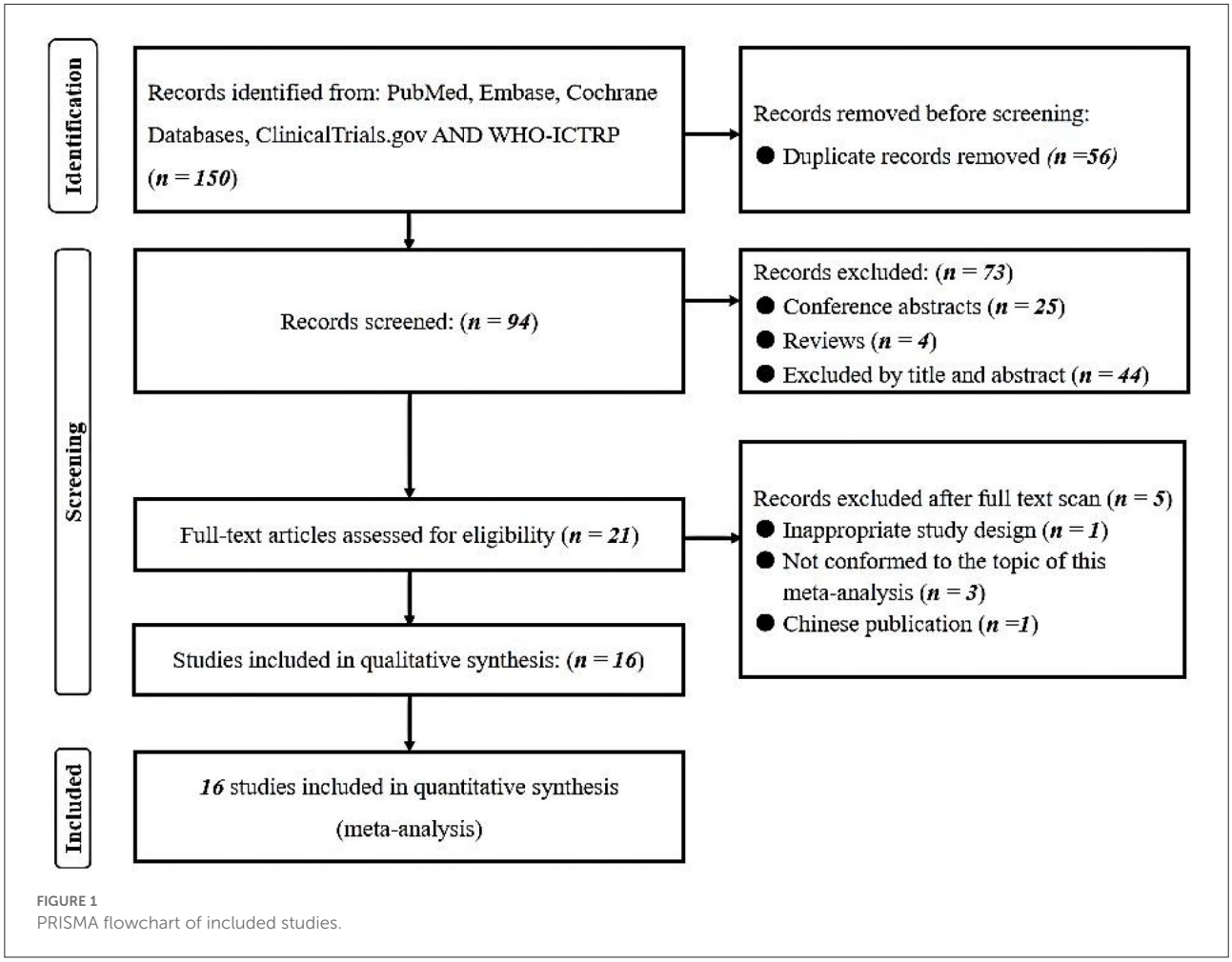

FIGURE 1
PRISMA flowchart of included studies.

Results

A comprehensive literature search of the PubMed, Embase, Cochrane Library databases, clinicaltrials.gov and WHO-ICTRP was performed. A total of 150 records were identified. Twenty-one articles underwent a full-text evaluation, five of which were excluded (one for inappropriate study design, three for inappropriate topic, and one for Chinese publication), leaving altogether sixteen studies in this systematic review and meta-analysis (18–33). The flowchart based on PRISMA is summarized in Figure 1. We identified five multi-center retrospective and 11 single-center retrospective studies. A number of 183,588 patients were included and the results are summarized in Table 1.

Heterogeneity

According to the results of the studies, a moderate statistical heterogeneity was found with poor outcome (P = 0.0008 for Cochran Q, I^2 = 68%), mortality (P = 0.004 for Cochran Q, I^2 = 61%), neurological deficit (P = 0.0005 for Cochran Q, I^2 = 75%), infectious complications (P = 0.13 for Cochran Q I^2 = 57%). Therefore, a random-effect model was used in these endpoints. The results are summarized in Table 2.

Meta-analysis of different outcomes

The results are summarized in Table 2. The meta-analysis demonstrated a significant increase in the incidence of poor outcome (mRS $\geq$ 3 points) [odds ratio (OR) 2.53, 95% confidence interval (CI) 1.99–3.22, $P < 0.00001$, I^2 = 68%; Figure 2A], mortality (OR 1.96, 95% CI 1.58–2.44, $P < 0.00001$, I^2 = 61%; Figure 2B), neurological deficit (OR 1.99, 95% CI 1.47–2.70, $P < 0.00001$, I^2 = 75%; Figure 3A), hemorrhagic transformation (HT) (OR 3.70, 95% CI 2.69–5.08, $P < 0.00001$, I^2 = 0%; Figure 3B), and infectious complications [(Pneumonia) OR 2.06, 95% CI 1.57–2.72, $P < 0.00001$, I^2 = 24%; Figure 4A; (Urinary tract infection) OR 2.53, 95% CI 1.45–4.42, P =

TABLE 1 Baseline characteristic description of the included studies.

References	Country	Study design	Participants	Male-%	Type of stroke	Operation	Primary endpoint	Secondary endpoint	Clinical follow-up
Chen et al. (18)	China	Retrospectively single-center	160	67.5	Ischemic stroke	Mechanical thrombectomy	Poor outcome	—	3 months
Wang et al. (19)	China	Retrospectively single-center	321	61.1	Ischemic stroke	Mechanical thrombectomy	Mortality	SICH Infectious complications	3 months
Zhu et al. (20)	China	Retrospectively multi-center	999	64.4	Ischemic stroke	—	Mortality	Stroke recurrence	12 months
Li et al. (21)	China	Retrospectively multi-center	8,622	62.8	Ischemic stroke	—	Mortality	Neurological deficit	Discharge 3 months
Merlino et al. (22)	Italy	Retrospectively single-center	414	53.4	Ischemic stroke	Intravenous thrombolysis	Poor outcome Mortality SICH	Neurological deficit in-hospital mortality ICH	3months
Merlino et al. (23)	Italy	Retrospectively single-center	204	49.0	Ischemic stroke	Mechanical thrombectomy	Poor outcome Mortality SICH	Neurological deficit in-hospital mortality ICH	3months
Roberts et al. (24)	Australia	Retrospectively single-center	300	53.0	Ischemic stroke	—	Poor outcome	—	discharge
Shen et al. (25)	China	Retrospectively single-center	341	70.7	Ischemic stroke	Intravenous thrombolysis	Poor outcome	Mortality Neurological deficit SICH HT	3months
Yuan et al. (26)	China	Retrospectively single-center	572	68.4	Ischemic stroke	—	HT	—	—
Cai et al. (27)	China	Retrospectively single-center	846	61.7	Ischemic stroke/hemorrhagic stroke	—	Poor outcome Mortality	Infectious complications	3 months 12 months
Chen et al. (28)	China	Retrospectively single-center	230	62.2	Ischemic stroke	Intravenous thrombolysis	Poor outcome Neurological deficit	Mortality	3 months
Chu et al. (29)	China	Retrospectively multi-center	313	72.5	Hemorrhagic stroke	—	Poor outcome	Neurological deficit Mortality	3 months
Li et al. (30)	China	Retrospectively multi-center	586	70.3	Hemorrhagic stroke	—	Poor outcome	—	3 months
Merlino et al. (31)	Italy	Retrospectively single-center	501	53.9	Ischemic stroke	Intravenous thrombolysis	Poor outcome Mortality SICH	Neurological deficit in-hospital mortality ICH	3 months
Mi et al. (32)	China	Retrospectively multi-center	168,381	57.0	Ischemic stroke	—	Mortality	—	12 months
Wang et al. (33)	China	Retrospectively single-center	798	64.2	Ischemic stroke	Intravenous thrombolysis	Poor outcome	Neurological deficit in-hospital mortality SICH	discharge

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TABLE 2 Heterogeneity and meta-analysis of included studies.

Items	Trials, *n*	Results		
		OR (95% CI)	*p*-value	Heterogeneity (I^2, *p* for Cochran Q)
Poor outcome	10	2.53 (1.99–3.22)	$p < 0.00001$	$I^2 = 68\%$, $P = 0.0008$
Mortality	11	1.96 (1.58–2.44)	$p < 0.00001$	$I^2 = 61\%$, $P = 0.004$
Neurological deficit	7	1.99 (1.47–2.70)	$p < 0.00001$	$I^2 = 75\%$, $P = 0.0005$
Hemorrhagic transformation	7	3.70 (2.69–5.08)	$p < 0.00001$	$I^2 = 0\%$, $P = 0.69$
Pneumonia	3	2.06 (1.57–2.72)	$p < 0.00001$	$I^2 = 24\%$, $P = 0.27$
Urinary tract infection	2	2.53 (1.45–4.42)	$p = 0.001$	$I^2 = 57\%$, $P = 0.13$
Recanalization rate	2	0.86 (0.54–2.04)	$p = 0.53$	$I^2 = 0\%$, $P = 0.32$

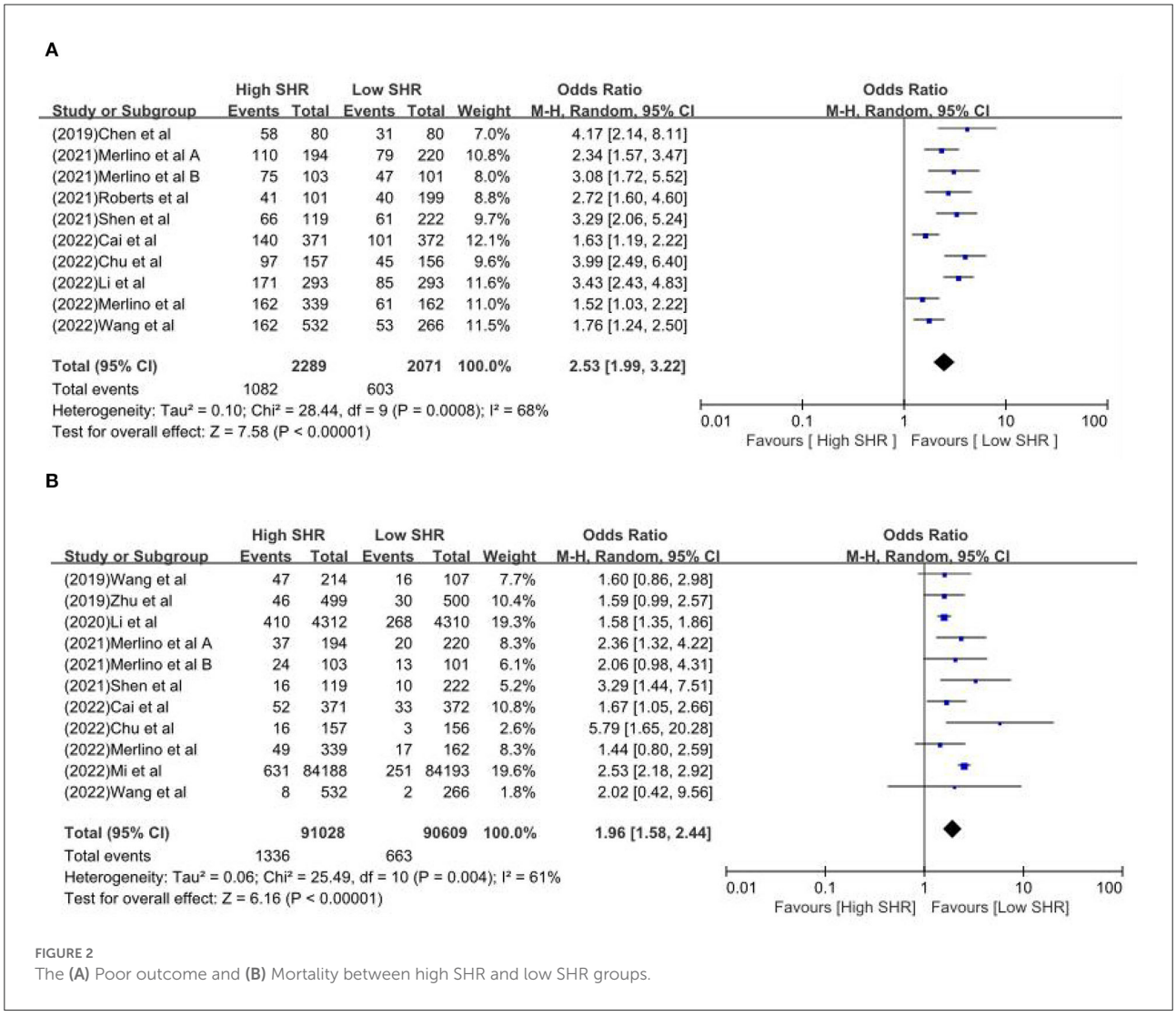

FIGURE 2
The **(A)** Poor outcome and **(B)** Mortality between high SHR and low SHR groups.

0.001, $I^2 = 57\%$; Figure 4B] in patients with higher SHR. However, no significant benefit was observed for re-canalization rate (OR 0.86, 95% CI 0.54–1.38, $P = 0.53$, $I^2 = 0\%$; Figure 5).

Risk of bias assessment

All these studies were marked as having low levels of RoB according to the NOS tool within the following items:

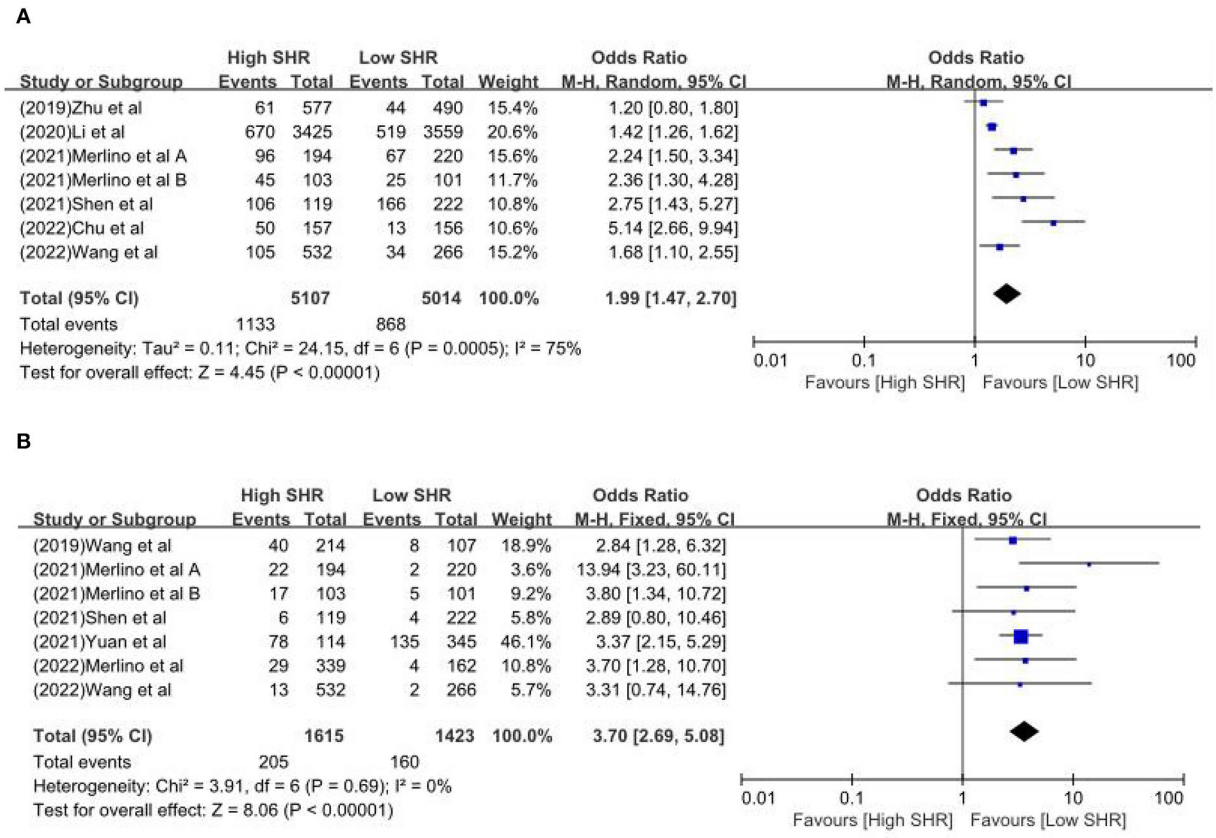

FIGURE 3
The **(A)** Neurological deficit and **(B)** hemorrhagic transformation between high SHR and low SHR groups.

selection bias, detection bias, and reporting bias. All studies were retrospective and with a mean of 7.69 stars and a standard deviation (SD) of 0.98 stars. The methodological quality of the included studies is presented in Supplementary Table S3.

Discussion

It is generally believed that the key points of SH are the activation of the hypothalamic-pituitary axis and sympatho-adrenal system causing the increases in the release of epinephrine, norepinephrine, and pro-inflammatory cytokines (TNF-α, IL-1, and IL-6) (39). The underlying mechanism of SH is as follows: first, strong inflammatory and neurohormonal responses caused increased induction of endothelial apoptosis and oxidative stress (OS) (6). In detail, activation of matrix metalloproteinase gelatinase B (MMP-9), breakdown of the blood-brain barrier (BBB), and BBB leakage increased brain edema and hemorrhage causing severe neurological deficits (40). Second, stress hormones can stimulate hepatic gluconeogenesis and inhibit glucose uptake in peripheral tissues (39). Pro-inflammatory cytokines, by upregulating expression and membrane localization of glucose transporters GLUT-1 and GLUT-3, facilitated the glucose uptake. They are used by the peripheral and central nervous systems (41). Besides, cellular glucose overload caused an increase in brain lactate production and further transformed asymptomatic tissue into symptomatic tissue (42). Third, no matter acute or chronic hyperglycemia, all play a particularly critical role in prothrombotic shift (43) and may facilitate thrombus extension (44). Fourth, SH may reflect the transient glycemic change. The glucose fluctuations exhibited a more specific triggering effect on OS (45). Finally, the degree of SH, named SHR, may reflect the severity of diseases. In patients with stroke, SHR can represent the extent of ischemic damage and cause poor clinical outcomes.

One study investigated by Chen et al. (18) demonstrated that increased SHR is strongly correlated with poor outcome at 3 months after MT for proximal artery occlusion in the anterior circulation (high SHR 72.5% vs. low SHR 38.8%). But the result was limited to being significant in non-diabetic stroke patients, not in stroke patients with diabetes. Poor glycemic control seemed to be associated with poor functional

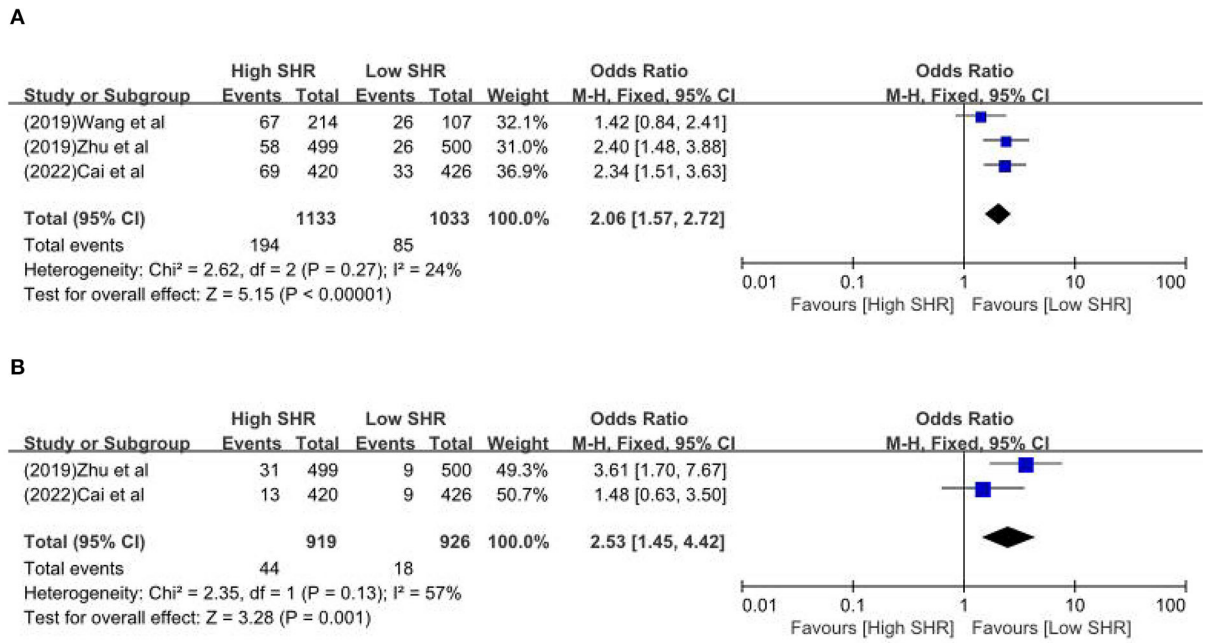

FIGURE 4
The **(A)** Pneumonia and **(B)** urinary tract infection between high SHR and low SHR groups.

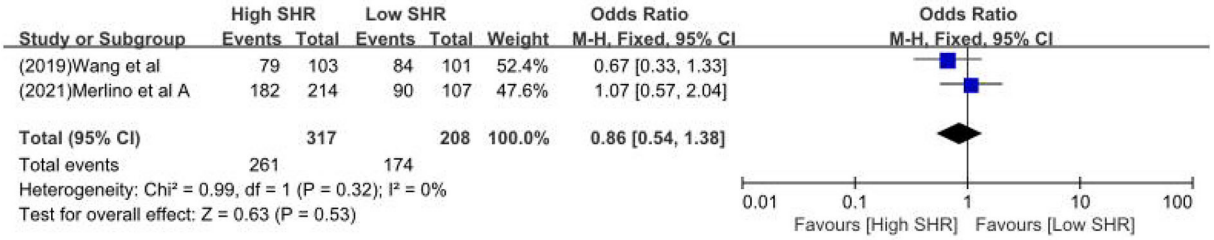

FIGURE 5
The recanalization rate between high SHR and low SHR groups.

outcomes after stroke. That meant long-term glycemic stress and damage are involved in the functional prognosis of stroke, while acute hyperglycemia after stroke might be a predictor of death. Another relevant study conducted by Wang et al. (19) focused on the mortality risk, and they found that higher SHR was associated with higher mortality risk after MT in acute ischemic stroke patients (high SHR 22.0% vs. low SHR 15%). Zhu et al. (20) performed a study focusing on non-diabetic stroke patients and showed that SHR was related to an elevated risk of stroke recurrence and all-cause death. Li et al.'s (21) study found that SHR was associated with an increased risk of severe neurological deficit and mortality within 1 year in acute ischemic stroke people with and without diabetes. In 2021, two studies from Italy demonstrated that SHR is associated with worse outcomes and detrimental effects in stroke patients undergoing intravenous thrombolysis or mechanical thrombectomy (22, 23). Another two relevant studies focus on hemorrhagic stroke and demonstrated that SHR is a reliable predictor for early hematoma expansion and poor outcomes and SHR was independently correlated with worse functional outcomes at discharge and 3 months in patients with ICH (29, 30). Li et al. (30) showed that SHR was independently correlated with worse functional outcomes at discharge and 3 months in patients with ICH. Besides, SHR could be used as a simple and readily available index to predict clinical outcomes of ICH. The study of SH provides meaningful insight into optimal glucose levels among ICH patients and develops tailored glucose-lowering strategies (30). Chen et al.'s investigation suggested that SHR is expected to replace random or fasting glucose concentration as a novel generation of prognostic indicator and

a potential therapeutic target (28). However, Merlino et al. (31) found that SHR was not associated with the clinical outcome of diabetic patients receiving intravenous thrombolysis for acute ischemic stroke. Mi et al. (32) conducted a massive sample and multi-center study involving 168,381 stroke patients from the Chinese Stroke Center Alliance (CSCA) database. Based on their findings, they considered that the SHR may serve as an accessory parameter for the prognosis of patients with diabetes after acute ischemic stroke, and hyperglycemia in stroke patients with diabetes mellitus is associated with a higher risk of in-hospital death. One has confirmed that SH has a certain predictive value for hemorrhagic transformation in patients with AIS (26).

SH is a common manifestation found in patients with critical illnesses, especially in stroke patients. As Li et al. (30) said, SHR was a simple and readily available index to predict clinical outcomes. In clinical practice, we need such an index that is easy to use and appraise possible clinical outcomes of stroke patients. For instance, some imaging markers, such as island sign (46) and blend sign (47) on the baseline computed tomography scan, identify the high-risk patients of hematoma expansion by this non-invasive to provide earlier clinical intervention aiming to decrease mortality and disability. In fact, the SHR is similar to this. Because of its convenience and non-invasive, SHR may be widely used for our screening of high-risk stroke patients and earlier identification of the adverse results. If further studies in the future aim to establish the prediction model or artificial intelligence algorithm for predicting the clinical outcomes of stroke patients, the SHR may serve as an important component of the associated model or algorithm. Altogether, SHR is an important prognosis factor for stroke patients and is helpful for clinicians to identify the high-risk population for stroke.

Our meta-analysis has comprehensively and systematically reviewed the currently available literature that compared different SHR in stroke patients with/without diabetes, and we obtained three major findings. First, in patients with stroke, higher SHR indicated poor outcome, mortality, neurological deficit, HT, and infectious complications. But the studies on infectious complications are limited. Second, no matter whether undergoing intravenous thrombolysis or mechanical thrombectomy, there was no statistically significant recanalization rate between the two groups. Third, studies on hemorrhagic strokes are urgent, as we know, hemorrhagic strokes are often more deadlier and devastating. By appraising SHR, earlier identification of the adverse results, such as hematoma expansion, is much vital for the neurosurgeon.

Limitations

Some limitations to this meta-analysis are as follows: first, available studies are mainly retrospective studies other than randomized even though massive sample; second, most of the included studies were from Chinese scholars, and the articles from other countries are required. Despite these limitations, we believe that the results of our meta-analysis may be useful to the clinicians in their choice of treatment for stroke patients; third, heterogeneity in outcomes reporting is also significant due to the highly variable duration of postoperative follow-up and different SHR groupings.

Conclusion

To our knowledge, this is the first meta-analysis assessing the association of different SHR and clinical outcomes in patients with stroke. With or without diabetes, no matter whether undergoing intravenous thrombolysis or mechanical thrombectomy, higher SHR significantly increased the occurrence of poor outcomes, mortality, neurological deficit, HT, and infectious complications. No statistically significant difference in recanalization was observed between the two groups. More attention must be paid to clinical practice. Future investigation should focus on the diagnostic value of SHR and the early control of hyperglycemia. Meanwhile, whether SHR could be a novel target for early intervention is worthy of attention in future research. Besides, the impact of the dynamic change of glucose-to-HbA1c ratio on ICH outcomes requires further investigation in future research. Although no randomized double-blind studies have been conducted, the available massive sample studies reflect the actual situation in the clinic and assist clinical decision makers.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

Y-WH and X-SY developed the initial idea for this study, formulated the study design, and contributed to the original draft. Z-PL developed and revised the search strategy and responsible for the revision of the draft. All authors approved the final version of the manuscript before submission.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.999536/full#supplementary-material

References

1. Li Q, Warren AD, Qureshi AI, Morotti A, Falcone GJ, Sheth KN, et al. Ultra-early blood pressure reduction attenuates hematoma growth and improves outcome in intracerebral hemorrhage. *Ann Neurol.* (2020) 88:388–95. doi: 10.1002/ana.25793

2. Cordonnier C, Demchuk A, Ziai W, Anderson CS. Intracerebral haemorrhage: current approaches to acute management. *Lancet.* (2018) 392:1257–68. doi: 10.1016/S0140-6736(18)31878-6

3. Tu WJ, Hua Y, Yan F, Bian HT, Yang Y, Lou M, et al. Prevalence of stroke in China, 2013–2019: a population-based study. *Lancet Regional Health Western Pacific.* (2022) 20:100550. doi: 10.1016/j.lanwpc.2022.100550

4. Wu S, Wu BO, Liu M, Chen Z, Wang W, Anderson CS, et al. Stroke in China: advances and challenges in epidemiology, prevention, and management. *Lancet Neurol.* (2019) 18:394–405. doi: 10.1016/S1474-4422(18)30500-3

5. Wang W, Jiang B, Sun H, Ru X, Sun D, Wang L, et al. Prevalence, incidence, and mortality of stroke in China: results from a nationwide population-based survey of 480 687 adults. *Circulation.* (2017) 135:759–71. doi: 10.1161/CIRCULATIONAHA.116.025250

6. Dungan KM, Braithwaite SS, Preiser JC. Stress hyperglycaemia. *Lancet.* (2009) 373:1798–807. doi: 10.1016/S0140-6736(09)60553-5

7. Capes SE, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. *Lancet.* (2000) 355:773–8. doi: 10.1016/S0140-6736(99)08415-9

8. Melamed E. Reactive hyperglycaemia in patients with acute stroke. *J Neurol Sci.* (1976) 29:267–75. doi: 10.1016/0022-510X(76)90176-3

9. Krinsley JS. Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients. *Mayo Clin Proc.* (2003) 78:1471–8. doi: 10.4065/78.12.1471

10. Roberts GW, Quinn SJ, Valentine N, Alhawassi T, O'Dea H, Stranks SN, et al. Relative hyperglycemia, a marker of critical illness: introducing the stress hyperglycemia ratio. *J Clin Endocrinol Metab.* (2015) 100:4490–7. doi: 10.1210/jc.2015-2660

11. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C assay into estimated average glucose values. *Diabetes Care.* (2008) 31:1473–8. doi: 10.2337/dc08-0545

12. Tziomalos K, Dimitriou P, Bouziana SD, Spanou M, Kostaki S, Angelopoulou SM, et al. Stress hyperglycemia and acute ischemic stroke in- hospital outcome. *Metabolism.* (2017) 67:99–105. doi: 10.1016/j.metabol.2016.11.011

13. Szczudlik A, Slowik A, Turaj W, Wyrwicz-Petkow U, Pera J, Dziedzic T, et al. Transient hyperglycemia in ischemic stroke patients. *J Neurol Sci.* (2001) 189:105–11. doi: 10.1016/S0022-510X(01)00566-4

14. Hu GC, Hsieh SF, Chen YM, Hsu HH, Hu YN, Chien KL. Relationship of initial glucose level and all- cause death in patients with ischaemic stroke: the roles of diabetes mellitus and glycated hemoglobin level. *Eur J Neurol.* (2012) 19:884–91. doi: 10.1111/j.1468-1331.2011.03647.x

15. Roquer J, Giralt-Steinhauer E, Cerdà G, Rodríguez-Campello A, Cuadrado-Godia E, Jiménez-Conde J, et al. Glycated hemoglobin value combined with initial glucose levels for evaluating mortality risk in patients with ischemic stroke. *Cerebrovasc Dis.* (2015) 40:244–50. doi: 10.1159/000440735

16. Kim JT, Jahan R, Saver JL, Investigators S. Impact of glucose on outcomes in patients treated with mechanical thrombectomy: a post hoc analysis of the solitaire flow restoration with the intention for thrombectomy study. *Stroke.* (2016) 47:120–7. doi: 10.1161/STROKEAHA.115.010753

17. Ye XH, Cai XL, Nie DL, Chen YJ, Li JW, Xu XH, et al. Stress- induced hyperglycemia and remote diffusion-weighted imaging lesions in primary intracerebral hemorrhage. *Neurocrit Care.* (2019) 32:427–36. doi: 10.1007/s12028-019-00747-y

18. Chen X, Liu Z, Miao J, Zheng W, Yang Q, Ye X, et al. High stress hyperglycemia ratio predicts poor outcome after mechanical thrombectomy for ischemic stroke. *J Stroke Cerebrovasc Dis.* (2019) 28:1668–73. doi: 10.1016/j.jstrokecerebrovasdis.2019.02.022

19. Wang L, Zhou Z, Tian X, Wang H, Yang D, Hao Y, et al. Impact of relative blood glucose changes on mortality risk of patient with acute ischemic stroke and treated with mechanical thrombectomy. *J Stroke Cerebrovasc Dis.* (2019) 28:213–9. doi: 10.1016/j.jstrokecerebrovasdis.2018.09.036

20. Zhu B, Pan Y, Jing J, Meng X, Zhao X, Liu L, et al. Stress hyperglycemia and outcome of non-diabetic patients after acute ischemic stroke. *Front Neurol.* (2019) 10:1003. doi: 10.3389/fneur.2019.01003

21. Li J, Quan K, Wang Y, Zhao X, Li Z, Pan Y, et al. Effect of stress hyperglycemia on neurological deficit and mortality in the acute ischemic stroke people with and without diabetes. *Front Neurol.* (2020) 11:576895. doi: 10.3389/fneur.2020.576895

22. Merlino G, Pez S, Gigli GL, Sponza M, Lorenzut S, Surcinelli A, et al. Stress hyperglycemia in patients with acute ischemic stroke due to large vessel occlusion undergoing mechanical thrombectomy. *Front Neurol.* (2021) 12:725002. doi: 10.3389/fneur.2021.725002

23. Merlino G, Smeralda C, Gigli GL, Lorenzut S, Pez S, Surcinelli A, et al. Stress hyperglycemia is predictive of worse outcome in patients with acute ischemic stroke undergoing intravenous thrombolysis. *J Thrombosis Thrombolysis.* (2021) 51:789–97. doi: 10.1007/s11239-020-02252-y

24. Roberts G, Sires J, Chen A, Thynne T, Sullivan C, Quinn S, et al. A comparison of the stress hyperglycemia ratio, glycemic gap, and glucose to assess the impact of stress-induced hyperglycemia on ischemic stroke outcome. *J Diabetes.* (2021) 13:1034–42. doi: 10.1111/1753-0407.13223

25. Shen CL, Xia NG, Wang H, Zhang WL. Association of stress hyperglycemia ratio with acute ischemic stroke outcomes post-thrombolysis. *Front Neurol.* (2021) 12:785428. doi: 10.3389/fneur.2021.785428

26. Yuan C, Chen S, Ruan Y, Liu Y, Cheng H, Zeng Y, et al. The stress hyperglycemia ratio is associated with hemorrhagic transformation in patients with acute ischemic stroke. *Clin Interventions Aging.* (2021) 16:431–42. doi: 10.2147/CIA.S280808

27. Cai ZM, Zhang MM, Feng RQ, Zhou XD, Chen HM, Liu ZP, et al. Fasting blood glucose-to-glycated hemoglobin ratio and all-cause mortality among chinese in-hospital patients with acute stroke: a 12-month follow-up study. *BMC Geriatr.* (2022) 22:508. doi: 10.1186/s12877-022-03203-3

28. Chen G, Ren J, Huang H, Shen J, Yang C, Hu J, et al. Admission random blood glucose, fasting blood glucose, stress hyperglycemia ratio, and functional outcomes in patients with acute ischemic stroke treated with intravenous thrombolysis. *Front Aging Neurosci.* (2022) 14:782282. doi: 10.3389/fnagi.2022.782282

29. Chu H, Huang C, Tang Y, Dong Q, Guo Q. The stress hyperglycemia ratio predicts early hematoma expansion and poor outcomes in patients with spontaneous intracerebral hemorrhage. *Therapeutic Adv Neurol Disord.* (2022) 15:17562864211070681. doi: 10.1177/17562864211070681

30. Li S, Wang Y, Wang W, Zhang Q, Wang A, Zhao X. Stress hyperglycemia is predictive of clinical outcomes in patients with spontaneous intracerebral hemorrhage. *BMC Neurol.* (2022) 22:236. doi: 10.1186/s12883-022-02760-9

31. Merlino G, Pez S, Tereshko Y, Gigli GL, Lorenzut S, Surcinelli A, et al. Stress hyperglycemia does not affect clinical outcome of diabetic patients receiving intravenous thrombolysis for acute ischemic stroke. *Front Neurol.* (2022) 13:903987. doi: 10.3389/fneur.2022.903987

32. Mi D, Li Z, Gu H, Jiang Y, Zhao X, Wang Y, et al. Stress hyperglycemia is associated with in-hospital mortality in patients with diabetes and acute ischemic stroke. *CNS Neurosci Therap.* (2022) 28:372–81. doi: 10.1111/cns.13764

33. Wang L, Cheng Q, Hu T, Wang N, Wei X, Wu T, et al. Impact of stress hyperglycemia on early neurological deterioration in acute ischemic stroke patients treated with intravenous thrombolysis. *Front Neurol.* (2022) 13:870872. doi: 10.3389/fneur.2022.870872

34. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol.* (2009) 62:e1–34. doi: 10.1016/j.jclinepi.2009.06.006

35. Huang YW, Yin XS, Li ZP. Association of the stress hyperglycemia ratio and clinical outcomes in patients with stroke: a systematic review and meta-analysis. *PROSPERO.* (2022) CRD42022345587. Available online at: https://www.crd.york.ac.uk/prospero/display_record.php?ID$=$CRD42022345587 (accessed July 24, 2022).

36. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomized Studies in Meta-Analyses*. Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm (accessed February 27, 2020).

37. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* (1986) 7:177–88. doi: 10.1016/0197-2456(86)90046-2

38. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* (2002) 21:1539–58. doi: 10.1002/sim.1186

39. Marik PE, Bellomo R. Stress hyperglycemia: an essential survival response! *Crit Care.* (2013) 17:305. doi: 10.1186/cc12514

40. Gasche Y, Copin JC, Sugawara T, Fujimura M, Chan PH. Matrix metalloproteinase inhibition prevents oxidative stress-associated blood-brain barrier disruption after transient focal cerebral ischemia. *J Cereb Blood Flow Metab.* (2001) 21:1393–400. doi: 10.1097/00004647-200112000-00003

41. Vanhorebeek I, Van den Berghe G. Diabetes of injury: novel insights. *Endocrinol Metab Clin North Am.* (2006) 35:859–72. doi: 10.1016/j.ecl.2006.09.002

42. Parsons MW, Barber PA, Desmond PM, Baird TA, Darby DG, Byrnes G, et al. Acute hyperglycemia adversely affects stroke outcome: a magnetic resonance imaging and spectroscopy study. *Ann Neurol.* (2002) 52:20–8. doi: 10.1002/ana.10241

43. Lemkes BA, Hermanides J, Devries JH, Holleman F, Meijers JC, Hoekstra JB. Hyperglycemia: a prothrombotic factor? *J Thromb Haemost.* (2010) 8:1663–9. doi: 10.1111/j.1538-7836.2010.03910.x

44. Seners P, Turc G, Oppenheim C, Baron JC. Incidence, causes and predictors of neurological deterioration occurring within 24 h following acute ischaemic stroke: a systematic review with pathophysiological implications. *J Neurol Neurosurg Psychiatry.* (2015) 86:87–94. doi: 10.1136/jnnp-2014-308327

45. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *J Am Med Assoc.* (2006) 295:1681–7. doi: 10.1001/jama.295.14.1681

46. Li Q, Liu QJ, Yang WS, Wang XC, Zhao LB, Xiong X, et al. Island sign: an imaging predictor for early hematoma expansion and poor outcome in patients with intracerebral hemorrhage. *Stroke.* (2017) 48:3019–25. doi: 10.1161/STROKEAHA.117.017985

47. Li Q, Zhang G, Huang YJ, Dong MX, Lv FJ, Wei X, et al. Blend sign on computed tomography: novel and reliable predictor for early hematoma growth in patients with intracerebral hemorrhage. *Stroke.* (2015) 46:2119–23. doi: 10.1161/STROKEAHA.115.009185

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High systemic immune-inflammation index is associated with carotid plaque vulnerability: New findings based on carotid ultrasound imaging in patients with acute ischemic stroke

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Vulnerable carotid plaque is closely related to the occurrence of Ischemic stroke. Therefore, accurate and rapid identification of the nature of carotid plaques is essential. AS is a chronic immune inflammatory process. Systemic immune-inflammation index (SII) is a novel index of immune inflammation obtained from routine whole blood cell count analysis, which comprehensively reflects the state of inflammation and immune balance in the body. This study sought to explore the relationship between SII level and carotid plaque vulnerability, plaque composition characteristics, and acute ischemic stroke (AIS) severity. A total of 131 patients diagnosed with AIS presenting with a carotid atherosclerotic plaque were enrolled in this study. Using carotid ultrasound (CDU) to assess the carotid-responsible plaque properties, we divided the patients into stable plaques group and vulnerable plaques group, and analyzed the correlation between SII levels and plaque vulnerability. And we further analyzed to evaluate the correlation between high SII levels and plaque characteristics and AIS severity. In addition, Cohen's Kappa statistics was used to detect the consistency of Carotid ultrasound (US) and cervical High-resolution magnetic resonance imaging (HRMRI) in evaluating plaque vulnerability. The findings showed that the vulnerable group had higher levels of SII compared with the stable group. The high SII group had more vulnerable plaques and a high frequency of plaque fibrous cap rupture compared with the low SII group. Logistic analysis showed that a high SII level was an independent risk factor for vulnerable plaques (odds ratio [OR] = 2.242) and plaque fibrous cap rupture (OR=3.462). The results also showed a high consistency between Carotid US and HRMRI methods in the assessment of plaque vulnerability

[Cohen's kappa value was 0.89 (95% CI = 0.78–0.97)] and the level of SII was positively associated with NIHSS score ($r = 0.473$, $P < 0.001$). Our study suggests that elevated levels of SII may have adverse effects on the vulnerability of carotid plaques, especially in stroke patients with vulnerable plaques with ruptured fibrous caps, which may aggravate the severity of AIS.

KEYWORDS

carotid ultrasound, systemic immune-inflammation index, acute ischemic stroke, vulnerable plaque, high-resolution magnetic resonance imaging

Introduction

Ischemic stroke (IS) is the most common clinical subtype of stroke. It is associated with high morbidity, disability, and mortality. Thus, it is a health burden worldwide. Atherosclerosis (AS) is a major cause of atherosclerotic ischemic stroke (AIS). Studies report that AS is a chronic immune inflammatory process associated with a variety of immune-inflammatory cells and mediators and causes instability of plaque structure (1). The risk of AS increases with an increase in atherosclerotic plaque vulnerability (2). Therefore, the nature of carotid plaque should be identified in a timely and accurate manner. It is imperative to explore immuno-inflammatory markers for early and accurate prediction of carotid plaque, thus improving the prevention and treatment of AIS. The systemic immune-inflammation index (SII) is a novel index of immune inflammation, which comprehensively reflects the state of inflammation and immune balance in the body (3, 4). SII can be obtained from routine whole blood cell count analysis.

Previous studies have extensively explored the application of SII in cardio-cerebrovascular diseases, tumors, and other diseases (3–6). However, few studies have explored the relationship between SII and vulnerability of carotid plaque and the severity of AIS. The present study sought to determine the associations between SII and plaque vulnerability and AIS severity.

Methods

Study population

This study was approved by the Ethics Committee of the First People's Hospital of Yancheng. All participants provided an informed consent before participating in the study. The study was a bidirectional cohort study. A total of 131 patients diagnosed with AIS presenting with carotid atherosclerotic plaque admitted to the Stroke Center of the First People's Hospital of Yancheng between June 2020 and May 2021 were enrolled in this study. CDU was used to evaluate the atherosclerotic plaque properties of the responsible vessels. Inclusion criteria: (1) patients with acute internal carotid artery system (ICA) ischemic stroke, aged ≥ 18 years; (2) time from onset to admission <3 days; (3) brain MRI and the carotid US performed within 1 week after onset of neurovascular symptoms, and conditional patients underwent cervical HRMRI. Exclusion criteria: (1) patients with intracranial arterial stenosis and other causes such as vasculitis and moyamoya disease; (2) patients with severe cardiovascular disease, hematological disorder, and hepatorenal insufficiency; (3) patients with severe infection, sepsis, malignant tumor, or autoimmune disease, and patients who were taking immunosuppressants, glucocorticoids, or cytotoxic drugs during the time of recruitment; (4) patients whose two carotid arteries (common carotid artery and internal carotid artery) had no plaque or patients without at least one carotid artery occlusion; and (6) patients with incomplete clinical baseline, laboratory examination, and imaging data (poor image quality could not be distinguished).

Clinical baseline data were collected within 24 h of admission including sex, age, hypertension, diabetes, coronary heart disease, history of stroke, history of smoking, National Institutes of Health Stroke scale (NHISS) score, and Laboratory data. Laboratory data recorded in this study included routine blood test, SII, fasting blood glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, hypersensitive C-reactive protein, homocysteine, fibrinogen, and uric acid level. SII value was defined as follows: SII = P × N/L, whereby P, N, and L indicated the peripheral blood platelet, neutrophil, and lymphocyte counts (7) at admission, respectively. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, and patients were undergoing treatment with antihypertensive drugs (8). Diabetes mellitus was defined as fasting blood glucose level ≥ 7.0 mmol/L or at any time and (or) glucose tolerance test 2H plasma glucose level ≥ 11.0 mmol/L (9). Coronary heart disease was defined as coronary angiography showing the left main coronary artery, left anterior descending branch, circumflex branch, right coronary artery, and main branches of any lumen diameter stenosis ≥50% (10). History of smoking was defined as smoking more than one cigarette a day for more than 1 year (11). Dyslipidemia was defined as total cholesterol >5.20 mmol/L, triglyceride >1.70

mmol/L, high-density lipoprotein cholesterol >2.00 mmol/L or <0.94 mmol/L, and low-density lipoprotein cholesterol >3.36 mmol/L (12).

Carotid US protocol

Diagnosis in all patients was performed using a Resona8 scanner (Mindray Medical System). L14-5WE linear array probe and 3C5S convex array probe were used to extend the blood vessels from the proximal to the distal segment. Continuous cross-sectional and longitudinal scans were then performed. Bilateral common carotid arteries (CCA), carotid artery bulb (CAB), and internal carotid arteries (ICA) were explored through grayscale imaging, color flow imaging, and spectral Doppler analysis. Multi-section and multi-angle imaging were performed to identify the presence of plaques, determine the plaque size, shape, echo, integrity, and degree of vascular stenosis, as well as the peak systolic velocity, end-diastolic velocity, and resistance index (13). All images of carotid atherosclerotic plaques were saved and analyzed.

Carotid HRMRI protocol

Patients underwent HRMRI examinations through a 3.0T MRI system (Signa Pioneer, GE Healthcare, Fairfield). Head and neck joint coil and ECG gating were selected and the standard carotid artery multi-sequence contrast imaging scheme was scanned. The 2D time of flight MR angiography (TOFMRA) imaging was performed to determine the bifurcation position of the common carotid artery. The 3DTOF MRA imaging was performed after locating the common carotid artery bifurcation, followed by rotational imaging reconstruction using maximum intensity projection (MIP). The location of the plaque was determined by combining the cross-sectional position of TOF and the reconstructed image. High-resolution target scanning of black blood sequence [including Double IR T1-weighted imaging (DIRT1WI), T2-weighted imaging (T2WI), and Proton density-weighted imaging (PDWI)] were performed to explore cervical vessels and targeted plaques. Gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA) enhanced SET1WI, and Gd-DTPA were administered into the cubital vein at a concentration of 0.1 mmol/kg.

Carotid US and HRMRI interpretation

An experienced vascular radiologist with 10-year experience evaluated the carotid plaque images. The radiologist was blinded to the clinical information and HRMRI scan. A second experienced vascular radiologist with a 10-year of experience explored the vulnerability of the plaque within a month after the initial evaluation, to determine the reproducibility of the process. Carotid US criteria for judging the nature of atherosclerotic plaque were as follows: vulnerable plaque: hypoechoic plaque, with or without irregular plaque surface shape or had incomplete fibrous cap and presence of plaque blood flow signal (ulcerative plaque); and Stable plaque: plaque was isoechoic or hyperechoic, plaque surface was regular, or the fibrous cap was intact (14–16).

Two experienced radiologists (>5 years of experience in neuroradiology) blinded to the clinical information and CDU scans independently analyzed the carotid plaque images. The two radiologists conducted all data analyses separately. The consensus of the two experts was used as the final result in case of any inconsistencies.

American Heart Association (AHA) was used as HRMRI classification criteria for the determination of atherosclerotic plaques as follows (17): vulnerable plaques: type IV-V: plaques with large necrotic fat nuclei and fibrous caps and plaques with a small amount of calcification; type VI: plaque with surface ulcers, or intra-plaque bleeding, thrombosis; and stable plaques: type II: diffuse intimal thickening or small non-calcified eccentric plaques; type VII: calcified plaque; type VIII: fibrous plaque without a fat nucleus, and with a low level of calcification. HRMRI results for the determination of plaque properties were highly consistent with plaque histopathological results; therefore, the present study used HRMRI results as the non-invasive "gold standard" for the determination of plaque properties (18).

The echogenicity of carotid plaques was divided into three groups: hypoechoic, isoechoic, and hyperechoic regarding the echogenicity of the vessel lumen and adventitia. Hypoechogenicity was defined as echogenicity similar to the vessel lumen. It looks black or almost black. Hyperechogenicity was defined as echogenicity similar to the adjacent adventitia. Isoechogenicity was defined as intermediate between hypoechogenicity and hyperechogenicity (19). Irregular plaque was defined as the plaque depth variation between 0.4 and 2 mm along the contour of the lesion (20). Carotid plaque fibrous cap rupture was defined as an arcuate line-like hyperechoic discontinuity at the surface of the plaque. An ulcerated plaque was defined as a plaque surface with depression (length and depth of the depression $\geq$ 2 mm) on at least two sonographically accessible surfaces and a well-defined back wall at its base. Color Doppler (CDFI) shows a "crater" - like filling defect (20). Calcification was defined as a hyperechoic area posteriorly within the plaque with acoustic-shadowing (21). Thrombus was defined as an isoechogenic material that partly or totally filled the carotid artery lumen (22).

Diffusion-weighted imaging (DWI) results were interpreted and the recent acute cerebral infarction (AIS) was evaluated according to the consensus of two neuroradiologists. American Heart Association/American Stroke Association guidelines were used for ischemic stroke diagnosis (8).

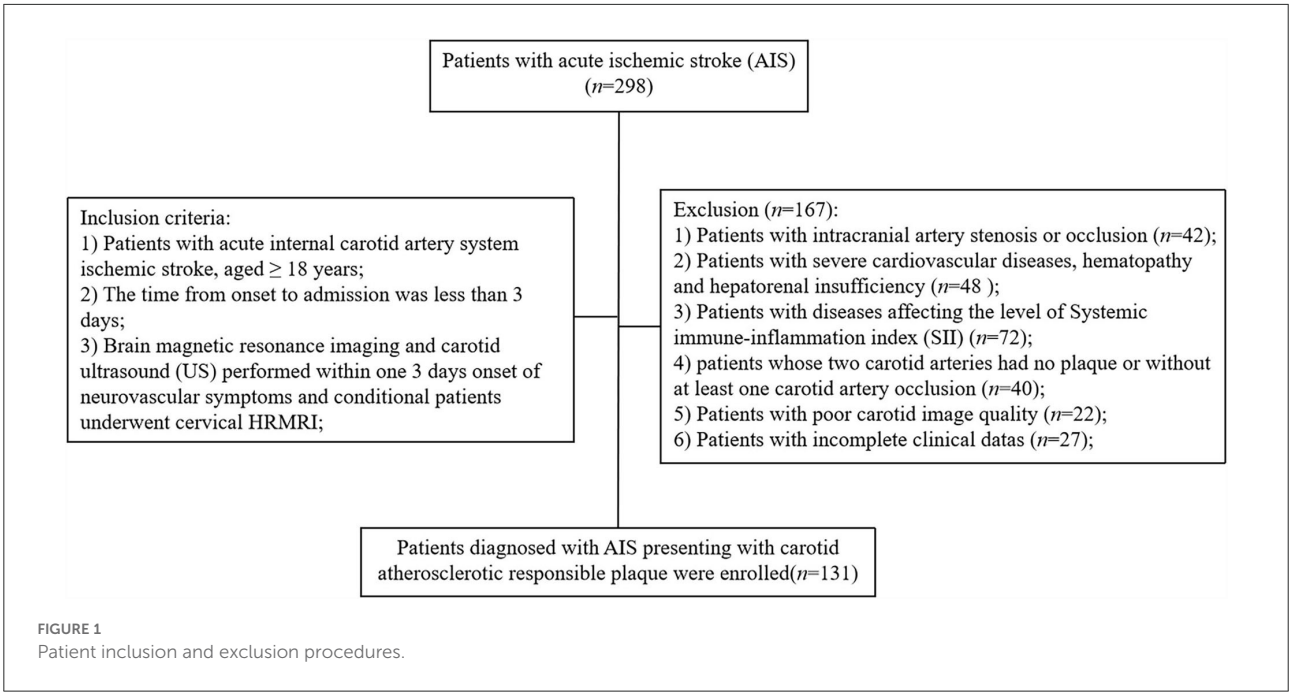

FIGURE 1
Patient inclusion and exclusion procedures.

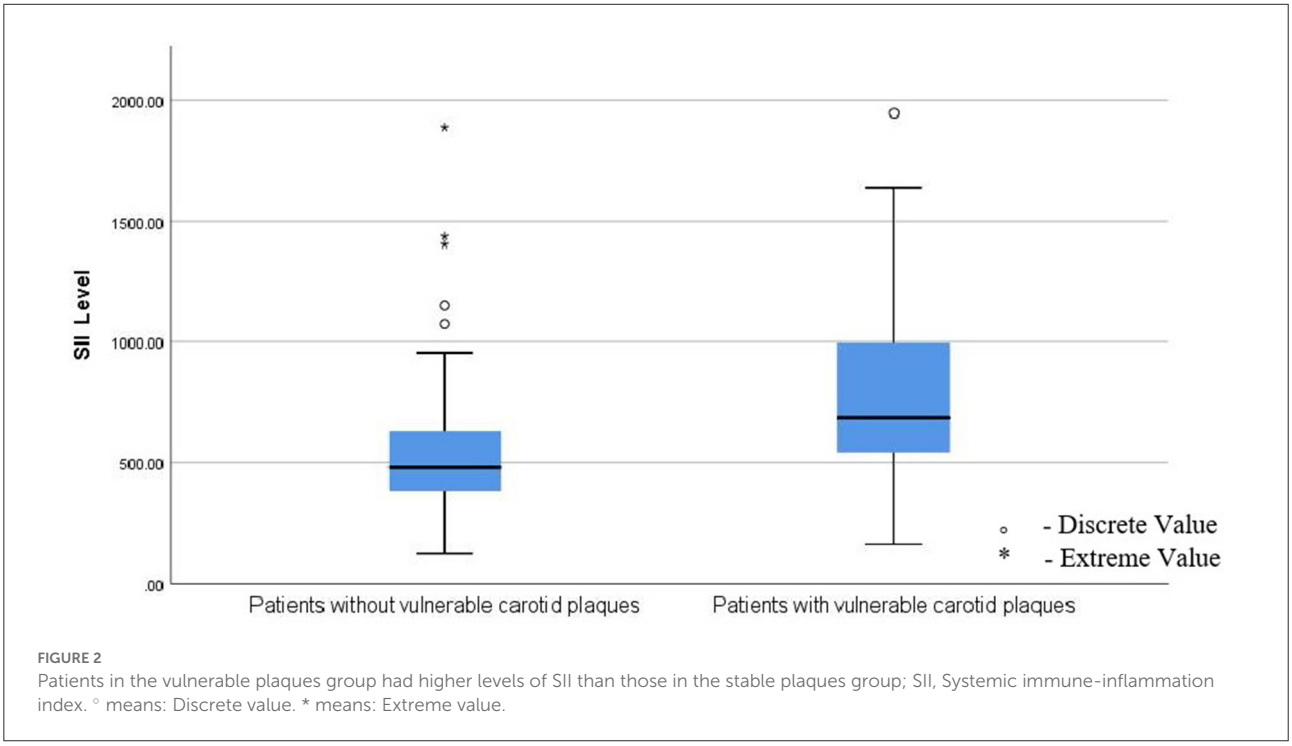

FIGURE 2
Patients in the vulnerable plaques group had higher levels of SII than those in the stable plaques group; SII, Systemic immune-inflammation index. ◦ means: Discrete value. * means: Extreme value.

Evaluation of the severity of acute cerebral infarction

NIHSS Stroke scale is a widely used scoring index for the determination of stroke severity in clinics. The scoring method is simple and easy and can be used to objectively and comprehensively evaluate dysfunction after stroke. The evaluation result criteria and objectivity can be used as a reliable tool to determine the severity and prognosis of stroke (23, 24). The total score is 42; a higher score denotes severe neurological impairment and consequently severe stroke. A score of 0 indicates the absence of neurological impairment symptoms, <6

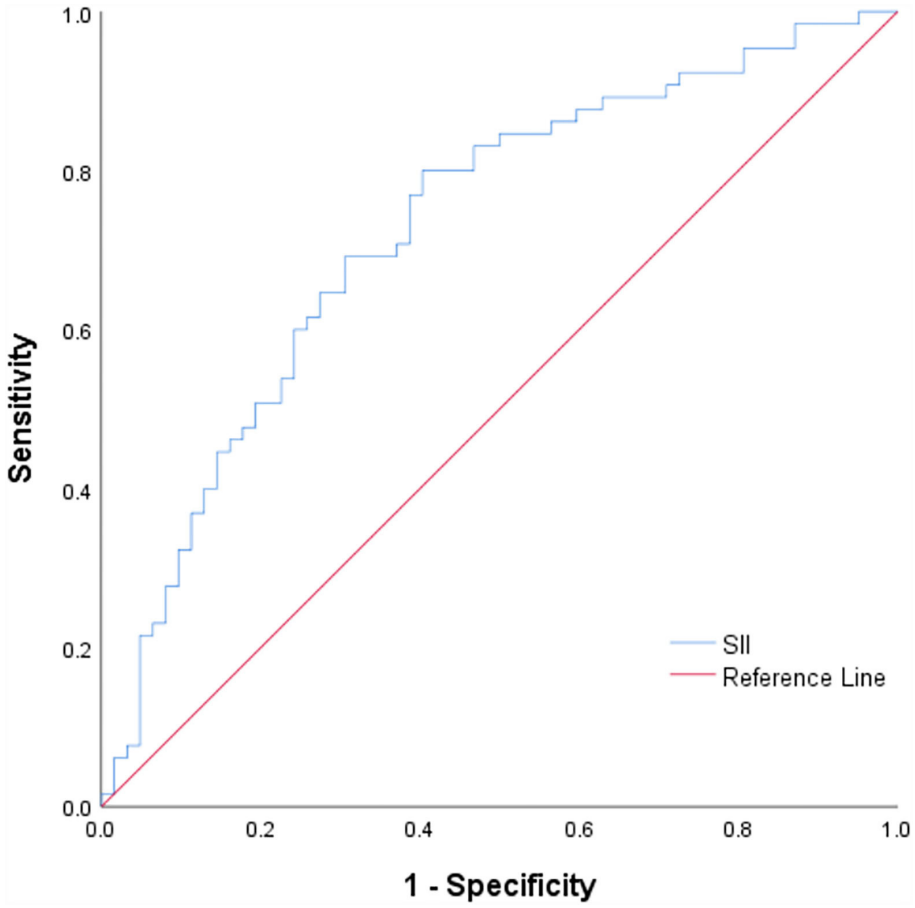

FIGURE 3
ROC curve for the SII values to predict plaque vulnerability. ROC, receiving operating characteristic curves; SII, Systemic immune-inflammation index.

score defined as mild stroke, and ≥6 score defined as moderate-severe stroke (25).

Statistical analysis

First, CDU was used to evaluate the nature of carotid responsible plaque, and the patients were divided into stable and vulnerable groups. Then, the best cut-off value of SII to evaluate plaque vulnerability was calculated through the analysis of the receiver operating characteristic (ROC) curve, and the patients were then assigned to the high SII group [≥541.27 (10^9/L)] and low SII group [< 541.27 (10^9/L)] according to the cut-off value of SII. Continuous data were expressed as mean ± standard deviation or median [quartile range (IQR)]. Classified data were expressed as frequencies (percentage). The normality of continuous variable distribution was determined using the Kolmogorov–Smirnov test. Data of the two groups were compared using the chi-square test and Mann–Whitney U test. Logistic regression analysis was performed to evaluate the correlation between the carotid plaque vulnerability, the characteristics of vulnerable plaques, and SII (including parameters with significant values < 0.10 in univariate analysis) using odds ratio (OR) and 95% confidence interval (CI). Cardiovascular and cerebrovascular risk factors and demographic factors were adjusted for as confounding factors during logistic regression analysis. Spearman test was used to evaluate the relationship between SII and NIHSS scores (Correlation coefficient(r)<0.4 was low correlation, 0.4 ≤ r <0.7 was medium correlation, and ≥0.7 was high correlation). CDU and HRMRI were used to determine the properties of carotid plaque and the consistency between observers (kappa ≤ 0.40 for poor consistency, 0.40–0.75 for good consistency, ≥0.75 for excellent consistency). All statistical analyses were performed using SPSS software (version 26.0, IBM company, Armonk). All tests were two-tailed, with a *p*-value threshold of 0.05 for statistical significance.

TABLE 1 Baseline data of patients according to SII level ($N = 131$).

Characteristics	SII ≥541.27(10^9/L) ($N = 79$)	SII <541.27(10^9/L) ($N = 52$)	*P*-value*
Age (years)	62.26 ± 10.91	61.96 ± 12.10	0.739
Sex, male	55(83.1%)	43(86.0%)	0.614
Hypertension	37(46.8%)	17(32.7%)	0.056
Systolic blood pressure (mmHg)	143.84 ± 18.25	133.06 ± 17.21	0.001*
Diastolic blood pressure (mmHg)	85.04 ± 13.03	83.53 ± 11.20	0.423
Dyslipidemia	25(31.6%)	19(36.5%)	0.537
Diabetes mellitus	26(32.9%)	16(30.8%)	0.494
Coronary heart disease	30(37.9%)	18(34.6%)	0.302
History of alcohol intake	24(30.4%)	9(17.3%)	0.443
Current or former smokers	28(35.4%)	11(21.2%)	0.086
History of stroke	21(26.6%)	20(38.5%)	0.153
NHISS	11.0 [8.0–14.3]	4.0 [4.0–5.0]	<0.001*
Neurological symptoms			
Unilateral limb symptoms	38(48.1%)	29(55.8%)	0.617
Indistinct speech	21(26.6%)	14(26.9%)	0.931
Blurred vision	14(17.7%)	11(21.2%)	0.731
Dizzy	12(15.2%)	11(21.2%)	0.759
Headache	10(12.7%)	8(15.4%)	0.486
Laboratory tests			
Fasting blood-glucose(mmol/l)	5.74 ± 1.92	5.58 ± 1.21	0.178
Total cholesterol(mmol/l)	3.93 ± 0.94	3.99 ± 0.91	0.723
Triglycerides(mmol/l)	1.2[1.0–1.9]	1.5[1.1–1.5]	0.070
HDL-C(mmol/l)	0.97 ± 0.20	0.99 ± 0.21	0.184
LDL-C(mmol/l)	2.4[1.6–3.2]	2.3[1.9–3.1]	0.382
hs-CRP (mmol/l)	3.2[2.0–4.7]	2.7[1.5–5.9]	0.057
Uric acid(mmol/l)	324.55 ± 77.35	295.27 ± 85.15	0.269
Fibrinogen(mmol/l)	3.3[2.2–4.3]	3.1[2.2–4.7]	0.066
HCY (mmol/l)	9.8[6.7–12.4]	9.7[7.8–11.6]	0.845
Cardiovascular medication			
Statin treatment	35(44.3%)	20(38.5%)	0.688
Antiplatelet treatment	30(37.9%)	21(40.4%)	0.637

*means P-values indicating statistically significant. SII, systemic immune-inflammation index; NIHSS, National Institutes of Health Stroke Scale; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, high-sensitive C-reactive protein; HCY, homocysteine.

Results

Patient characteristics

A total of 298 patients with AIS admitted at the Stroke Center of the First People's Hospital of Yancheng between June 2020 and May 2021 were included in this study (Figure 1). Clinical and imaging examination of the 298 patients showed that 42 (14.1%) patients had intracranial artery stenosis or occlusion, 48 (16.1%) patients were diagnosed with severe cardiovascular disease, and 72 (24.2%) patients were diagnosed with diseases affecting the level of SII. Notably, 40 (13.4%) patients had at least one carotid artery occlusion, 22 (7.4%) patients presented with poor carotid image quality, and 27 (9.1%) patients were excluded due to incomplete clinical data. After exclusion and inclusion screening, a total of 131 (43.9%) patients were enrolled in the present two-way cohort study. The mean age of the 131 patients was 61.86 ± 12.37 years with 98 (74.8%) male patients. Out of the 131 patients, 57 patients (43.5%) were diagnosed with hypertension and 42 patients (32.1%) presented with diabetes; 41 (31.3%) patients reported a history of stroke and 62 (47.3%) patients were on medication (42.0% were on satin whereas 38.9% were on antiplatelet medication). Ultrasonographic findings of 131 responsible plaques and HRMRI findings of 103 (78.6%) plaques were recorded.

TABLE 2 CDU imaging findings of carotid responsible plaques in patients with different SII levels.

Characteristics	SII≥541.27(10^9/L) ($N = 79$)	SII<541.27(10^9/L) ($N = 52$)	*P*-value*
Plaque presence	64(81.0%)	39(75.0%)	0.412
Vulnerable plaque	53(67.1%)	23(44.2%)	0.013*
Plaque features			
Irregular surface morphology	32(40.5%)	16(30.8%)	0.093
Ruptured fibrous cap	30(37.9%)	10(19.2%)	0.032*
Ulcerative plaque	13(16.5%)	9(17.3%)	0.624
LRNC or IPH prevalence	54(68.4%)	32(61.5%)	0.467
CA prevalence	37(46.8%)	30(57.7%)	0.076
Thrombus	8(10.1%)	6(11.5%)	0.732

*means P-values indicating statistically significant. CDU, color duppler ultrasound; SII, systemic immune-inflammation index; LRNC, lipid-rich necrotic core; IPH, intraplaque hemorrhage; CA, calcification.

Comparison of SII level between stable plaques group and vulnerable plaques group

The baseline serum SII level was 541.27 [407.23–846.27] (10^9/L), and the patients in the vulnerable plaques group had higher SII levels than those in the stable plaques group (Figure 2). The difference was statistically significant ($P < 0.001$).

Comparison of baseline data between high and low SII groups

The cut-off value of SII was calculated through ROC curve analysis (Figure 3). The 131 patients were assigned to two groups: SII < 541.27(10^9/L) ($n = 52$, low) and SII ≥ 541.27 (10^9/L) ($n = 79$, high) following a method described previously (26). Baseline demographic characteristics, clinical characteristics, and laboratory results of the two groups are presented in Table 1. The results showed that admission systolic blood pressure ($P = 0.001$) and NHISS score ($P < 0.001$) were significantly different between the two groups. However, there was no significant difference in other characteristics between the two groups. Characteristics of 131 responsible plaques in the high SII group and the low SII group are presented in Table 2. The prevalence of vulnerable plaques was higher in the high SII group relative to the prevalence in the low SII group (67.1 vs. 44.2%, $P = 0.013$). The rate of rupture of plaque fibrous cap in patients with anterior circulation infarction was higher in the high SII group compared with the rate in the patients with low SII (37.9 vs. 19.2%, $p = 0.032$). The results showed no significant difference in characteristics of other plaques between the two groups.

Relationship between the level of SII and characteristics of symptomatic carotid plaque

Univariate logistic regression and multivariate logistic analysis after adjusting for possible confounding factors showed that high SII level [odds ratio (OR) = 2.242, 95% confidence interval (CI) = 1.378–4.024, $P = 0.023$] was an independent risk factor for vulnerable plaques (Table 3). In addition, coronary heart disease [odds ratio (OR) = 4.774, 95% confidence interval (CI) = 1.337–17.049] and high SII level [odds ratio (OR) = 3.462, 95% confidence interval (CI) = 2.031–6.374] were independent risk factors for ruptured fibrous cap (Table 4).

Comparison of consistency between CDU and HRMRI imaging of the same carotid plaque

The consistency of the two imaging techniques was explored through a comparison of detecting characteristics of vulnerable plaques and vulnerable plaques (Figure 4, Table 2). The Cohen's kappa value of the two methods for detecting vulnerable plaques was 0.89 (95% CI = 0.78–0.97) (Table 5). The Cohen's kappa values for detecting characteristics of vulnerable plaques (irregular surface morphology, ruptured fibrous cap, ulcerative plaque, lipid-rich necrotic core (LRNC) or intraplaque hemorrhage (IPH), calcification (CA) and thrombus) were 0.77 (95% CI = 0.62–0.91), 0.78 (95% CI = 0.63–0.92), 0.80 (95% CI = 0.63–0.97), 0.86 (95% CI = 0.75–0.98), 0.70 (95% CI = 0.60–0.86), and 0.82 (95% CI = 0.62∼1.00), respectively.

TABLE 3 Univariate and multivariate logistic regression analysis of factors related to vulnerability plaque ($N = 131$).

	Vulnerability plaques presence					
	Univariate analysis		Multivariate analysis			
			Modle1		Modle2	
Characteristics	OR [95% CI]	*P*-value*	OR [95% CI]	*P*-value*	OR [95% CI]	*P*-value*
Clinical parameters						
Age (years)	1.248[0.978–3.041]	0.701	0.999[0.958–1.043]	0.989	1.001[0.957–1.047]	0.991
Sex, male	0.945[0.364–2.457]	0.908	0.760[0.175–3.311]	0.715	0.864[0.183–4.086]	0.853
Hypertension	0.796[0.395–1.600]	0.521				
Systolic blood pressure(mmHg)	1.022[1.002–1.043]	0.039*			1.018[0.989-1.049]	0.237
Diastolic blood pressue(mmHg)	1.016[0.987–1.045]	0.288				
Hyperlipemia	2.083[0.512–3.248]	0.852				
Diabetes mellitus	1.071[0.507–2.266]	0.857				
Coronary heart disease	2.003[0.953–4.212]	0.067				
History of alcohol intake	0.982[0.444–2.171]	0.964				
Current or former smokers	0.738[0.346–1.576]	0.433				
History of stroke	1.806[0.841–3.878]	0.129				
Laboratory tests						
SII≥529.87 (10^9/L)	2.316[1.201–4.184]	0.005*	2.302[1.124–4.004]	0.005*	2.242[1.378–4.024]	0.023*
Fasting blood-glucose(mmol/l)	1.035[0.840–1.275]	0.746				
Total cholesterol(mmol/l)	0.666[0.446–0.994]	0.047*			0.687[0.182–2.590]	0.687
Triglycerides(mmol/l)	0.753[0.456–1.246]	0.270				
HDL-C(mmol/l)	0.844[0.208–3.435]	0.813				
LDL-C(mmol/l)	0.669[0.434–1.032]	0.069			1.043[0.247–4.399]	0.579
hs-CRP (mmol/l)	0.997[0.938–1.058]	0.913				
Uric acid(mmol/l)	1.000[0.996–1.004]	0.928				
Fibrinogen(mmol/l)	1.100[0.906–1.337]	0.336				
HCY (mmol/l)	1.021[0.950–1.097]	0.572				
Cardiovascular medication						
Statin treatment	0.745[0.370–1.501]	0.410				
Antiplatelet treatment	1.081[0.530–2.204]	0.830				

*means P-values indicating statistically significant. OR, odds ratio; CI, confidence interval; SII, systemic immune-inflammation index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, high-sensitive C-reactive protein; HCY, homocysteine. Model 1, adjusted for age and sex; model 2, adjusted for age, sex, systolic blood pressure, Total cholesterol, LDL-C.

Relationship between the level of SII and severity of acute cerebral infarction

Spearman test showed that SII was positively associated with NIHSS score ($r = 0.473$, $P < 0.001$), suggesting a medium correlation between SII and NHISS score (Figure 5).

Interobserver agreement

The Cohen's kappa value of carotid US for evaluation of the vulnerability of plaques by the two observers was 0.94 (95%CI = 0.92–0.98), and the Cohen's kappa values for evaluation characteristics of vulnerable plaques (irregular surface morphology, ruptured fibrous cap, ulcerative plaque, lipid-rich necrotic core (LRNC) or intraplaque hemorrhage (IPH), calcification (CA) and thrombus) by the two observers were 0.96 (95% CI = 0.92–0.98), 0.96 (95% CI = 0.91–0.97), 0.92 (95% CI = 0.89∼0.95), 0.97 (95%CI = 0.94–0.99), 0.97 (95%CI = 0.94–0.0.99), and 0.97 (95% CI = 0.94–1.00), respectively.

Discussion

Pathological features of atherosclerosis (AS) include chronic, low-grade inflammatory vascular diseases, which mainly occur in the major arteries (1). Ischemic stroke is a disease that is caused by various factors and is associated with high morbidity, disability, and mortality. Studies report that immune inflammation is involved in the occurrence, progression, and

TABLE 4 Univariate and multivariate logistic regression analysis of factors related to ruptured fibrous cap ($N = 131$).

	Ruptured fibrous cap					
	Univariate analysis		Multivariate analysis			
			Modle1		Modle2	
Characteristics	OR [95% CI]	*P*-value*	OR [95% CI]	*P*-value*	OR [95% CI]	*P*-value*
Clinical parameters						
Age (years)	1.001[0.968–1.035]	0.946	1.000[0.954–1.049]	0.998	1.004[0.953–1.058]	0.892
Sex, male	0.948[0.335–2.685]	0.920	0.429[0.072–2.543]	0.351	0.482[0.070–3.319]	0.459
Hypertension	1.827[0.840–3.977]	0.129				
Systolic blood pressure(mmHg)	1.024[1.003–1.046]	0.028*			1.015[0.981–1.051]	0.386
Diastolic blood pressure(mmHg)	1.022[0.991–1.055]	0.167				
Hyperlipemia	0.847[0.383–1.875]	0.683				
Diabetes mellitus	1.845[0.777–4.380]	0.165				
Coronary heart disease	2.611[1.196–5.700]	0.016*			4.774[1.337–17.049]	0.016*
History of alcohol intake	1.270[0.526–3.064]	0.595				
Current or former smokers	1.754[0.738–4.172]	0.203				
History of stroke	1.667[0.700–3.971]	0.249				
Laboratory tests						
SII≥529.87 (10^9/L)	3.532[1.981–5.014]	0.013*	3.417[1.879–5.804]	0.019*	3.462[2.031–6.074]	0.011*
Fasting blood-glucose(mmol/l)	0.913[0.716–1.166]	0.467				
Total cholesterol(mmol/l)	0.545[0.343–0.866]	0.010*			1.137[0.199–6.480]	0.885
Triglycerides(mmol/l)	0.893[0.517–1.544]	0.686				
HDL-C(mmol/l)	0.299[0.058–1.536]	1.148				
LDL-C(mmol/l)	0.565[0.345–0.925]	0.023*			0.436[0.064–2.981]	0.436
hs-CRP (mmol/l)	0.985[0.921–1.053]	0.655				
Uric acid(mmol/l)	1.003[0.999–1.007]	0.144				
Fibrinogen(mmol/l)	1.051[0.878–1.257]	0.591				
HCY (mmol/l)	1.029[0.953–1.110]	0.464				
Cardiovascular medication						
Statin treatment	0.634[0.297–1.356]	0.240				
Antiplatelet treatment	0.572[0.266–1.231]	0.153				

*means P-values indicating statistically significant. OR, odds ratio; CI, confidence interval; SII, systemic immune-inflammation index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, high-sensitive C-reactive protein; HCY, homocysteine. Model 1, adjusted for age and sex; model 2, adjusted for age, sex, coronary heart disease, systolic blood pressure, Stroke history.

prognosis of ischemic stroke and atherosclerosis (27, 28). Several studies have explored the relationship between peripheral blood immune cell profile and AIS because the method of peripheral blood immune cell detection is simple and the results are easily obtained. Luo et al. (29), reported that neutrophil-to-lymphocyte ratio (NLR) is associated with poor early prognosis in patients with AIS or transient ischemic attack (TIA). In addition, other studies report that the platelet-to-lymphocyte ratio (PNR) is a potential independent protective factor for predicting the prognosis of AIS (30). Systemic immune-inflammation index (SII) is a novel systemic immune-inflammation index obtained through the sum of lymphocyte count (L), neutrophil count (N), and platelet count (P). SII partially represents the balance of inflammation and immunity in the body. An increase in SII indicates that inflammatory response is enhanced and the immune response is weakened. This value was initially used in predicting the prognosis of various tumor diseases (7, 31). The present study sought to investigate the relationship between the level of SII and the vulnerability of carotid plaque and the severity of AIS.

Previous studies indicate that neutrophils play a key role in the inflammatory response associated with atherosclerosis (32–34). Neutrophils secrete high levels of inflammatory mediators, chemoattractants, and oxygen-free radicals to induce endothelial cell injury and subsequent tissue ischemia. Activation of monocytes and their transformation into lipid-rich macrophages is the key process resulting in atherosclerotic lesion formation (35). On the contrary, lymphocytes play a regulatory role. Moreover, platelets play a central role in

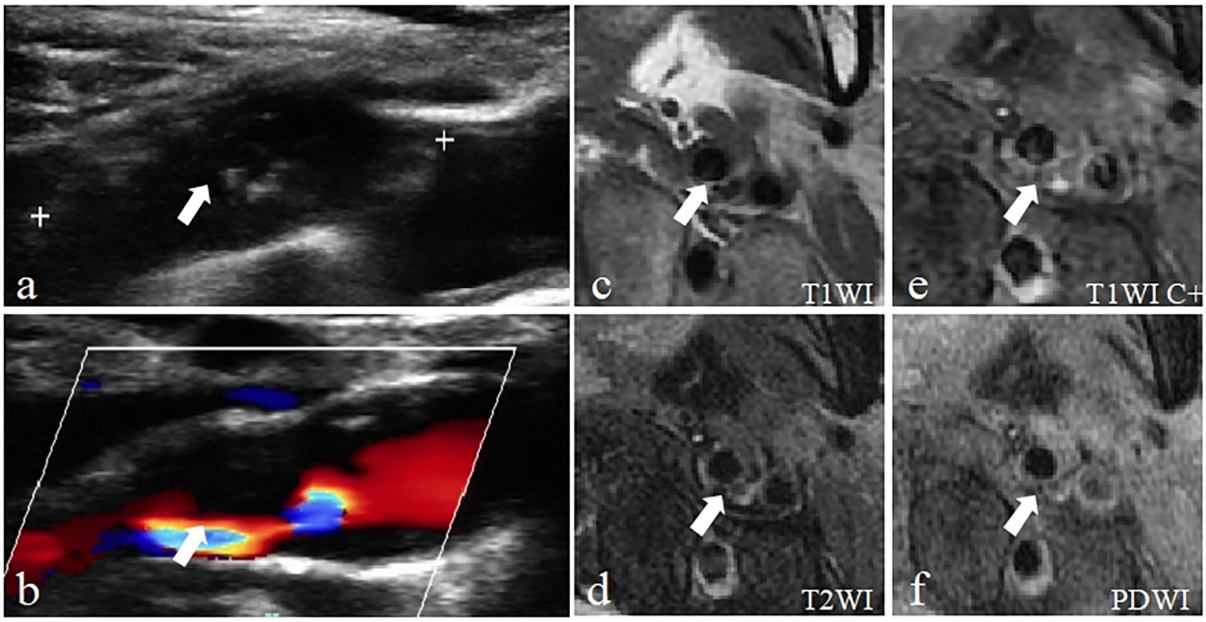

FIGURE 4
Example of a vulnerable carotid plaque of a patient using Carotid ultrasound (CDU) and High-resolution magnetic resonance imaging (HRMRI). **(a)** Gray-scale longitudinal section scan shows heterogeneous plaques in the posterolateral wall of the left carotid bulb extended to the internal carotid artery with a large hypoechoic area inside (arrow). **(b)** CDFI longitudinal section scan showed that blood flow signals were visible in the residual lumen (arrow). **(c)** The plaque is characterized by the isointensity on T1-weighted imaging (T1WI) (arrow). **(d)** T2-weighted imaging (T2WI) showing slightly high signal intensity (arrow). **(e)** Enhanced T1-weighted imaging (T1WI C+) showing regional enhancement within the AP (arrow), defined as neovascularization or inflammation, indicate the plaque is more likely to be vulnerable. **(f)** Proton density-weighted imaging (PDWI) showing slightly high signal intensity within the AP (arrow).

thrombosis and are correlated with the prognosis of cardio-cerebrovascular diseases (36). These findings imply that SII is associated with plaque vulnerability. The results of the present study showed that high levels of SII were an independent risk factor for cervical vulnerable plaques in patients with acute anterior circulation stroke. In addition, the findings indicated that a high level of SII was an independent risk factor for fibrous cap rupture of vulnerable plaques. The possible mechanism is that in the early stage of plaque formation which comprises the fatty streak stage and fibrous plaque stage, the plaque is relatively stable with the deposition of lipids. As a result, it aggravates ischemia and hypoxia of the lesion site. The surface of atherosclerotic plaque is gradually covered by lipids with the development of atherosclerotic plaque during hypoxia. Toxins and inflammatory mediators stimulate the deposition of membrane complexes owing to a lack of adventitia formation, which further reduces the diffusion of oxygen to the vessel wall, leading to the release of vascular endothelial growth factor (VEGF). The release of these vascular growth factors promotes sprouting migration and proliferation of the original microvascular endothelial cells and induces matrix remodeling and other changes such as budding to form new capillaries. Neovascularization in plaque is characterized by high permeability. Moreover, abundant nutrient vessels increase the infiltration of lipid and inflammatory cells into carotid atherosclerotic plaque, which is the molecular channel for inflammatory cells to enter plaques. The formation of nutrient vessels further accelerates the deposition of blood lipids in plaques, aggravates infiltration of inflammatory cells, and formation of cytokines. Production of various cytokines gradually weakens the fibrous cap of the plaque, leading to interruption of the continuity of the fibrous cap of the plaque and exposure to the surface.

TABLE 5 Comparison of the consistency of CDU and HRMRI for the detection of vulnerability plaques ($N = 76$).

Item	Detection of vulnerability plaques via HRMRI		
	Not detected	Detected	Total
Detection of vulnerability plaques via CDU			
Not detected	40(52.6%)	5(6.6%)	45(59.2%)
Detected	1(1.3%)	30(39.5%)	31(40.8%)
K-value	0.894		
P-value*	<0.05		

*means P-values indicating statistically significant. CDU, color doppler ultrasound; HRMRI, High-resolution magnetic resonance imaging.

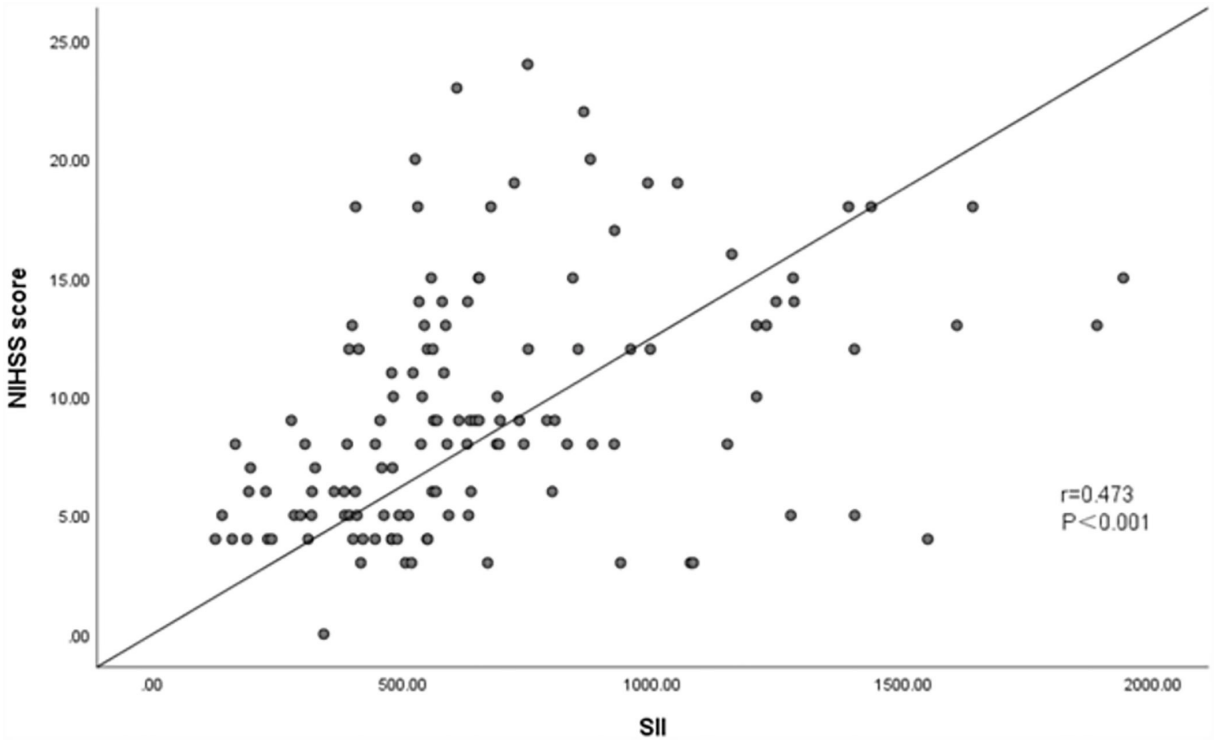

FIGURE 5
Association between SII and NHISS score. Spearman test showed that SII was positively associated with NHISS Score ($r = 0.473$, $P < 0.001$).

The results of the present study indicated that a high SII level is a potential predictor of the severity of an acute ischemic stroke, which is consistent with findings from previous studies (31). Neutrophils and lymphocytes are the main immune inflammatory cells and they play an important role in inducing ischemic brain injury. The expression level of inflammatory mediators in normal brain tissue is very low. The occurrence of ischemic injury in brain tissue can induce the release of pro-inflammatory cytokines, and promote the recruitment and accumulation of inflammatory factors and immune cells. Further, ischemic injury can aggravate neurological dysfunction by destroying the blood-brain barrier and inducing a loss of adaptive immune response (37).

A variety of imaging methods are available for evaluating the vulnerability of carotid plaque, including carotid US, HRMRI, Computed Tomography (CT), intravascular ultrasound (IVUS), and optical coherence tomography (OCT). CT, IVUS, and OCT methods are invasive or radiative imaging techniques limiting their clinical application. HRMRI is a non-invasive method with high spatial resolution, high tissue resolution, and good reproducibility. HRMRI uses multi-sequence contrast-enhanced imaging to clearly display the substructure of blood vessels from adventitia to lumen. Therefore, it allows the identification of the internal components of carotid plaque and thus the nature of the carotid plaque can be determined. A previous study compared plaque pathological detection and HRMRI and the findings showed high consistency in the evaluation of plaque properties (38). In addition, HRMRI was more effective compared with pathological detection in timeliness. Therefore, HRMRI can be used as a "non-invasive gold standard" for the evaluation of vulnerable plaques. Carotid US has several advantages such as being non-invasive, inexpensive, simple operation, does not require radiation, and has a few contraindications. Carotid US has wide clinical application owing to these characteristics and has become the first choice method for imaging examination. Carotid US provides information on distinguishing "vulnerable" and "stable" plaques through internal echoes and morphological characterization. The echoes of plaques vary with internal components, for instance, the lipid-rich core (LRNC) is hypoechoic (39) due to the high level of fat composition. The echo of intra-plaque hemorrhage (IPH) varies with the amount of oxygenated hemoglobin resulting from varying bleeding periods, and maybe sometimes similar to that of a fat nucleus (39, 40). Notably, hypoechoic (LRNC and IPH) may overlap on carotid US sonograms, making it difficult to distinguish them, and they have low sensitivity and specificity; therefore, the two were combined in this study. Calcification showed a strong echo and the fibrous cap (FC) was a thin line-like hyperechoic layer between the plaque and blood. Heterogeneous hypoechoic or moderately hypoechoic plaques, plaques with

or without irregular surface shape or incomplete fibrous cap, and ulcerative plaques with internal blood flow signals were defined as vulnerable plaques in the present study. Cohen's kappa value for carotid US and HRMRI methods for plaque vulnerability was 0.89, indicating a high consistency between the two techniques. This finding confirms the reliability of carotid US in evaluating plaque vulnerability. Cohen's kappa values of plaque vulnerability-related characteristics (such as LRNC or IPH, thrombus, ulcerative plaque, ruptured fibrous cap, irregular surface morphology, Ca) of carotid US and HRMRI were 0.86, 0.82, 0.80, 0.78, 0.77, and 0.70, respectively. The results show that the Cohen's kappa value of the remaining plaque characteristics was above 0.75 except for CA prevalence, indicating an excellent consistency between the two methods. The low Cohen's kappa value for calcification prevalence may be because the calcium component is mainly calcium hydroxyapatite, which gives a low signal in all HRMRI sequences due to its magnetic sensitivity and low proton density. Studies have not fully explored whether calcification components can lead to plaque instability. In addition, calcification of the plaque is not correlated with the texture of the plaque.

The study was a two-way cohort study conducted to explore the relationship between systemic immune-inflammation index (SII) levels, carotid plaque characteristics, and AIS severity in patients with acute anterior circulation stroke. Further, the consistency of HRMRI and carotid US in assessing carotid plaque vulnerability was explored in this study. Currently, only a few studies have explored the relationship among SII, the vulnerability of atherosclerotic plaques, and the severity of acute stroke. The main findings of this study were as follows: patients with acute internal carotid artery stroke with high SII levels had a higher number of vulnerable carotid plaque as shown by the carotid US compared with patients with low SII levels. Moreover, these patients had a high risk of rupturing the fibrous cap of plaque; a high SII level was an independent risk factor for vulnerable plaque and ruptured fibrous cap. In addition, the SII level was correlated with the characteristics of carotid plaque. Spearman's test showed that the SII level was a potential predictor of AIS severity. Carotid US and HRMRI techniques showed high consistency in evaluating the vulnerability of carotid plaques.

The current study had a few limitations: (1) This was a single-center study, thus further multicenter large-sample prospective follow-up studies should be conducted to verify the findings on the correlation among SII levels, carotid atherosclerotic disease progression, and the severity of acute cerebral infarction events. (2) Levels of neutrophils, platelets, and lymphocytes vary with time. In the present study, the SII level was only measured during admission, thus further analysis should be conducted using SII levels from different time points. (3) It was challenging to get the full cooperation of patients with severe symptoms due to the long period required for MRI examination.

Conclusion

The results of this study indicate that increase in SII value is associated with adverse effects on carotid plaque lesions in stroke patients with carotid atherosclerosis. The effects are significant for patients with vulnerable plaques with ruptured fibrous caps and may aggravate the severity of AIS in the ICA region. These findings indicate that predicting carotid atherosclerotic plaque vulnerability and stroke severity by carotid US wall imaging and SII level detection can help in choosing effective treatment options for stroke patients. Further research is needed to perform for exploring their exact underlying mechanisms between SII and vulnerable plaques.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the First People's Hospital of Yancheng. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

GS and SJ: guarantor of the article. LZ, QL, and WZ: conception, design, collection, and assembly of data. XL and QN: data analysis and interpretation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Liu XR, Xu Q, Xiao J, Deng YM, Tang ZH, Tang YL, et al. Role of oral microbiota in atherosclerosis. *Clin Chim Acta*. (2020) 506:191–5. doi: 10.1016/j.cca.03033

2. Saba L, Saam T, Jäger HR, Yuan C, Hatsukami TS, Saloner D, et al. Imaging biomarkers of vulnerable carotid plaques for stroke risk prediction and their potential clinical implications. *Lancet Neurol*. (2019) 18:559–72. doi: 10.1016/S1474-4422(19)30035-3

3. Famakin BM. The immune response to acute focal cerebral, ischemia, and associated post-stroke immunodepression,: a focused, review. *Aging Dis*. (2014) 5:307–26. doi: 10.14336/AD.2014.0500307

4. Ladecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med*. (2011) 17:796–808. doi: 10.1038/nm.2399

5. Fann DY, Lee SY, Manzanero S, Chunduri P, Sobey CG, Arumugam TV. Pathogenesis of acute stroke and the role of inflammasomes. *Ageing Res Rev*. (2013) 12:941–66. doi: 10.1016/j.arr.09004

6. Chen ZQ, Mou RT, Feng DX, Wang Z, Chen G. The role of nitric oxide in stroke. *Med Gas Res*. (2017) 7:194–203. doi: 10.4103/2045-9912.215750

7. Hu B, Yang XR, Xu Y, Sun YF, Sun C, Guo W, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. *Clin Cancer Res*. (2014) 20:6212–22.doi: 10.1158/1078-0432.CCR-14-0442

8. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. Correction to: an updated definition of stroke for the 21st century: a statement for healthcare professionals from the American heart association/American stroke association. *Stroke*. (2019) 50:e239. doi: 10.1161/STR.0000000000000205

9. Obayashi, K., and Saeki, K. Kurumatani, N. Higher melatonin secretion is associated with lower leukocyte and platelet counts in the general elderly population: the HEIJO-KYO cohort. *J Pineal Res*. (2015) 58:227–33. doi: 10.1111/jpi.12209

10. Mariampillai JE, Liestøl K, Kjeldsen SE, Prestgaard EE, Engeseth K, Bodegard J, et al. Exercise systolic blood pressure at moderate workload is linearly associated with coronary disease risk in healthy men. *Hypertension*. (2020) 75:44–50. doi: 10.1161/HYPERTENSIONAHA.119.13528

11. Young JC, Paul NJ, Karatas TB, Kondrasov SA, McGinigle KL, Crowner JR, et al. Cigarette smoking intensity informs outcomes after open revascularization for peripheral artery disease. *J Vasc Surg*. (2019) 70:1973–83. doi: 10.1016/j.jvs.02066

12. Kuwabara M, Kuwabara R, Niwa K, Hisatome I, Smits G, Roncal-Jimenez CA, et al. Different risk for hypertension, diabetes, dyslipidemia, and hyperuricemia according to level of body mass index in japanese and american subjects. *Nutrients*. (2018) 10:1011. doi: 10.3390/nu10081011

13. Lyu Q, Tian X, Ding Y, Yan Y, Huang Y, Zhou P, et al. Evaluation of carotid plaque rupture and neovascularization by contrast-enhanced ultrasound imaging: an exploratory study based on histopathology. *Translational Stroke Res*. (2021) 12:49–56. doi: 10.1007/s12975-020-00825-w

14. Jang CW, Kim YK, Kim KH, Chiara A, Lee MS, Bae JH. Predictors for high-risk carotid plaque in asymptomatic Korean population. *Cardiovasc Ther*. (2020) 30:6617506. doi: 10.1155/2020/6617506

15. Ten Kate GL, van den Oord SC, Sijbrands EJ, van der Lugt A, de Jong N, Bosch JG, et al. Current status and future developments of contrast-enhanced ultrasound of carotid atherosclerosis. *J Vasc Surg*. (2013) 57:539–46. doi: 10.1016/j.jvs.2012.07.028

16. Johnsen SH, Mathiesen EB. Carotid plaque compared with intimamedia thickness as a predictor of coronary and cerebrovascular disease. *Curr Cardiol Rep*. (2009) 11:21–7. doi: 10.1007/s11886-009-0004-1

17. Lubner MG, Smith AD, Sandrasegaran K, Sahani DV, Pickhardt PJ. CT texture analysis: definitions, applications, biologic correlates, and challenges. *Radiographics*. (2017) 37: 483–1503. doi: 10.1148/rg.2017170056

18. Brinjikji W, Huston J, Rabinstein AA, Kim GM, Lerman A, Lanzino G. Contemporary carotid imaging: from degree of stenosis to plaque vulnerability. *J Neurosurg*. (2016) 124:27–42.doi: 10.1016/j.atherosclerosis.12028

19. Sabetai MM, Tegos TJ, Nicolaides AN, Dhanjil S, Pare GJ, Stevens JM. Reproducibility of computer-quantified carotid plaque echogenicity. *Stroke*. (2000) 31:2189–96.doi: 10.1161/01.str.31.9.2189

20. Zhou F, Hua Y, Ji X, Jia L, Zhang K, Li Q, et al.Ultrasound-based carotid plaque characteristics help predict new cerebral ischemic lesions after endarterectomy. *Ultrasound Med Biol*. (2021) 47:244251. doi: 10.1016/j.ultrasmedbio.09025

21. Jashari F, Ibrahimi P, Johansson E, Ahlqvist J, Arnerlöv C, Garoff M, et al. Atherosclerotic calcification detection: a comparative study of carotid ultrasound and cone beam CT. *Int J Mol Sci*. (2015) 16:19978–88. doi: 10.3390/ijms160819978

22. Stein L, Liang JW, Weinberger J. Resolution of occlusive carotid artery thrombus treated with anticoagulation as demonstrated on duplex ultrasonography. *J Neuroimaging*. (2017) 27:376–80. doi: 10.1111/jon.12447

23. Jayaraj RL, Azimullah S, Beiram R, Jalal FY, Rosenberg GA. Neuroinflammation: friend and foe for ischemic stroke. *J Neuroinflammation*. (2019) 16:142. doi: 10.1186/s12974-019-1516-2

24. Jeyaseelan, R. D., and Vargo, M. M. (2014). Chae, J. National institutes of health stroke scale (NIHSS) as an early predictor of post-stroke dysphagia. *PM and R: J Injury Funct Rehabil*. (2015) 7:593–8.doi: 10.1016/j.pmrj.12007

25. Zhao H, Cao Y, Chen H, Xu W, Sun X, Pan X. The association between OPG rs3102735 gene polymorphism, microembolic signal and stroke severity in acute ischemic stroke patients. *Gene*. (2017) 613:25–9. doi: 10.1016/j.gene.02029

26. Xiang Y, Xu Y, Bhandari A, Sindan N, Hirachan S, Yang Q, et al. Serum TSH levels are associated with postoperative recurrence and lymph node metastasis of papillary thyroid carcinoma. *Am J Transl Res*. (2021) 13:6108–6116.eCollection 2021

27. Planas AM. Role of immune cells migrating to the ischemic brain. *Stroke*. (2018) 49:2261–7.doi: 10.1161/STROKEAHA.118.021474

28. Fani L, van Dam-Nolen DH, Vernooij M, Kavousi M, van der Lugt A, Bos D. Circulatory markers of immunity and carotid atherosclerotic plaque. *Atherosclerosis*. (2021) 325:69–74. doi: 10.1016/j.atherosclerosis.03040

29. Luo Y, Xia LX, Li ZL, Pi DF, Tan XP, Tu Q. Early neutrophil-to-lymphocyte ratio is a prognostic marker in acute minor stroke or transient ischemic attack. *Acta Neurol Belg*. (2020) 8:1289. doi: 10.1007./s13760-020-01289-3

30. Jin P, Li X, Chen J, Zhang Z, Hu W, Chen L, et al. Platelet-to-neutrophil ratio is a prognostic marker for 90-days outcome in acute ischemic stroke. *J Clin Neurosci*. (2019) 63:110–5. doi: 10.1016/j.jocn.01028

31. Jomrich G, Gruber ES, Winkler D, Hollenstein M, Gnant M, Sahora K, et al. Systemic immune-inflammation index (SII) predicts poor survival in pancreatic cancer patients undergoing resection. *J Gastrointest Surg*. (2020) 24:610–8.doi: 10.1007/s11605-019-04187-z

32. Huh JY, Ross GW, Chen R, Abbott RD, Bell C, Willcox B, et al. Total and differential white blood cell counts in late life predict 8-year incident stroke: the Honolulu heart program. *J Am Geriatr Soc*. (2015) 63:439–46.doi: 10.1111/jgs.13298

33. Shah AD, Denaxas S, Nicholas O, Hingorani AD, Hemingway H. Neutrophil counts and initial presentation of 12 cardiovascular diseases: a caliber cohort study. *J Am Coll Cardiol*. (2017) 69:1160–9. doi: 10.1016/j.jacc.12022

34. Ghattas A, Griffiths HR, Devitt A, Lip GY, Shantsila E. Monocytes in coronary artery disease and atherosclerosis: where are we now? *J Am Coll Cardiol*. (2013) 62: 1541–51.doi: 10.1016/j.jacc.07043

35. Kim JH, Lee YJ, Park B. Higher monocyte count with normal white blood cell count is positively associated with 10-year cardiovascular disease risk determined by Framingham risk score among community- dwelling Korean individuals. *Medicine*. (2019). 98:e15340. doi: 10.1097/MD.0000000000015340

36. Kurtul A, Ornek E. Platelet to lymphocyte ratio in cardiovascular diseases: a systematic review. *Angiology*. (2019) 70:802–18.doi: 10.1177/0003319719845186

37. Brea D, Sobrino T, Blanco M, Fraga M, Agulla J, Rodríguez-Yáñez M, et al. Usefulness of haptoglobin and serum amyloid A proteins as biomarkers for atherothrombotic ischemic stroke diagnosis confirmation. *Atherosclerosis*. (2009) 205:561–7. doi: 10.1016/j.atherosclerosis.2008.12.028

38. Cai JM, Hatsukami TS, Ferguson MS, Small R, Polissar NL, Yuan C, et al. Classification of human carotid atherosclerotic lesions with *in vivo* multicontrast magnetic resonance imaging. *Circulation*. (2002) 106:1368–73. doi: 10.1161/01.cir.0000028591.44554.f9

39. Saba L, Anzidei M, Marincola BC, Piga M, Raz E, Bassareo PP, et al. Imaging of the carotid artery vulnerable plaque. *Cardiovasc Intervent Radiol*. (2014). 37:572–585.doi: 10.1007/s00270-013-0711-2

40. Mura M, Della Schiava N, Long A, Chirico EN, Pialoux V, Millon A. Carotid intraplaque haemorrhage: pathogenesis, histological classification, imaging methods and clinical value. *Ann Transl Med*. (2020) 8:1273. doi: 10.21037/atm-20-1974

Glossary

AHA, American Heart Association; AIS, Acute ischemic stroke; AS, Atherosclerosis; AP, Atherosclerotic plaque; AUC, Area under curve; CA, Calcification; CAB, Carotid artery bulb; CCA, Common carotid artery; CDFI, Color Doppler; CDU, Carotid Ultrasound; CI, Confidence Interval; CT, Computed Tomography; DIRT1WI, Double IR T1-weighted imaging; DWI, Diffusion-weighted imaging; FC, Fibrous cap; Gd-DTPA, Gadolinium diethylenetriamine penta-acetic acid; HCY, Homocysteine; HDL-C, High-density lipoprotein cholesterol; HRMRI, High-resolution magnetic resonance imaging; hs-CRP, High-sensitive C-reactive protein; ICA, Internal carotid artery; IPH, Intraplaque hemorrhage; IS, Ischemic stroke; IVUS, Intravascular ultrasound; L, Lymphocyte; LASSO, Least absolute shrinkage and selection operator; LDL-C, Low-density lipoprotein cholesterol; LRNC, Lipid-rich necrotic core; MIP, Maximum Intensity Projection; N, Neutrophil; NHISS, National Institutes of Health Stroke scale; NLR, Neutrophil-to-lymphocyte Ratio; OCT, Optical coherence tomography; OR, Odds Ratio; P, Platelet count; PDWI, Proton density weighted imaging; PNR, Platelet-to-lymphocyte Ratio; ROC, Receiving operating characteristic; ROI, Region of interest; SII, Systemic immune-inflammation index; T1WI T1-, weighted imaging; T2WI T2-, weighted imaging; TC, Total cholesterol; TG, Triglycerides; TIA, Transient ischemic attack; TOF MRA, Time of flight MR angiography; US, Ultrasound; VEGF, Vascular Endothelial Growth Factor.

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Optic nerve sheath diameter and optic nerve sheath diameter/eyeball transverse diameter ratio in prediction of malignant progression in ischemic stroke

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Background: The optic nerve sheath diameter (ONSD)/eyeball transverse diameter (ETD) ratio has been suggested in the evaluation of intracranial pressure (ICP). The aim of this study was to evaluate the predictive value of ONSD and ONSD/ETD in relation to risk for secondary malignant middle cerebral artery infarction (MMI).

Methods: A total of 91 patients with MCA occlusion were included in this study. Data were divided into two groups based on development of MMI or not. ONSD and ETD were measured by unenhanced computed tomography (CT). The differences in ONSD and the ONSD/ETD ratios between the MMI and non-MMI groups were compared. Receiver operating characteristic curve analyses were used to test the diagnostic value of ONSD and ONSD/ETD independently, to predict MMI.

Results: The ONSD in the MMI group and non-MMI group were 5.744 ± 0.140 mm and 5.443 ± 0.315 mm, respectively ($P = 0.001$). In addition, the ONSD/ETD ratios in the MMI group and non-MMI group were 0.258 ± 0.008 and 0.245 ± 0.006, respectively ($P = 0.001$). The receiver operating characteristic (ROC) curve demonstrated an area under the curve (AUC) for ONSD of 0.812 [95% confidence interval (CI): 0.718–0.906, $P = 0.001$], with a sensitivity of 97.4% and a specificity of 66.0% at the cut-off value of 5.520 mm. The AUC for ONSD/ETD ratio in predicting occurrence of MMI was 0.895 (95% CI: 0.823–0.968, $P = 0.001$), with a sensitivity of 84.2% and a specificity of 92.5% at a cut-off value of 0.250.

Conclusion: In acute stroke patients with massive cerebral infarction, an increased ONSD or ONSD/ETD ratio increases the odds of malignant progression and may be used as an indicator for emergent therapeutic interventions. In addition, the ONSD/ETD ratio may be more valuable than ONSD in predicting the malignant progression of acute stroke patients.

KEYWORDS

optic nerve sheath diameter, eyeball transverse diameter, intracranial pressure, secondary malignant middle cerebral artery infarction, ischemic stroke

Introduction

Acute cerebral infarction is a common disease in neurology. The prognosis of patients is typically related to the infarct size and location. Partial or complete obstruction of the middle cerebral artery (MCA) leads to severe cerebral edema, increased intracranial pressure (ICP), midline displacement of brain tissue, and even the formation of cerebral hernia, otherwise known as malignant middle cerebral artery infarction (MMI). Usually, the most severe brain swelling develops within 1–5 days after stroke (1). Recent studies have shown that early rather than late decompressive interventions can improve clinical outcomes in patients at risk for secondary MMI (2, 3). The fatality rate can be as high as 70–80% if conservative medical treatment is adopted instead of active surgical intervention (4, 5). Therefore, the early identification of the patients who are likely to develop the MMI is crucial.

In patients with MMI, hemispheric brain swelling may lead to shifting brain tissue, while patients undergoing cerebral infarction may observe early elevation of ICP (6). The optic nerve, enveloped by cerebrospinal fluid and the arachnoid membrane, is an important component of the central nervous system. The subarachnoid space surrounding the optic nerve is connected with the intracranial mass, and the change in cerebrospinal fluid pressure can be transmitted along the optic nerve sheath. The presence of an increased optic nerve sheath diameter (ONSD) serves as an indirect marker of changes in ICP due to the direct influence of the ICP on the diameter of the subarachnoid space around the optic nerve (7). Studies assessing the ultrasound-based evaluation of the optic nerve have supported the notion that ONSD might accurately identify patients at risk for developing MMI (8, 9). However, ultrasound-based ONSD measurements require technical expertise to obtain adequate images, which limits its ubiquitous clinical utility. Alternatively, ONSD measurements obtained by computed tomography (CT) are strongly correlated with ICP in patients with brain injury and showed excellent agreement both between raters and between sides in the same patient (10, 11). The thickness of the optic nerve is proportional to the size of the eyeball (12). It has been suggested that the ratio of ONSD/eyeball transverse diameter (ETD) may thus reduce the variation of ONSD and provide an alternative measure for ICP monitoring, with more accurate results (13, 14). The measured parameters of ONSD and ETD can be referred to Figure 1. So far, few studies have evaluated the predictive value of ONSD and ONSD/ETD measured by CT for development of MMI.

The aim of this study was to evaluate the predictive value of ONSD and ONSD/ETD in relation to risk for secondary MMI in a cohort of patients with MCA infarction.

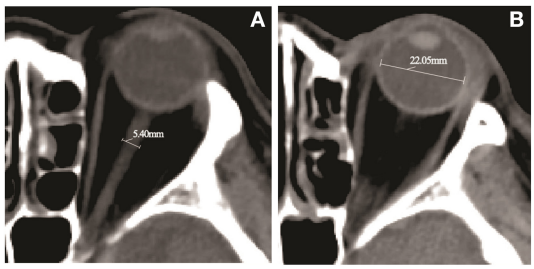

FIGURE 1
Computed tomography (CT) image of a 69-year-old female patient with a middle cerebral artery (MCA) infarction due to an occlusion of the proximal M1 segment. **(A)** The measurement of the optic nerve sheath diameter (ONSD) indicates diameter of 5.40 mm. **(B)** The eyeball transverse diameter (ETD) retina to retina measurement by head CT scan indicates diameter of 22.05 mm.

Methods

Patient selection

This study was approved by the ethics committee of Longyan First Affiliated Hospital of Fujian Medical University and performed according to the ethical standards of the Declaration of Helsinki. For the integrity of case data, we retrospectively collected all MCA infarction-related data available in our Neurology department from July 2016 to December 2021. Inclusion criteria were as follows: (1) ischemic stroke is listed as the primary diagnosis, identified using the International Classification of Diseases, 10th Revision, Clinical Modification (ICD-10CM) diagnostic codes I63, I64, I65, and I66; (2) age >18 years; (3) acute hemispheric infarction involving MCA region (covering more than two thirds of the MCA territory); (4) non-enhanced CT scan and computed tomography angiography (CTA) scan were performed in the emergency room; (5) availability of CT imaging 12–36 h after stroke onset; (6) MCA main trunk occlusion with or without internal carotid artery (ICA) occlusion confirmed on CTA images; (7) infarction confirmed by CT scan; (8) availability of follow-up CT imaging. Exclusion criteria were: (1) the patients with the diagnoses of posterior intracranial circulation occlusion (ICD-10CM diagnostic codes G45.0, I65.0, I65.1, I66.3, and I63.904); (2) age < 18 years; (3) previous ocular pathology (as glaucoma or cataract) and optic nerve diseases (ICD-10CM diagnostic codes H26, H40, H46, H47); (4) concurrent hemorrhagic stroke (ICD-10CM diagnostic codes I60, I61); (5) concurrent vascular territory infarction other than MCA; (6) other comorbidities that affect the state of the nervous system, such as seizures or acute respiratory distress syndrome.

The study screened 286 patients by ICD code, and a total of 91 patients were included in the final analysis after further

screening by inclusion criteria and exclusion criteria. Data were divided into 2 groups based on patients who had or had not developed MMI, hereto referred as the MMI group and the non-MMI group. Diagnosis of MMI was defined as in Thomalla's study (15) according to the following criteria: (1) secondary neurological deterioration including decline of consciousness by 1 or more points on the level of consciousness item of the National Institutes of Health Stroke Scale (NIHSS) and (2) large space-occupying MCA infarction on follow-up CT (covering more than two thirds of the MCA territory with compression of ventricles or midline shift) assessed in consensus by an experienced neurologist and neuroradiologist.

Baseline data

The following data were collected: gender, age, smoking, and drinking history, history of diabetes mellitus, hypertension, heart disease, Hyperlipidemia, C-reactive protein (CRP), D-dimer, NIHSS score, and latency between CT scan and stroke onset.

ONSD and ETD measurement

All CT images were obtained with a 64-slice CT scanner (Siemens, Munich, Germany), with a single slice section of 0.6 mm. To analyze CT-based factors for the prediction of impending herniation, CT was taken 12–36 h after onset of cerebral infarction. The ONSD and ETD were measured using the middle third spine window (window width 60, window level 360), with identical contrast and brightness. The ONSD was measured 10 mm behind the globe, perpendicular to the linear axis of the optic nerve (Figure 1). The ETD was defined as the maximal transverse diameter of the eyeball from retina to retina (Figure 1). The values were averaged from measurements independently obtained by two neuroradiologists. All of the measurements were performed bilaterally, and the mean value was used to calculate the ONSD/ETD ratio.

Statistical analysis

The analyses of the data were performed using IBM SPSS Statistical 20.0 software (IBM Corporation, NY, USA). Normally distributed continuous variables were expressed as mean ± standard deviation (SD) and the differences were analyzed using Student's *t*-tests. Categorical variables were expressed as percentages and the differences were analyzed using chi-square tests. The optimal threshold values of the ONSD and ONSD/ETD ratio for predicting MMI were determined by the area under the curve (AUC) of receiver operating characteristic (ROC) curve, and their sensitivity and specificity were calculated. A *P*-value of 0.05 was considered statistically significant.

TABLE 1 Baseline characteristics.

Characteristics	MMI group ($n = 38$)	Non-MMI group ($n = 53$)	*P*-value
Male, *N* (%)	24 (63.16)	26 (49.06)	0.182
Mean age, years	62.68 ± 6.68	66.75 ± 9.26	0.023
Smoke, *N* (%)	15 (39.47)	14 (26.42)	0.187
Alcohol consumption, *N* (%)	15 (39.47)	20 (37.74)	0.867
Diabetes mellitus, *N* (%)	16 (42.10)	23 (43.40)	0.902
Hypertension, *N* (%)	22 (57.89)	27 (50.94)	0.512
Atrial fibrillation, *N* (%)	5 (11.16)	12 (22.64)	0.252
Hyperlipidemia, *N* (%)	19 (50.00)	29 (54.72)	0.657
C-reactive protein, mg/L	3.84 ± 6.2	5.69 ± 8.52	0.258
D-dimer, mg/L	0.66 ± 0.97	0.61 ± 0.54	0.786
Admission NIHSS score	17.66 ± 2.40	17.58 ± 1.56	0.870
Latency between CT scan and stroke onset, h	22.13 ± 4.76	23.17 ± 5.84	0.370

MMI, malignant middle cerebral artery infarction; NIHSS, national institutes of health stroke scale; CT, computed tomography.

Results

Baseline characteristics

A total of 38 MMI patients and 53 non-MMI patients were included in this study. The demographic and clinical characteristics of the MMI patients and non-MMI patients are shown in Table 1. The gender composition, number of smokers, drinkers, rates of diabetes mellitus, hypertension, atrial fibrillation, hyperlipidemia, levels of C-reactive protein, D-dimer, admission NIHSS score and latency between CT scan and stroke onset were not significantly different between MMI patients and non-MMI patients ($P > 0.05$). Patients developing MMI were younger (mean age 62.68 ± 6.68 vs. 66.75 ± 9.26 years, $P = 0.023$).

Measurements of ONSD and ETD

The ONSD in the MMI group and non-MMI group were 5.744 ± 0.140 mm and 5.443 ± 0.315 mm, respectively ($P = 0.001$). In addition, the ONSD/ETD ratios in the MMI group and non-MMI group were 0.258 ± 0.008 and 0.245 ± 0.006, respectively ($P = 0.001$). The parameters are detailed in Table 2.

TABLE 2 ONSD and ETD measurements by CT and the ONSD/ETD ratio in patients.

Variables	MMI group ($n = 38$)	Non-MMI group ($n = 53$)	*P*-value
ONSD (mm)	5.744 ± 0.140	5.443 ± 0.315	0.001
ETD(mm)	22.307 ± 0.819	22.168 ± 0.974	0.476
ONSD/ETD	0.258 ± 0.008	0.245 ± 0.006	0.001

MMI, malignant middle cerebral artery infarction; ONSD, optic nerve sheath diameter; ETD, eyeball transverse diameter; CT, computed tomography.

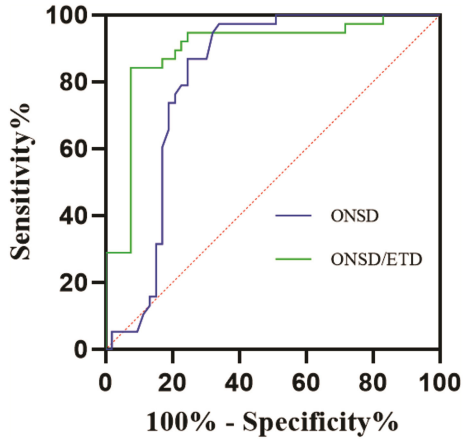

FIGURE 2
ROC curves for the efficiency of ONSD and ONSD/ETD ratio in predicting occurrence of MMI. ROC, receiver operating characteristic; MMI, malignant middle cerebral artery infarction; ONSD, optic nerve sheath diameter; ETD, eyeball transverse diameter.

Predictive efficiency of ONSD/ETD ratio for MMI

The efficiency of ONSD and ONSD/ETD ratio in predicting the occurrence of MMI is shown in Figure 2. The ROC curve demonstrated an AUC for ONSD in predicting the occurrence of MMI was 0.812 [95% confidence interval (CI): 0.718–0.906, $P = 0.001$], with a sensitivity of 97.4% and specificity of 66.0% at a cut-off value of 5.520 mm. The AUC for ONSD/ETD ratio in predicting the occurrence of MMI was 0.895 [95% (CI): 0.823–0.968, $P = 0.001$], with a sensitivity of 84.2% and a specificity of 92.5% at a cut-off value of 0.250.

Discussion

Patients with MMI have a poor prognosis due to space-occupying and life-threatening edema formation in the brain. Identifying patients at risk of developing fatal edema is critical for the earliest performance of decompressive hemicraniectomy. At present, there is a lack of clinical tools that can effectively predict the occurrence of malignant cerebral infarction. While many studies have attempted the early prediction of MMI, the gold standard for measuring ICP remains invasive monitoring. One study has even suggested that ICP monitoring is of little value in the vast majority of patients with acute ischemic stroke (16). On the other hand, infarct volume has been demonstrated as a reliable predictor of MMI (17). This study found that patients with MMI were more likely to be younger than those without MMI. One explanation may be rooted in the shrinkage of brain volume that increases with age, allowing greater space for brain swelling.

The ONSD is a reliable, non-invasive radiological marker of ICP, whether measured by magnetic resonance imaging (MRI), ultrasound or CT scan (11, 14, 18). Liu et al. (11) suggest that ONSD can reliably predict the requirement for surgery in patients with traumatic brain injury following admission to the emergency department (AUC = 0.920, 95% CI, 0.877–0.962). Similarly, Goel et al. (19) reported that ONSD can predict surgical intervention with a sensitivity and specificity of 98.3 and 62.5%, respectively. Even in non-traumatic cases, Amini et al. (20) reported that a cut-off value of 5.5 mm could be used to detect increased ICP with a sensitivity and specificity of 100%.

Due to the strong correlation between ONSD and ETD (12), the ONSD/ETD ratio has been introduced as a surrogate metric of ONSD accounting for interindividual variability due to orbit size (13, 21). Several researches have reported that changes in the ONSD/ETD ratio are more effective than ONSD in detecting increased ICP (13, 14, 22, 23). Albert et al. (24) found that an ONSD of more than 5.25 mm and an ONSD/ETD ratio of more than 0.232 on initial CT may identify MCA stroke patients at high risk of developing malignant MCA syndrome. Lee et al. (22) similarly described that the rate of ONSD/ETD changes can predict late malignant progression and midline shifting. The ONSD/ETD ratio may even reliably predict intracranial hypertension in traumatic brain injury patients (14).

Current research suggests that standard deviation of the ONSD measurements varies from 0.62 to 1.51, while the standard deviation of the ONSD/ETD index is 0.01–0.02, yielding more precise, normative data (13). Standard procedures involve measuring the ONSD from 3 mm behind the globe, though new studies suggest that for ICP monitoring, the most stable results can be obtained if the diameter is measured 10 mm from the globe (13). The rationale is that this depth is shielded from affects by tremor, gaze deviations, and involuntary movements of the eyes after trauma or stroke. To determine the value of the ONSD/ETD ratio in predicting, MMI, we performed a single-center retrospective cohort study.

In this study, ONSD was measured 10 mm behind the globe, and ROC curve was used to analyze the predictive value of ONSD/ETD for MMI. The results showed that the ONSD in the MMI group was 5.744 ± 0.140 mm, compared

to 5.443 ± 0.315 mm in the non-MMI group. In addition, the ONSD/ETD ratio in the MMI group was 0.258 ± 0.008, compared to 0.245 ± 0.006 in the non-MMI group. The ROC curve demonstrated an AUC for ONSD in predicting the occurrence of MMI was 0.812 (95% CI: 0.718–0.906, $P = 0.001$), with sensitivity of 97.4% and specificity of 66.0% at a cut-off value of 5.520 mm. The AUC for the ONSD/ETD ratio in predicting the occurrence of MMI was 0.895 (95% CI: 0.823–0.968, $P = 0.001$), with sensitivity of 84.2% and specificity of 92.5% at a cut-off value of 0.250. The main finding of this study was that ONSD/ETD was an effective predictor of the development of MMI, with a higher accuracy than ONSD alone. The ONSD/ETD was also a much earlier predictor of ICP than the CT findings of cisternal effacement, sulcal effacement, ventricular compression, and cerebral herniation. Thus, ONSD/ETD measured on CT could serve as a non-invasive predictor of intracranial hypertension in patients, allowing for non-invasive monitoring that can be used in therapeutic decision making.

One of the limitations of this study was its single-center, retrospective design, which yielded a small sample size that was more vulnerable to bias. Second, invasive ICP monitoring was not performed as a control intervention. This is due to current practice guidelines, which do not recommend invasive ICP monitoring of ischemic stroke patients (25). Lastly, while we assessed the value of the ONSD/ETD ratio in predicting MMI, the relationship between ONSD/ETD and long-term ischemic stroke outcomes remain uncertain.

Conclusion

In acute stroke patients with massive cerebral infarction, an increased ONSD or ONSD/ETD ratio may signal the increased odds of malignant progression, and may be used as an indicator for patients who may more likely benefit from emergent therapeutic interventions. We also report that the ONSD/ETD ratio may yield more clinical value than traditional ONSD in detecting elevated ICP and predicting the malignant progression of acute stroke patients. A multicenter study including different imaging devices with a larger sample size is necessary to confirm our results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Longyan First Hospital Affiliated to Fujian Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YG, YiC, and CS designed and performed the experiments and wrote the manuscript. XH, JD, DF, and YaC collected and analyzed the data. All authors have read and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Huttner HB, Schwab S. Malignant middle cerebral artery infarction: clinical characteristics, treatment strategies, and future perspectives. *Lancet Neurol.* (2009) 8:949–58. doi: 10.1016/S1474-4422(09)70224-8

2. Manawadu D, Butcher K, Findlay JM. Repeated hemicraniectomy for malignant cerebral edema: getting it right first time. *Can J Neurol Sci.* (2008) 35:652–4. doi: 10.1017/S0317167100009471

3. Vahedi K, Hofmeijer J, Juettler E, Vicaut E, George B, Algra A, et al. Early decompressive surgery in malignant infarction of the middle cerebral artery: a pooled analysis of three randomised controlled trials. *Lancet Neurol*. (2007) 6: 215–22. doi: 10.1016/S1474-4422(07)70036-4

4. Hacke W, Schwab S, Horn M, Spranger M, De Georgia M, von Kummer R. 'Malignant' middle cerebral artery territory infarction: clinical course and prognostic signs. *Arch Neurol*. (1996) 53:309–15. doi: 10.1001/archneur.1996.00550040037012

5. Berrouschot J, Sterker M, Bettin S, Köster J, Schneider D. Mortality of space-occupying ('malignant') middle cerebral artery infarction under conservative intensive care. *Intensive Care Med*. (1998) 24:620–3. doi: 10.1007/s001340050625

6. Frank JI. Large hemispheric infarction, deterioration, and intracranial pressure. *Neurology*. (1995) 45:1286–90. doi: 10.1212/WNL.45.7.1286

7. Hansen HC, Helmke K. The subarachnoid space surrounding the optic nerves. An ultrasound study of the optic nerve sheath. *Surg Radiol Anat*. (1996) 18:323–8. doi: 10.1007/BF01627611

8. Lochner P, Fassbender K, Andrejewski A, Behnke S, Wagenpfeil G, Fousse M, et al. Sonography of optic nerve sheath diameter identifies patients with middle cerebral artery infarction at risk of a malignant course: a pilot prospective observational study. *J Neurol*. (2020) 267:2713–20. doi: 10.1007/s00415-020-09906-0

9. Oliveira BDD, Lima FO, Homem HDC, Figueirêdo AA, Freire VMB, Maia Carvalho FM. Optic nerve sheath diameter detects intracranial hypertension in acute malignant middle cerebral artery infarction. *J Stroke Cerebrovasc Dis*. (2022) 31:106276. doi: 10.1016/j.jstrokecerebrovasdis.2021.106276

10. Sekhon MS, Griesdale DE, Robba C, McGlashan N, Needham E, Walland K. Optic nerve sheath diameter on computed tomography is correlated with simultaneously measured intracranial pressure in patients with severe traumatic brain injury. *Intensive Care Med*. (2014) 40:1267–74. doi: 10.1007/s00134-014-3392-7

11. Liu M, Yang ZK, Yan YF, Shen X, Yao HB, Fei L, et al. Optic nerve sheath measurements by computed tomography to predict intracranial pressure and guide surgery in patients with traumatic brain injury. *World Neurosurg*. (2020) 134:e317–24. doi: 10.1016/j.wneu.2019.10.065

12. Kim DH, Jun JS, Kim R. Ultrasonographic measurement of the optic nerve sheath diameter and its association with eyeball transverse diameter in 585 healthy volunteers. *Sci Rep*. (2017) 7:15906. doi: 10.1038/s41598-017-16173-z

13. Vaiman M, Gottlieb P, Bekerman I. Quantitative relations between the eyeball, the optic nerve, and the optic canal important for intracranial pressure monitoring. *Head Face Med*. (2014) 10:32. doi: 10.1186/1746-160X-10-32

14. Du J, Deng Y, Li H, Qiao S, Yu M, Xu Q, et al. Ratio of optic nerve sheath diameter to eyeball transverse diameter by ultrasound can predict intracranial hypertension in traumatic brain injury patients: a prospective study. *Neurocrit Care*. (2020) 32:478–85. doi: 10.1007/s12028-019-00762-z

15. Thomalla GJ, Kucinski T, Schoder V, Fiehler J, Knab R, Zeumer H, et al. Prediction of malignant middle cerebral artery infarction by early perfusion- and diffusion-weighted magnetic resonance imaging. *Stroke*. (2003) 34:1892–9. doi: 10.1161/01.STR.0000081985.44625.B6

16. Poca MA, Benejam B, Sahuquillo J, Riveiro M, Frascheri L, Merino MA, et al. Monitoring intracranial pressure in patients with malignant middle cerebral artery infarction: is it useful? *J Neurosurg*. (2010) 112:648–57. doi: 10.3171/2009.7.JNS081677

17. Lehrieder D, Layer K, Müller HP, Rücker V, Kassubek J, Juettler E, et al. Association of infarct volume before hemicraniectomy and outcome after malignant infarction. *Neurology*. (2021) 96:e2704–13. doi: 10.1212/WNL.0000000000011987

18. Onder H, Goksungur G, Eliacik S, Ulusoy EK, Arslan G, et al. The significance of ONSD, ONSD/ETD ratio, and other neuroimaging parameters in idiopathic intracranial hypertension. *Neurol Res*. (2021) 43:1098–106. doi: 10.1080/01616412.2021.1949688

19. Goel RS, Goyal NK, Dharap SB, Kumar M, Gore MA. Utility of optic nerve ultrasonography in head injury. *Injury*. (2008) 39:519–24. doi: 10.1016/j.injury.2007.09.029

20. Amini A, Kariman H, Dolatabadi AA, Hatamabadi HR, Derakhshanfar H, Mansouri B, et al. Use of the sonographic diameter of optic nerve sheath to estimate intracranial pressure. *Am J Emerg Med*. (2013) 31:236–9. doi: 10.1016/j.ajem.2012.06.025

21. Bekerman I, Sigal T, Kimiagar I, Ben Ely A, Vaiman M. The quantitative evaluation of intracranial pressure by optic nerve sheath diameter/eye diameter CT measurement. *Am J Emerg Med*. (2016) 34:2336–42. doi: 10.1016/j.ajem.2016.08.045

22. Lee SJ, Choi MH, Lee SE, Park JH, Park B, Lee JS, et al. Optic nerve sheath diameter change in prediction of malignant cerebral edema in ischemic stroke: an observational study. *BMC Neurol*. (2020) 20:354. doi: 10.1186/s12883-020-01931-w

23. Zhu S, Cheng C, Zhao D, Zhao Y, Liu X, Zhang J, et al. The clinical and prognostic values of optic nerve sheath diameter and optic nerve sheath diameter/eyeball transverse diameter ratio in comatose patients with supratentorial lesions. *BMC Neurol*. (2021) 21:259. doi: 10.1186/s12883-021-02285-7

24. Albert AF, Kirkman MA. Clinical and radiological predictors of malignant middle cerebral artery infarction development and outcomes. *J Stroke Cerebrovasc Dis*. (2017) 26:2671–9. doi: 10.1016/j.jstrokecerebrovasdis.2017.06.041

25. Jeon SB, Koh Y, Choi HA, Lee K. Critical care for patients with massive ischemic stroke. *J Stroke*. (2014). 16:146–60. doi: 10.5853/jos.2014.16.3.146

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Reduced plasma levels of RGM-A predict stroke-associated pneumonia in patients with acute ischemic stroke: A prospective clinical study

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Background: Stroke-induced immunodepression syndrome is considered the major etiology of stroke-associated pneumonia (SAP). Repulsive guidance molecule A (RGM-A) is an immunomodulatory protein that is closely related to inflammation and immune responses. To explore the relationship between RGM-A and SAP and facilitate the early identification of patients at high risk of developing SAP, we investigated the predictive value of RGM-A in SAP.

Methods: We enrolled 178 patients with acute ischemic stroke (AIS) and finally analyzed 150 patients, among whom 69 had SAP and 81 had non-SAP. During the same period, 40 patients with community-acquired pneumonia and 40 healthy participants were included as controls. SAP was defined according to the modified US Centers for Disease Control and Prevention criteria. Blood samples were collected at 24 h, 48 h, 3 days, 4 to 7 days, and 8 to 14 days after stroke onset. An enzyme-linked immunosorbent assay was used to detect the plasma levels of RGM-A and interleukin-6.

Results: The plasma RGM-A levels were significantly decreased in both patients with community-acquired pneumonia and those with AIS, and the decline was most pronounced in patients with SAP ($P < 0.001$). RGM-A started to decline within 24 h after stroke in the SAP group, and the lowest levels were detected on day 3 and days 4 to 7 ($P < 0.001$). The RGM-A levels in the SAP group were lower than those in the non-SAP group at all blood collection time points ($P < 0.05$). In the logistic regression analyses, RGM-A was a protective factor for SAP after adjusting for confounders (adjusted odds ratio = 0.22, 95% confidence interval = 0.091–0.538, $P = 0.001$). Receiver operating

characteristic curve analysis showed that the area under the curve for RGM-A was 0.766 (0.091–0.538; $P = 0.001$), the cutoff value was 4.881 ng/mL, and the sensitivity and specificity were 80.00 and 76.36%, respectively.

Conclusions: We demonstrated that reduced plasma levels of RGM-A might help in the early identification of high-risk patients with SAP and predict the occurrence of SAP in patients with AIS. RGM-A might provide new clues to a potential alternative therapy for SAP.

KEYWORDS

repulsive guidance molecule A, ischemic stroke, pneumonia, inflammation, immunomodulation, prediction

Introduction

Stroke is the second leading cause of death globally (1, 2). Stroke-associated pneumonia (SAP) is the most common complication after stroke and is significantly associated with death (3, 4), and with the incidence varying from 6.7 to 47% (5–9). SAP not only worsens stroke outcomes but also prolongs hospitalization and increases the economic burden on families and society (2, 5). Initial concepts on the etiology of SAP were mainly focused on aspiration, and a variety of studies have focused on age, stroke severity, stroke volume, and dysphagia in patients with SAP (4, 10). However, both nasogastric tubes and prophylactic antibiotic use have failed to reduce the incidence of SAP and the stroke mortality rate (10–12). The failure of these clinical trials has caused researchers to reconsider the etiology and therapeutic strategies of SAP. In recent years, accumulating experimental and clinical studies have confirmed that stroke-induced immunodepression syndrome (SIDS) is the main cause of SAP. Acute stroke causes a rapid and persistent deterioration in cellular immune function, inducing decreases in lymphocytes and natural killer cells and deactivation of monocytes, Th1 cells, and Th-mediated lymphocytes (3, 13, 14); this weakens the resistance of the human body against pathogens and leads to an increased susceptibility to SAP. Along these lines, immunomodulation has been explored as an alternative therapy for the prevention of SAP. However, highly sensitive and specific immunodepression biomarkers that can predict the occurrence of SAP are still lacking.

Repulsive guidance molecule A (RGM-A) is a 33-kDa glycosylphosphatidylinositol-linked membrane glycoprotein. It is the first molecule found to guide axons to their final location through a balance of chemoattractive or chemorepulsive signals in the developing nervous system (15–17). Recent evidence has identified RGM-A as a versatile immunoregulatory protein that is involved in a variety of inflammatory and immune diseases including autoimmune encephalomyelitis (18), multiple sclerosis (19), cerebrovascular atherosclerosis (20), peritonitis (21, 22), and acute lung injury (23, 24). RGM-A is strongly expressed in peripheral tissues, especially the surface of immune cells and tissues sensitive to infection, and it plays crucial roles in the regression of acute inflammation and tissue regeneration *via* its receptor neogenin (21, 22). *In vitro*, RGM-A restores MΦ chemotaxis and enhances macrophage phagocytosis, inhibiting polymorphonuclear leukocyte (PMN) migration (21, 22). *In vivo*, systemic application of RGM-A peptides was shown to attenuate the inflammatory response and infiltration of inflammatory cell traffic in a mouse model of zymosan-A-induced peritonitis (21, 22). In other studies involving an acute lung injury model, the RGM-A receptor neogenin was strongly induced within injured pulmonary tissue, and the binding of RGM-A to its receptor inhibited leukocyte migration, decreasing the production of proinflammatory cytokines and promoting inflammation resolution in acute lung injury (23, 24). Moreover, in our previous study, we found that RGM-A was involved in vascular inflammation and cerebrovascular atherosclerosis (20, 25). RGM-A levels were reduced in atherosclerotic aortas and in macrophages isolated from plaques (20).

Therefore, we hypothesized that RGM-A is associated with the development of SAP. To test this hypothesis, we designed the current study to investigate whether RGM-A is associated with SAP in patients with acute ischemic stroke (AIS). We found that reduced RGM-A levels might facilitate early prediction of the occurrence of SAP.

Methods

Patient selection

In this prospective clinical study, we recruited all patients with AIS from the Department of Neurology at Yongchuan Hospital of Chongqing Medical University from July 2020 to February 2022. During the same registration period, 40 healthy participants and 40 patients with community-acquired pneumonia (CAP)

were recruited from the Physical Examination Center and Respiratory Department, respectively. This research was approved by the Ethics Committee of Yongchuan Hospital of Chongqing Medical University. All participants involved in this study provided written informed consent.

The inclusion criteria for patients with AIS were an age of ≥18 years, acute stroke onset within 72 h, confirmation of AIS by cranial computed tomography and magnetic resonance imaging at admission, and provision of informed consent. The exclusion criteria were a National Institutes of Health Stroke Scale (NIHSS) score of <1; active infection or pyrexia within 2 weeks before admission; cancer or autoimmune disease; unstable conditions such as renal failure, hepatic failure, heart failure, or immunosuppressant treatment; and loss to follow-up within 3 months. SAP was defined according to the modified United States Centers for Disease Control and Prevention criteria (26). SAP was diagnosed by two experienced neurologists during the first 7 days after the onset of stroke. CAP was defined as an acute lower respiratory infection associated with clinical signs and symptoms according to the British Thoracic Society (27), and the diagnosis of CAP was confirmed by pulmonary infiltrates on a chest computed tomography scan and laboratory indicators of acute lower respiratory infection at the time of hospitalization (28, 29).

In total, 178 patients with AIS were enrolled, and 28 were excluded. The remaining 150 patients with AIS were analyzed, among whom 69 had SAP and 81 had non-SAP (Figure 1).

Clinical data

The baseline demographic and clinical data analyzed in this study were age, sex, and preexisting comorbidities including a history of smoking and drinking, hypertension, diabetes mellitus, hyperlipidemia, coronary heart disease, atrial fibrillation, previous stroke, and chronic obstructive pulmonary disease. Clinical parameters included the admission NIHSS score, the admission Glasgow Coma Scale score, dysphagia, infarct volume, and Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. The NIHSS scores were divided into three categories: mild impairment (NIHSS score of 1–4), moderate impairment (NIHSS score of 5–15), and severe impairment (NIHSS score of ≥16) (13). Dysphagia was identified using bedside non-instrumented swallowing tests at admission. Glasgow Coma Scale assessment involves evaluation of eye-opening, motor, and verbal responses to speech. The etiological subtype of ischemic stroke was described according to the TOAST criteria. Large stroke volume was defined as an infarction involving two-thirds or more of the middle cerebral artery territory or internal carotid artery regions (30). All patients were followed up within 3 months after stroke onset. The follow-up was conducted by two experienced neurologists *via* a structured telephone interview to assess the modified Rankin Scale (mRS) score. A good outcome was defined as an mRS score of <3, and a poor outcome was defined as an mRS score of ≥3 (3–6), including death (31). The 30-day mortality rate and hospitalization duration were also included in the outcome assessment.

Laboratory data

Fasting blood samples were collected at 24 h, 48 h, 3 days, 4 to 7 days, and 8 to 14 days after stroke onset. Because some patients were discharged or died within 14 days, blood samples were not collected at a fixed time point for every patient. Blood samples were collected in a calcium ethylenediaminetetraacetic acid tube (5 mL), and then centrifuged at low temperature and high speed (450×*g*, 4°C, 10 min). The plasma was collected and stored at −80°C for future use (31, 32). The plasma RGM-A and interleukin (IL)-6 concentrations were detected in accordance with the instructions for the enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA; 4 A BIOTECH, Beijing, China), and the absorbance value was detected with a multifunctional fluorescent luminescence analyzer (Varioskan Flash; Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis

Categorical variables were expressed as a number (%), and continuous variables with a normal distribution were expressed as the mean ± standard deviation. Data that did not fit a normal distribution were expressed as the median and interquartile range. Multiple group comparisons among the four different groups were made using the Kruskal-Wallis test. Differences in continuous variables between patients with and without SAP were analyzed using the chi-square test, Mann-Whitney U-test, or Kruskal-Wallis test, as appropriate. To investigate the association of RGM-A with SAP, adjusted odds ratios (aOR) and 95% confidence intervals (CIs) were calculated. Logistics regression analysis was used to analyze the predictive value of different variables for SAP. The diagnostic value of RGM-A was evaluated using the receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC). Sensitivity and specificity were given under the maximal Youden's Index (sensitivity+specificity−1). Statistical analyses were performed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered highly statistically significant. Graphs were drawn using Graph Pad Prism 8 (Graph Pad Software, San Diego, CA, USA).

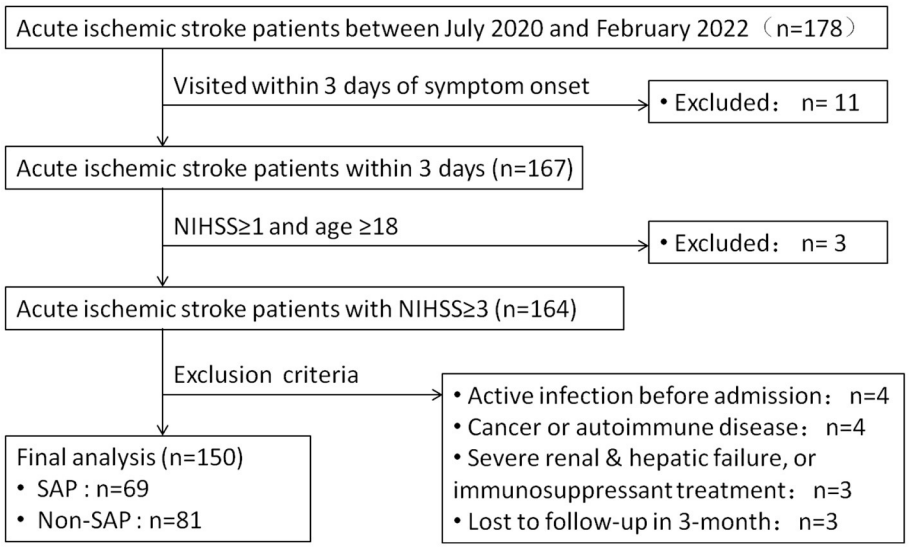

FIGURE 1
Flow-chart of inclusion and exclusion criteria. SAP, stroke-associated pneumonia; NIHSS, National Institutes of Health Stroke Scale.

Results

Participant characteristics and clinical data

After recruitment, we preliminarily enrolled 178 patients with AIS in this study. Of these 178 patients, 28 were excluded (symptom onset >3 days previously, $n = 11$; NIHSS score of <1, $n = 3$; active infection within 2 weeks before admission, $n = 4$; cancer and autoimmune disease, $n = 4$; renal failure and hepatic failure, $n = 3$; and loss to follow-up within 3 months, $n = 3$) (Figure 1). The remaining 150 patients with AIS were analyzed, among whom 69 (46%) patients were assigned to the SAP group and 81 (54%) to the Non-SAP group. We also included 40 patients with CAP and 40 healthy subjects as controls.

As shown in Table 1, there were no differences in sex ($P = 0.316$), hypertension ($P = 0.642$), diabetes mellitus ($P = 0.915$), hyperlipidemia ($P = 0.545$), and chronic obstructive pulmonary disease ($P = 0.177$) between the SAP group and Non-SAP group. However, the patients' age and incidence of atrial fibrillation, coronary heart disease, and previous stroke were higher in the SAP group than in the Non-SAP group ($P < 0.05$). The incidence of severe neurological deficits (NIHSS score of ≥16), Glasgow Coma Scale score, dysphagia, infarct volume, and TOAST criteria were significantly different between the two groups ($P < 0.01$). Moreover, we evaluated the clinical outcomes of patients in the SAP and Non-SAP groups according to the 3-month mRS score, 30-day mortality, and hospitalization duration (Table 1). These results showed that the incidence of poor outcomes (3-month mRS score of 3–6) was significantly higher in the SAP group than in the Non-SAP group (75.4 vs. 32.1%, respectively; $P < 0.001$); similar results were observed for the 30-day mortality rate (29.0 vs. 3.7%, respectively; $P < 0.001$) and hospitalization duration (13.67 vs. 11.56 days, respectively; $P < 0.05$).

We also analyzed laboratory data including the C-reactive protein (CRP) level, interleukin-6 (IL-6) level, neutrophil-to-lymphocyte ratio (NLR), white blood cell (WBC) count, and percentage of neutrophils (NEUT%) in patients with and without SAP (Table 1). We found that all included inflammatory predictors were substantially increased in patients with SAP and were significantly higher than those in patients with Non-SAP ($P < 0.01$): the CRP level was 14.80 (6.45–38.30) vs. 1.40 (0.90–5.70) mg/L, respectively ($P < 0.001$); the IL-6 level was 41.20 (29.29–92.00) vs. 28.76 (21.48–37.44) ng/mL, respectively ($P = 0.002$); the WBC count was 10.38 ± 4.31 vs. 7.96 ± 2.84 × 109/L, respectively ($P < 0.001$); the NLR was 7.16 (4.84–12.10) vs. 3.65 (2.24–8.14), respectively ($P < 0.001$); and the NEUT% was 78.51 ± 13.75% vs. 69.84 ± 16.57%, respectively ($P = 0.001$). These results suggest that elevated inflammatory factors were associated with the occurrence of SAP and that the diagnosis of SAP in this study was credible. Notably, the RGM-A level was significantly lower in the SAP group than in the Non-SAP group (4.94 ± 1.40 vs. 6.33 ± 2.02, respectively; $P < 0.001$), indicating that a relationship might exist between RGM-A and SAP. Therefore, we next investigated the possibility of this relationship.

TABLE 1 Characteristics and clinical data of patients with AIS with and without SAP.

	Non–SAP	SAP	χ^2/t/z	*P*–value
n (%)	81 (54)	69 (46)		
Demographic parameters				
Age (SD), years	68.74 ± 11.60	75.04 ± 10.27	3.495	<0.001
Male, (*n* %)	50 (61.7)	37 (53.6)	1.005	0.316
Comorbidities				
Hypertension, (*n* %)	58 (71.6)	47 (68.1)	0.216	0.642
Diabetes mellitus, (*n* %)	24 (29.6)	21 (30.4)	0.012	0.915
Hyperlipidemia, (*n* %)	65 (80.2)	58 (84.1)	0.367	0.545
Atrial fibrillation, (*n* %)	22 (27.2)	32 (46.4)	5.972	0.015
Coronary artery disease, (*n* %)	20 (24.7)	32 (46.4)	7.736	0.005
Previous stroke, (*n* %)	8 (9.9)	16 (23.2)	4.913	0.027
COPD, (*n* %)	8 (9.9)	12 (17.4)	1.821	0.177
Clinical parameters				
Admission NIHSS score			20.918	<0.001
1–4, (*n* %)	24 (29.6)	6 (8.7)		
5–15, (*n* %)	46 (56.8)	33 (47.8)		
≥16, (*n* %)	11 (13.6)	30 (43.5)		
GCS score(IQR)	15 (13.5–15)	13 (10–15)	3.73	<0.001
dysphagia, (*n* %)	25 (30.9)	40 (58.0)	11.149	0.001
Large stroke volume, (*n* %)	45 (55.6)	62 (89.9)	21.436	<0.001
TOAST criteria			15.107	0.004
Large–artery atherosclerosis, (*n* %)	28 (34.6)	31 (44.9)		
Cardioembolism, (*n* %)	23 (28.4)	31 (44.9)		
Small–vessel occlusion, (*n* %)	23 (28.4)	6 (8.7)		
Other cause, (*n* %)	2 (2.5)	0 (0)		
Undefined cause, (*n* %)	5 (6.2)	1 (1.4)		
Outcomes				
3–month mRS			27.941	<0.001
good outcome (0–2), (*n* %)	55 (67.9)	17 (24.6)		
poor outcome (3–6), (*n* %)	26 (32.1)	52 (75.4)		
30–day mortality, (*n* %)	3 (3.7)	20 (29.0)	18.345	<0.001
Hospitalization duration (SD), days	11.56 ± 13.67	13.67 ± 7.70	2.067	0.04
Inflammatory predictors				
CRP (IQR), mg/L	1.40 (0.90–5.70)	14.80 (6.45–38.30)	6.632	<0.001
IL−6 (IQR), ng/mL	28.76 (21.48–37.44)	41.20 (29.29–92.00)	3.026	0.002
WBC (SD),10^9/L	7.96 ± 2.84	10.38 ± 4.31	4.043	<0.001
NLR, (*n* %)	3.65 (2.24–8.14)	7.16 (4.84–12.10)	3.965	<0.001
NEUT, (*n* %)	69.84 ± 16.57	78.51 ± 13.75	3.379	0.001
Immunodepression marker				
RGM–A (SD), ng/mL	6.33 ± 2.02	4.94 ± 1.40	4.813	<0.001

Data are presented as mean ± standard deviation (SD), median (interquartile range, IQR), or *n* (%). AIS, acute ischemic stroke; SAP, stroke–associated pneumonia; COPD, chronic obstructive pulmonary disease; NIHSS, National Institutes of Health Stroke Scale; GCS, Glasgow Coma Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment; mRS, modified Rankin Scale; CRP, C–reactive protein; IL–6, interleukin–6; WBC, white blood cell; NLR, neutrophil–to–lymphocyte ratio; NEUT%, percentage of neutrophils; RGM–A, repulsive guidance molecule A.

Plasma RGM-A levels were significantly decreased in patients with CAP and AIS

To further investigate the relationship between RGM-A and SAP, we included 40 patients with CAP and 40 healthy subjects as controls (Table 2). First, we found that the RGM-A levels were significantly lower in patients with CAP than in healthy controls [5.1 (5.1–5.3)c vs. 8.4 (6.5–11.6) ng/mL, respectively; $P < 0.001$] (Table 2, Figure 2). These results suggest that RGM-A is involved in the acute inflammatory reaction in the lung. In addition, the RGM-A levels were significantly lower in patients who had AIS without SAP than in healthy controls [5.5 (5.2–6.6) vs. 8.4

TABLE 2 Baseline characteristics and RGM–A levels among the four groups.

	Control ($n = 40$)	CAP ($n = 40$)	Non–SAP ($n = 81$)	SAP ($n = 69$)	*P*-value
Age (SD), years	64.0 ± 11.2	69.6 ± 13.5 [a]	68.7 ± 11.6 [a]	75.0 ± 10.3[a,b,c]	0.001
Male, (*n* %)	22(55.0)	28 (70.0)	50 (61.7)	37 (53.6)	0.344
Current smoking, (*n* %)	11 (27.5)	12 (30.0)	34 (42.0)	25 (36.2)	0.371
Current drinking, (*n* %)	10 (25.0)	14 (35.0)	21 (25.9)	19 (27.5)	0.721
Hypertension, (*n* %)	0 (0)	17 (42.5)	58 (71.6)	47 (68.1)	<0.001
Diabetes mellitus, (*n* %)	0 (0)	4 (10.0)	24 (29.6)	21 (30.4)	<0.001
Hyperlipidemia, (*n* %)	0 (0)	3 (7.5)	65 (80.2)	58 (84.1)	0.013
Systolic pressure (SD), mmHg	124.9 ± 18.6	130.2 ± 23.1	157.3 ± 27.3[a,b]	153.7 ± 32.1[a,b]	<0.001
Diastolic pressure (IQR), mmHg	72.0 (66.3–80.0)	72.0 (65.0–84.0)	90.0(77.0–98.5)[a,b]	87.0 (75.0–97.0)[a,b]	<0.001
RGM–A(IQR), ng/mL	8.4 (6.5–11.6)	5.1 (5.1–5.3)[a]	5.5 (5.2–6.6)[a,b]	5.2 (3.7–5.6)[a,c]	<0.001

Data are presented as mean ± standard deviation (SD), median (interquartile range, IQR), or *n* (%). RGM–A, repulsive guidance molecule A; CAP, community–acquired pneumonia; SAP, stroke–associated pneumonia. [a]$P < 0.05$ for CAP, Non–SAP, SAP vs. Control; [b]$P < 0.05$ for Non–SAP, SAP vs. CAP; [c]$P < 0.05$ for SAP vs. Non–SAP.

(6.5–11.6) ng/mL, respectively; $P < 0.001$]. Furthermore, among patients with AIS, these decreases were more pronounced in patients with than without SAP (5.2 [3.7–5.6] vs. 5.5 [5.2–6.6] ng/mL, respectively; $P < 0.001$). These results indicate that RGM-A is not only associated with acute lung infection but is also related to AIS, especially in patients with SAP.

Time course of RGM-A and inflammatory predictors in SAP and Non-SAP groups

To investigate the changes of RGM-A over time, we collected fasting blood samples at 24 h, 48 h, 3 days, 4 to 7 days, and 8 to 14 days after stroke onset. Figure 3A shows that the RGM-A expression started to decline during the initial 24 h after stroke in the SAP group ($P < 0.05$), and the lowest levels were reached on day 3 and days 4 to 7 ($P < 0.001$). These differences between the SAP and Non-SAP groups tended to shrink on days 8 to 14 ($P < 0.05$). Moreover, the RGM-A levels in the SAP group were lower than those in the Non-SAP group at all time points ($P < 0.05$) (Figure 3A). These results might mean that reduced RGM-A levels are associated with the development of SAP.

Furthermore, with the exception of the CRP level ($P < 0.05$) (Figure 3B), the other inflammatory predictors (IL-6 level, NLR, WBC count, and NEUT%) did not change significantly within 24 h after stroke ($P > 0.05$) (Figures 3B–F). The NLR and NEUT% were significantly increased on day 2, day 3, and days 4 to 7 in the SAP group ($P < 0.01$) (Figures 3C,E). The IL-6 level and WBC count were increased only on day 2 and day 3 ($P < 0.01$) (Figures 3D,F) and began to decrease on days 4 to 7 ($P > 0.05$) (Figures 3D,F). Finally, all the inflammatory predictors (CRP level, IL-6 level, NLR, WBC count, and NEUT%) were decreased on days 8 to 14 (Figures 3C–F). Based on these findings, we speculate that the variations of inflammatory predictors might be related to the disease process and antibiotic use in patients with SAP.

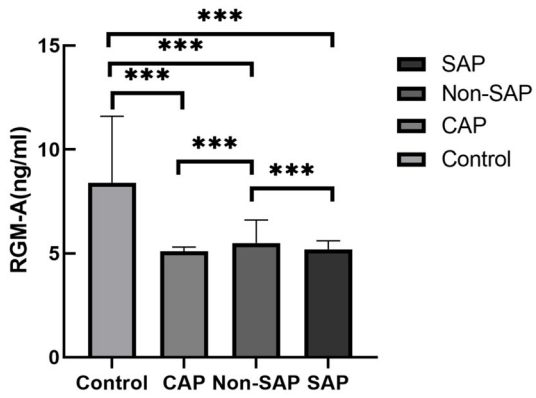

FIGURE 2
Comparison of plasma RGM-A levels among the Control, CAP, Non-SAP, and SAP groups.RGM-A, repulsive guidance molecule A; CAP, community-acquired pneumonia; SAP, stroke-associated pneumonia. ***$P < 0.001$.

Logistic regression analysis

To further understand the risk factors for SAP, we used logistic regression to analyze the predictive value of different confounding variables for SAP (Table 3). The results showed that RGM-A was a protective factor for SAP after adjusting for confounders (adjusted odds ratio: aOR = 0.221, 95% confidence interval: CI = 0.091–0.538, $P = 0.001$), whereas the CRP level (aOR = 1.157, 95% CI = 1.06–1.264, $P = 0.001$) and WBC count (aOR = 1.236, 95% CI=1.051–1.45, $P = 0.009$) were risk factors. Consistent with our hypothesis,

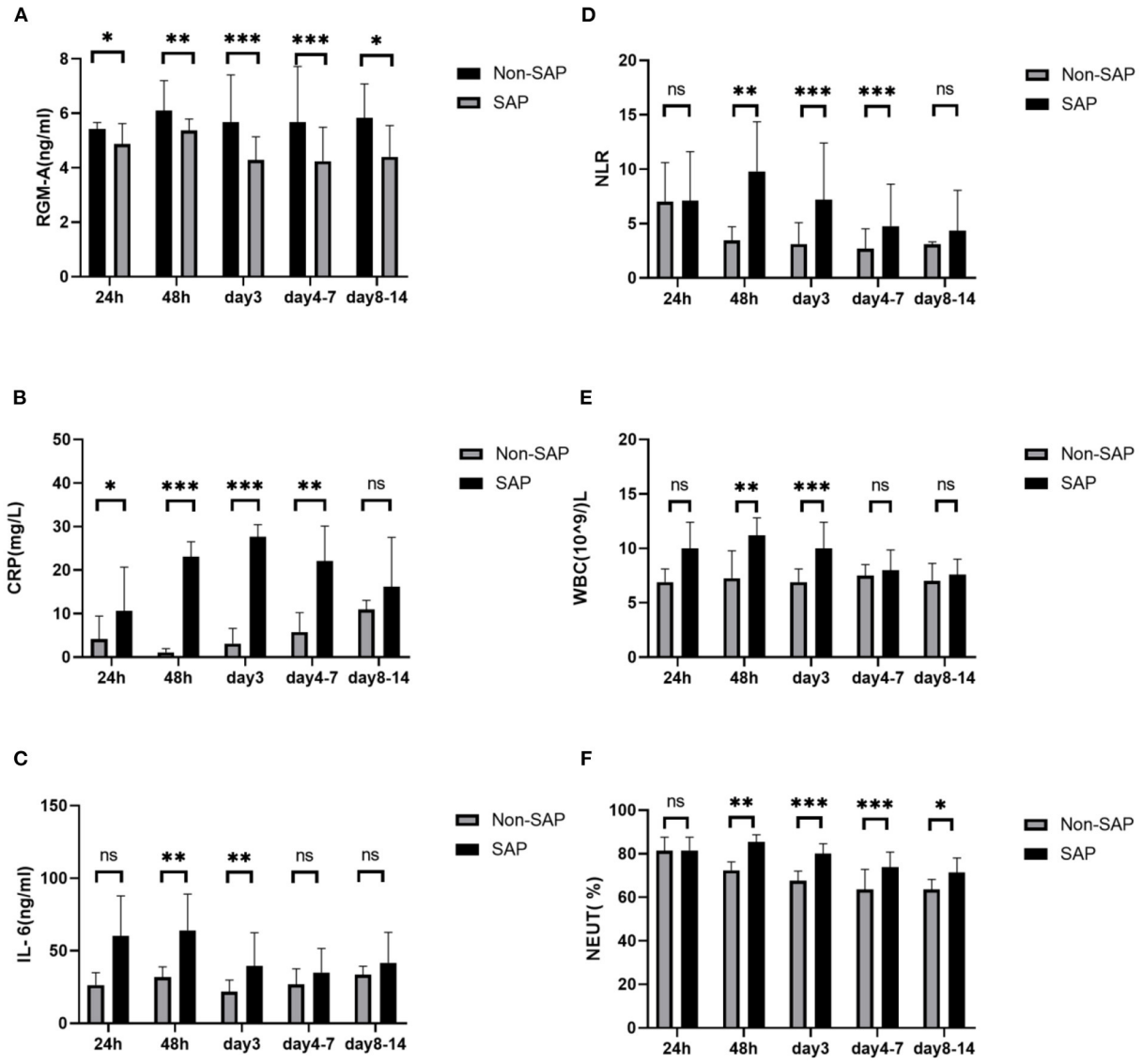

FIGURE 3
Levels of RGM-A and inflammatory predictors at 24 h, 48 h, 3 days, 4 to 7 days, and 8 to 14 days after stroke onset in patients with and without SAP. **(A)** RGM-A level, **(B)** CRP level, and **(C)** IL-6 level in patients with SAP and Non-SAP, **(D)** NLR, **(E)** WBC count, and **(F)** NEUT% in patients with SAP and Non-SAP. SAP: stroke-associated pneumonia; RGM-A: repulsive guidance molecule A; CRP: C-reactive protein; IL-6: interleukin-6; WBC: white blood cell; NLR: neutrophil-to-lymphocyte ratio; NEUT%: percentage of neutrophils. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$; ns, non-significant ($P > 0.05$).

RGM-A might be a protective factor for SAP, and patients with reduced plasma RGM-A levels may be more likely to develop SAP.

Receiver operating characteristic (ROC) analysis

ROC analysis was performed to evaluate the diagnostic value of RGM-A and other conventional inflammatory indicators for SAP (Table 4). The results showed that the AUC for RGM-A was 0.766 (95% CI: 0.686–0.847; $P < 0.001$), which was higher than that for IL-6 [0.758 (0.640–0.876); $P = 0.0002$], NLR [0.714 (0.629–0.799); $P<0.001$], WBC [0.709 (0.622–0.796); $P<0.001$], and NEUT% [0.696 (0.61–0.783); $P <0.001$] but slightly lower than the AUC for CRP [0.839 [0.761–0.916]; $P<0.001$). The cutoff value for RGM-A was 4.881 ng/mL, and the sensitivity and specificity were 80.00 and 76.36%, respectively. These results indicated that RGM-A had a good diagnostic value for SAP.

Discussion

In this study, we focused on identifying potential associations between the plasma RGM-A level and the development of SAP, while also investigating the changes of RGM-A over time. We found that a reduced plasma RGM-A level increased the risk of SAP in patients with AIS, and the earliest decline started as early as 24 h after stroke onset. We believe that a reduced plasma RGM-A level might help in the early identification of high-risk patients with SAP.

Stroke is an acute neurovascular disease with high morbidity, mortality, and disability rates (2). SAP is one of the most serious complications after stroke and is significantly associated with poor outcomes (3). Early identification of patients at high risk for SAP might help prevent the onset of SAP and ameliorate its consequences. Several studies have focused on age, stroke severity, stroke volume, and dysphagia in patients with SAP, and several clinical predictive models have been established to select patients at high risk for SAP (3, 4, 10, 33, 34). However, highly sensitive and specific immunodepression biomarkers that can predict the occurrence of SAP are still lacking.

To our knowledge, this study is the first to show that the plasma RGM-A level is decreased in patients with SAP. RGM-A is a promising target for the diagnosis and treatment of numerous diseases based on its versatility; it is involved in ischemic stroke (35), Parkinson's disease (36), autoimmune encephalomyelitis (18), multiple sclerosis (19), and cerebrovascular atherosclerosis (20). The evaluation of RGM-A as an immunoregulatory protein has recently increased in translational medical research, especially with regard to peritonitis (21, 22) and acute lung injury (23, 24). In our previous study, we found that the RGM-A level was reduced in atherosclerotic aortas and in macrophages isolated from plaques, suggesting that RGM-A has a potential protective effect in cerebrovascular atherosclerosis (20). Previous researchers have demonstrated that RGM-A is expressed in pathogenic Th17 cells in experimental autoimmune encephalomyelitis (18), and the binding of RGM-A to its receptor neogenin inhibits PMN migration and increases the attachment of CD4+ cells to intercellular adhesion molecule-1 to attenuate the inflammatory response (18, 21, 22). RGM-A restores MΦ chemotaxis and enhances macrophage phagocytosis, inducing human MΦ toward alternatively activated (M2) MΦs. One study showed that administering RGM-A peptides attenuated the inflammatory response and infiltration of inflammatory cell traffic in a mouse model of zymosan-A-induced peritonitis (21, 22). In recent years, researchers have found that RGM-A and its receptor neogenin are involved in the acute inflammatory response in pulmonary tissue (23). The binding of RGM-A to its receptor neogenin was shown to inhibit leukocyte migration, decrease the production of proinflammatory cytokines, and promote inflammation resolution in acute lung injury (23, 24). In the absence of RGM-A, neogenin has a damaging effect on lung tissue, resulting in PMN entry into the inflammatory site of lung tissue and exacerbation of the inflammatory response (37). However, when RGM-A binds to its receptor neogenin, it exerts anti-inflammatory activity to block the migration of PMNs to the inflammatory site (21, 22). Therefore, we are reasonably confident that the plasma RGM-A level is associated with the development of SAP.

Moreover, we found that the expression of RGM-A was decreased in patients with AIS (patients in the Non-SAP group). One explanation for the close relationship between the RGM-A and AIS may be the immunologic changes after stroke events. It is well known that stroke induces rapid and temporary immunodepression, inducing SIDS. SIDS leads

TABLE 3 Multivariable analysis of possible predictors of SAP.

Variable	β	S.E	aOR (95% CI)	*P*-value
RGM–A	−1.51	0.454	0.221 (0.091–0.538)	0.001
CRP	0.146	0.045	1.157 (1.06–1.264)	0.001
WBC	0.212	0.081	1.236 (1.051–1.45)	0.009
GCS	−0.246	0.105	0.782 (0.636–0.961)	0.019

Logistic regression analysis was performed to analyze the predictive value of different confounding variables for SAP. β, β-regression coefficient; S.E, standard error; aOR, adjusted odds ratio; CI, confidence interval; RGM–A, repulsive guidance molecule A; CRP, C–reactive protein; WBC, white blood cell; GCS, Glasgow Coma Scale.

TABLE 4 Comparison of predictive power of RGM–A vs. conventional inflammatory indicators in the prediction of SAP.

Variable	AUC	*P*-value	95% confidence interval		Cutoff value	Sensibility, %	Specificity, %
RGM–A	0.766	$P < 0.001$	0.686	0.847	4.881, ng/mL	80.00	76.36
CRP	0.839	$P < 0.001$	0.761	0.916	9.050, mg/L	79.16	82.19
IL–6	0.758	0.0002	0.64	0.876	32.557, ng/mL	72.97	74.19
NLR	0.714	$P < 0.001$	0.629	0.799	4.225	55.42	84.21
WBC	0.709	$P < 0.001$	0.622	0.796	7.90, 10^9/L	54.67	78.26
NEUT	0.696	$P < 0.001$	0.61	0.783	77.10,	57.74	79.45

Receiver operating characteristic analysis was performed to evaluate the diagnostic value of RGM–A and other conventional inflammatory indicators for SAP. AUC, area under the curve; RGM–A, repulsive guidance molecule A; CRP, C–reactive protein; IL–6, interleukin–6; WBC, white blood cell; NLR, neutrophil–to–lymphocyte ratio; NEUT%, percentage of neutrophils.

to a decrease in the number of lymphocytes and natural killer cells as well as deactivation of monocytes, Th1 cells, and Th-mediated lymphocytes (14). RGM-A is a membrane-binding immunomodulatory protein that is closely related to inflammation and immune responses. It is highly expressed in immune cells and is cleaved and presents in soluble form in serum under inflammatory conditions (21, 22).When SIDS occurs, immune cells decrease and RGM-A expression might be reduced, which would explain the reduction of RGM-A in patients with AIS.

Although biomarkers such as serum iron (38), intercellular adhesion molecule 1 (32), procalcitonin (39), and human leukocyte antigen–DR isotype (13) have been investigated to predict the development of SAP, highly sensitive and specific immunodepression biomarkers that might help in the early identification of high-risk patients with SAP are still lacking. Notably, our study showed that RGM-A started to decline within 24 h after stroke onset, and the lowest levels were observed on day 3 and days 4 to 7. This might have occurred because SAP is an early complication and most frequently occurs within the first 7 days after stroke, and SIDS usually begins within several hours after stroke and is even more pronounced during the following 3 days (13, 40). Moreover, the expression of RGM-A rather than other inflammatory indicators, such as CRP and IL-6, continued decreasing 8 to 14 days after stroke, indicating that SIDS might last longer than the inflammatory response. This may explain why antibiotic prophylaxis fails to reduce the frequency of SAP or improve 3-month outcomes in patients with stroke (11, 12). Thus, these results might provide clues for a more effective exploration of alternative therapy for the prevention of SAP.

Our research has several limitations. First, SAP was diagnosed according to clinical symptoms, chest computed tomography findings, and inflammatory biomarkers, but sputum cultures often remain negative without bacterial growth. Second, the incidence of SAP in our study was higher than that in other reports in the literature. This might be related to the fact that we performed a single-center study with a relatively small sample size, and our stroke center often receives patients in quite poor condition from local areas. Therefore, multicenter studies should be carried out to expand the sample size and further verify the experimental results. Third, because some patients were discharged or died within 14 days, blood samples were not collected at a fixed time point for every patient. Finally, to better understand the relationship between RGM-A and SAP, future studies should evaluate the very early changes in RGM-A that occur within the first hours after stroke onset.

In conclusion, this study is the first to identify a relationship between RGM-A and SAP, and our results suggest that a reduced plasma RGM-A level might help in the early identification of high-risk patients with SAP. RGM-A might provide new clues to a potential alternative therapy for SAP.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Yongchuan Hospital of Chongqing Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XQ and JZ participated in study design and study conception. JZ and JL performed data analysis and wrote the manuscript. RZ, CZ, ZW, SH, DH, and MY recruited patients and performed the laboratory analyses. XQ, JZ, LZ, and MY revised the manuscript. All authors provided critical review of the manuscript and approved the final draft for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.949515/full#supplementary-material

References

1. Campbell BCV, Khatri P. Stroke. *Lancet (London, England).* (2020) 396:129–42. doi: 10.1016/S0140-6736(20)31179-X

2. Feigin VL, Stark BA, Johnson CO, Roth GA, Bisignano C, Abady GG, et al. Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet Neurology.* (2021) 20:795–820. doi: 10.1016/S1474-4422(21)00252-0

3. Faura J, Bustamante A, Miró-Mur F, Montaner J. Stroke-induced immunosuppression: implications for the prevention and prediction of post-stroke infections. *J Neuroinflammation.* (2021) 18:127. doi: 10.1186/s12974-021-02177-0

4. Eltringham SA, Kilner K, Gee M, Sage K, Bray BD, Smith CJ, et al. Factors associated with risk of stroke-associated pneumonia in patients with dysphagia: a systematic review. *Dysphagia.* (2020) 35:735–44. doi: 10.1007/s00455-019-10061-6

5. Chen Y, Yang H, Wei H, Chen Y, Lan M. Stroke-associated pneumonia: a bibliometric analysis of worldwide trends from 2003 to 2020. *Medicine (Baltimore).* (2021) 100:e27321. doi: 10.1097/MD.0000000000027321

6. Ji R, Shen H, Pan Y, Wang P, Liu G, Wang Y, et al. Novel risk score to predict pneumonia after acute ischemic stroke. *Stroke.* (2013) 44:1303–9. doi: 10.1161/STROKEAHA.111.000598

7. Xu Y, Qiao H, Yang S, Zhou L, Zhao Y, Xu Q, et al. 15-Hydroxyprostaglandin dehydrogenase is a predictor of stroke-associated pneumonia. *Front Neurol.* (2022) 13:893624. doi: 10.3389/fneur.2022.893624

8. Smith CJ, Bray BD, Hoffman A, Meisel A, Heuschmann PU, Wolfe CD, et al. Can a novel clinical risk score improve pneumonia prediction in acute stroke care? A UK multicenter cohort study. *J Am Heart Assoc.* (2015) 4:e001307. doi: 10.1161/JAHA.114.001307

9. Cugy E, Sibon I. Stroke-Associated pneumonia risk score: validity in a French stroke unit. *J Stroke Cerebrovasc Dis.* (2017) 26:225–9. doi: 10.1016/j.jstrokecerebrovasdis.2016.09.015

10. Smith EE, Kent DM, Bulsara KR, Leung LY, Lichtman JH, Reeves MJ, et al. Effect of dysphagia screening strategies on clinical outcomes after stroke: a systematic review for the 2018 guidelines for the early management of patients with acute ischemic stroke. *Stroke.* (2018) 49:e123–e8. doi: 10.1161/STR.0000000000000159

11. Westendorp WF, Vermeij JD, Zock E, Hooijenga IJ, Kruyt ND, Bosboom HJ, et al. The Preventive Antibiotics in Stroke Study (PASS): a pragmatic randomised open-label masked endpoint clinical trial. *Lancet (London, England).* (2015) 385:1519–26. doi: 10.1016/S0140-6736(14)62456-9

12. Kalra L, Irshad S, Hodsoll J, Simpson M, Gulliford M, Smithard D, et al. Prophylactic antibiotics after acute stroke for reducing pneumonia in patients with dysphagia (STROKE-INF): a prospective, cluster-randomised, open-label, masked endpoint, controlled clinical trial. *Lancet (London, England).* (2015) 386:1835–44. doi: 10.1016/S0140-6736(15)00126-9

13. Hoffmann S, Harms H, Ulm L, Nabavi DG, Mackert BM, Schmehl I, et al. Stroke-induced immunodepression and dysphagia independently predict stroke-associated pneumonia - The PREDICT study. *J Cereb Blood Flow Metab.* (2017) 37:3671–82. doi: 10.1177/0271678X16671964

14. Liu DD, Chu SF, Chen C, Yang PF, Chen NH, He X. Research progress in stroke-induced immunodepression syndrome (SIDS) and stroke-associated pneumonia (SAP). *Neurochem Int.* (2018) 114:42–54. doi: 10.1016/j.neuint.2018.01.002

15. Fujita Y, Yamashita T. The roles of RGMa-neogenin signaling in inflammation and angiogenesis. *Inflamm Regen.* (2017) 37:6. doi: 10.1186/s41232-017-0037-6

16. Siebold C, Yamashita T, Monnier PP, Mueller BK, Pasterkamp RJ. RGMs: structural insights, molecular regulation, and downstream signaling. *Trends Cell Biol.* (2017) 27:365–78. doi: 10.1016/j.tcb.2016.11.009

17. Key B, Lah GJ. Repulsive guidance molecule A (RGMa): a molecule for all seasons. *Cell Adh Migr.* (2012) 6:85–90. doi: 10.4161/cam.20167

18. Muramatsu R, Kubo T, Mori M, Nakamura Y, Fujita Y, Akutsu T, et al. RGMa modulates T cell responses and is involved in autoimmune encephalomyelitis. *Nat Med.* (2011) 17:488–94. doi: 10.1038/nm.2321

19. Zhang L, Tang S, Ma Y, Liu J, Monnier P, Li H, et al. RGMa participates in the blood-brain barrier dysfunction through BMP/BMPR/YAP signaling in multiple sclerosis. *Front Immunol.* (2022) 13:861486. doi: 10.3389/fimmu.2022.861486

20. Hu Q, Chen Z, Yuan X, Li S, Zhang R, Qin X. Common polymorphisms in the RGMa promoter are associated with cerebrovascular atherosclerosis burden in chinese han patients with acute ischemic cerebrovascular accident. *Front Cardiovasc Med.* (2021) 8:743868. doi: 10.3389/fcvm.2021.743868

21. Mirakaj V, Brown S, Laucher S, Steinl C, Klein G, Kohler D, et al. Repulsive guidance molecule-A (RGM-A) inhibits leukocyte migration and mitigates inflammation. *Proc Natl Acad Sci U S A.* (2011) 108:6555–60. doi: 10.1073/pnas.1015605108

22. Korner A, Schlegel M, Kaussen T, Gudernatsch V, Hansmann G, Schumacher T, et al. Sympathetic nervous system controls resolution of inflammation via regulation of repulsive guidance molecule A. *Nat Commun.* (2019) 10:633. doi: 10.1038/s41467-019-08328-5

23. Mirakaj V, Jennewein C, Konig K, Granja T, Rosenberger P. The guidance receptor neogenin promotes pulmonary inflammation during lung injury. *FASEB.* (2012) 26:1549–58. doi: 10.1096/fj.11-200063

24. Konig K, Gatidou D, Granja T, Meier J, Rosenberger P, Mirakaj V. The axonal guidance receptor neogenin promotes acute inflammation. *PLoS ONE.* (2012) 7:e32145. doi: 10.1371/journal.pone.0032145

25. Zhang G, Wang R, Cheng K, Li Q, Wang Y, Zhang R, et al. Repulsive guidance molecule a inhibits angiogenesis by downregulating VEGF and phosphorylated focal adhesion kinase in vitro. *Front Neurol.* (2017) 8:504. doi: 10.3389/fneur.2017.00504

26. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control.* (2008) 36:309–32. doi: 10.1016/j.ajic.2008.03.002

27. Arnold FW, Summersgill JT, Lajoie AS, Peyrani P, Marrie TJ, Rossi P, et al. A worldwide perspective of atypical pathogens in community-acquired pneumonia. *Am J Respir Crit Care Med.* (2007) 175:1086–93. doi: 10.1164/rccm.200603-350OC

28. Ramirez JA, Wiemken TL, Peyrani P, Arnold FW, Kelley R, Mattingly WA, et al. Adults hospitalized with pneumonia in the United States: incidence, epidemiology, and mortality. *Clin Infect Dis.* (2017) 65:1806–12. doi: 10.1093/cid/cix647

29. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia. an official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am J Respir Crit Care Med.* (2019) 200:e45–67. doi: 10.1164/rccm.201908-1581ST

30. Torbey MT, Bösel J, Rhoney DH, Rincon F, Staykov D, Amar AP, et al. Evidence-based guidelines for the management of large hemispheric infarction: a statement for health care professionals from the neurocritical care society and the German Society for Neuro-intensive care and emergency medicine. *Neurocrit Care.* (2015) 22:146–64. doi: 10.1007/s12028-014-0085-6

31. Chen Z, Hu Q, Huo Y, Zhang R, Fu Q, Qin X. Serum interleukin-33 is a novel predictive biomarker of hemorrhage transformation and outcome in acute ischemic stroke. *J Stroke Cerebrovasc Dis.* (2021) 30:105506. doi: 10.1016/j.jstrokecerebrovasdis.2020.105506

32. Wang L, Chen Y, Feng D, Wang X. Serum ICAM-1 as a Predictor of prognosis in patients with acute ischemic stroke. *Biomed Res Int.* (2021) 2021:5539304. doi: 10.1155/2021/5539304

33. Harms H, Grittner U, Droge H, Meisel A. Predicting post-stroke pneumonia: the PANTHERIS score. *Acta Neurol Scand.* (2013) 128:178–84. doi: 10.1111/ane.12095

34. Hoffmann S, Malzahn U, Harms H, Koennecke HC, Berger K, Kalic M, et al. Development of a clinical score (A2DS2) to predict pneumonia in acute ischemic stroke. *Stroke.* (2012) 43:2617–23. doi: 10.1161/STROKEAHA.112.653055

35. Zhang R, Wu Y, Xie F, Zhong Y, Wang Y, Xu M, et al. RGMa mediates reactive astrogliosis and glial scar formation through TGFbeta1/Smad2/3 signaling after stroke. *Cell Death Differ.* (2018) 25:1503–16. doi: 10.1038/s41418-018-0058-y

36. Oda W, Fujita Y, Baba K, Mochizuki H, Niwa H, Yamashita T. Inhibition of repulsive guidance molecule-a protects dopaminergic neurons in a mouse model of Parkinson's disease. *Cell Death Dis.* (2021) 12:181. doi: 10.1038/s41419-021-03469-2

37. Mirakaj V, Rosenberger P. Immunomodulatory functions of neuronal guidance proteins. *Trends Immunol.* (2017) 38:444–56. doi: 10.1016/j.it.2017.03.007

38. Li J, Feng L, Huang Q, Ren W. An L-shaped relationship between serum iron and stroke-associated pneumonia. *Clinical Intervent Aging.* (2021) 16:505–11. doi: 10.2147/CIA.S301480

39. Shi G, Li M, Zhou R, Wang X, Xu W, Yang F, et al. Procalcitonin related to stroke-associated pneumonia and clinical outcomes of acute ischemic stroke after IV rt-PA treatment. *Cell Mol Neurobiol.* (2021). doi: 10.1007/s10571-020-01031-w

40. Chamorro Á, Meisel A, Planas AM, Urra X, van de Beek D, Veltkamp R. The immunology of acute stroke. *Nature Rev Neurol.* (2012) 8:401–10. doi: 10.1038/nrneurol.2012.98

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The dual function of microglial polarization and its treatment targets in ischemic stroke

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Stroke is the leading cause of disability and death worldwide, with ischemic stroke occurring in ~5% of the global population every year. Recently, many studies have been conducted on the inflammatory response after stroke. Microglial/macrophage polarization has a dual function and is critical to the pathology of ischemic stroke. Microglial/macrophage activation is important in reducing neuronal apoptosis, enhancing neurogenesis, and promoting functional recovery after ischemic stroke. In this review, we investigate the physiological characteristics and functions of microglia in the brain, the activation and phenotypic polarization of microglia and macrophages after stroke, the signaling mechanisms of polarization states, and the contribution of microglia to brain pathology and repair. We summarize recent advances in stroke-related microglia research, highlighting breakthroughs in therapeutic strategies for microglial responses after stroke, thereby providing new ideas for the treatment of ischemic stroke.

KEYWORDS

ischemic stroke, treatment target, microglia, polarization, dual function

Introduction

Stroke is the leading cause of mortality worldwide (1, 2) with poor curative effect, high lethality, and poor prognosis. Among all types of stroke, ischemic stroke caused by the occlusion of blood vessels represents the majority (3). Previous research has indicated that brain injury is caused not only by the hematoma mass effect and potential hematoma expansion (which are the main causes of primary brain injury) but also by secondary brain injury (SBI) (4). Cerebral ischemia can lead to a series of pathological processes including excitatory toxicity, calcium overload, oxygen free radical damage, inflammatory responses, necrosis/apoptosis, and blood–brain barrier (BBB) destruction, which ultimately lead to irreparable neuronal damage (5). It is now proposed that injury after stroke is a complex pathophysiological process involving several genes and signaling pathways. The BBB is important, and its permeability appears to follow a heterogeneous pattern of different stroke stages associated with

different biological substrates. In the hyperacute phase, sudden hypoxia damages the BBB, leading to cytotoxic edema and increased permeability; in the acute phase, neuroinflammatory responses exacerbate BBB damage, leading to higher permeability and subsequent risk, which can be stimulated by reperfusion therapy; and in the subacute phase (1–3 weeks), repair mechanisms, particularly neovascularization, occur. BBB leakage occurs in immature vessels, but this permeability is associated with improved clinical recovery. In the chronic phase (>6 weeks), an increase in the BBB restoration factor causes the barrier to begin to reduce its permeability (6). Manipulation of microglial polarization is a potential treatment strategy for patients with ischemic stroke, but small- and medium-sized glial cells in the potential molecular mechanisms of the polarization in ischemic stroke are still controversial. Despite the simplicity of the experiment, more work and clinical trials are needed to fully understand the mechanisms of microglial polarization (7). Evaluating the best time to intervene with microglia and monocyte/macrophage therapeutic strategies against ischemic stroke, as well as determining how to stimulate cells and to polarize their states, as well as the role of microRNAs (miRNA) and transplanted stem cells in mediating microglial activation and polarization during cerebral ischemia, are all important topics for future research (8, 9). Targeting specific miRNAs may provide major restorative therapy, and microglia-based therapy for ischemic stroke may become a future research area.

Abbreviations: SBI, Secondary brain injury; BBB, Blood–brain barrier; CNS, Central Nervous System; INF-γ, Interferon-γ; LPS, Lipopolysaccharide; TNF- α, Tumor necrosis factor-α; IL, interleukin; Ym1 [chitinase-like protein 3 (Chil3)]; Ym1/2, chitinase-3-like protein 3; ICAM1, intercellular adhesion molecule 1; STAT, Signal transducer and activator of transcription; JAK, janus kinase; NO, nitrous oxide; SDF-1/CXCL12, Stromal cell-derived factor-1; CD, Cluster of Differentiation; MHC II, Major histocompatibility complex class II; Arg-1, Arginase-1; TGF-β, Transforming growth factor-β; IGF-1, Insulin growth factor 1; A2a R, Adenosine A2a Receptor; iNOS, Inducible nitric oxide synthase; MCAO, Middle cerebral artery occlusion; MMP-9, Matrix Metalloproteinase 9; COX-2, Cyclooxygenase-2; EGFs, Epidermal growth factors; NRR, Negative Regulatory Region; HD, Heterodimerization domain; TLRs, Toll-like receptors; MAPK, Mitogen-activated protein kinase; MMP, Metalloproteinase; PParγ, Peroxisome proliferator-activated receptor γ; PNS, peripheral nervous system; Nrf2, Nuclear Factor erythroid 2-Related Factor 2; PPAR, Peroxisome proliferator-activated receptor; DEX, Dexmedetomidine; TSPO, Translocator protein; MCAO, Middle cerebral artery occlusion; NLRP3, Nod-like receptor pyrin domain-containing protein 3; ICAM-1, Intercellular cell adhesion molecule-1; Aβ, Amyloid-β; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; HTT, Huntingtin; mSOD1, Mutant human superoxide dismutase1; DHEA, Dehydroepiandrosterone; TrkA, tropomyosin-associated kinase A; Akt, protein kinase B; Jmjd3, Histone 3 Lysine 27 (H3K27) demethylase Jumonji D3; TREM2, trigger receptor 2; DHA, Docosahexaenoic acid; MANF, Mesencephalic astromarch-derived neurotrophic factor.

Recent studies have shown that there are still no effective therapeutic targets to improve the neurological function of patients after stroke, and potential treatment methods for SBI remain a hot point of research. Currently, an effective treatment for ischemic stroke is mainly intravenous thrombolysis and mechanical thrombectomy. However, these treatment options are limited by the recommended treatment window (10, 11). In addition, a series of reperfusion injuries caused by inflammation and oxidative stress may occur after ischemia-reperfusion (12); oxidative stress can induce inflammation (13, 14). There is increasing evidence that, during cerebral infarction, persistent neuroinflammation damages neurons and the BBB, leading to tissue destruction and impaired function (15–17). Neuroinflammation plays a crucial role in ischemic stroke-induced brain injury and affects disease prognosis**.** Future research will focus on controlling stroke-induced inflammation by targeted drugs and will be challenging.

Microglia are the permanent substantial macrophages in the central nervous system (CNS), and activated microglia typically behave "amoeba-like," primed for action (18). Several findings showed that almost five different types of microglia morphology were identified in control and experimental status epilepticus (SE) tissues, and were categorized as follows: (1) ramified; (2) hypertrophic; (3) bushy; (4) amoeboid; and (5) rod-shaped (19) (Figure 1). Microglial polarization plays a major role in promoting brain injury and nerve recovery (20). As the main source of inflammatory cells in ischemic brain injury, microglia play a key role in the inflammatory response after stroke (21). After stroke, microglia are polarized to the classical pro-inflammatory type (M1-like) or the alternative protective type (M2-like) under optimal conditions (8). Classical M1-like microglia are related to the induction of pro-inflammatory molecules, while other M2-like microglial activations are related to neuroprotection (22). In this review, advances in microglia and ischemic stroke, including the dual functions of phenotypic polarization of microglia/macrophages and polarization-related signaling pathways, have been studied. Future ischemic stroke treatments may target microglial polarization in the future.

Origin and function of microglial cells

Derived from primitive yolk sac progenitor cells, microglia are a type of fixed macrophages (9). The number of microglia showed a steady increase in the first 2 weeks after birth, and gradually decreased to 50% of the level at birth between 3 to 6 weeks later, after which the density gradually stabilized. A decrease in the rate of proliferation accompanied by an increase in apoptosis results in a decrease in the overall number of microglia, and mature microglia maintain their numbers in the CNS by self-renewal (23, 24). In the CNS, microglial cells in the brain of healthy adults are renewed to maintain their number

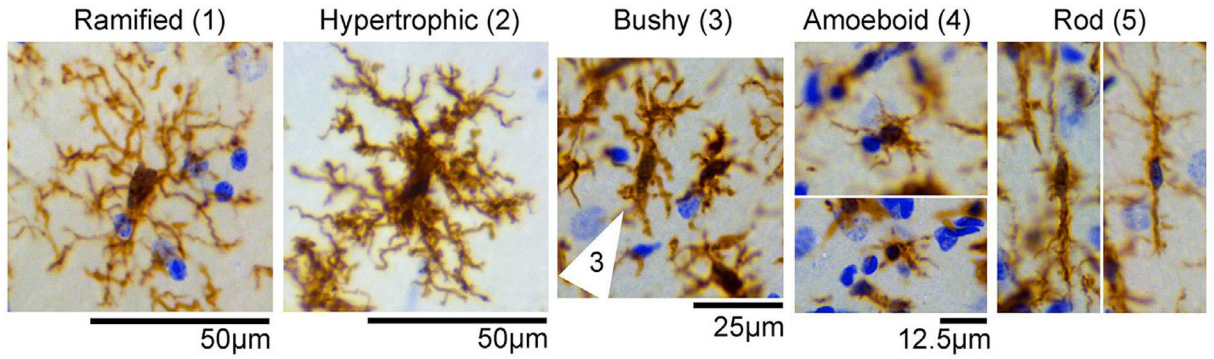

FIGURE 1
Representative images of microglial/macrophage cells (brown) with different morphologicalphenotypes observed in the control and status epilepticus (SE) groups, including (1) ramified, (2) hypertrophic, (3) bushy (cell indicated by arrows), (4) amoeboid, and (5) rod-shaped. Images were taken from the hippocampus of control or SE animals. Nissl-stained nuclei are indicated in blue [the figure is reproduced (19)].

and local expansion (25). In the physiological state, microglial cells present a typical branch-like state of small cell body and long branches and are referred to as "resting microglial cells." The protrusions have high mobility and can carry out extensive and continuous monitoring of the surrounding environment. In the pathological state, microglial cells are changed from the resting state to the active state. Polarization refers to the fact that microglia are affected by exogenous substances to achieve a specific phenotype, and there are one or more molecular markers and significant changes in molecular distribution (26). M1-like and M2-like microglia are essential in tissue damage and repair, respectively. Polarization of M1-like and M2-like microglia is also considered a functional manifestation of CNS disease, which is specifically manifested in the release of CNS disease-related inflammatory factors and the role of neuroinflammatory responses (27–29).

Microglial polarization

Polarization of the M1-like phenotype

M1-like microglia can secrete a variety of pro-inflammatory factors and chemokines, which can cause a neuroinflammatory response and induce neuronal apoptosis (30). As for the classic excitation type, it is mainly induced by interferon-γ (INF-γ), lipopolysaccharides (LPS), and tumor necrosis factor-alpha (TNF-α), which is characterized by the production of several pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, stromal cell-derived factor-1 (SDF-1/CXCL12), IL-1β, IL-12, and IL-23, and can be detected using cell surface markers such as cluster of differentiation 16 (CD16), CD32, major histocompatibility complex class II (MHCII), CD86, TNF-α, inducible nitric oxide synthase (iNOS), etc. During ischemia/hypoxia, nuclear factor-κB (NF-κB) is activated in microglia and transferred from the cytoplasm to the nucleus. This activates the release of pro-inflammatory cytokines that lead to SBI (31–33), such as IL-1β, IL-6, TNF-α, and iNOS. In addition, TNF-α secreted by M1-like microglia was identified to increase endothelial necrosis and BBB leakage after ischemic stroke in middle cerebral artery occlusion (MCAO) model mice. This further promotes neuroinflammation and cerebral edema, leading to poor outcomes (34, 35). Classically activated microglia can perform pro-inflammation, phagocytosis, cytotoxicity, present antigens, and kill intracellular pathogens to maintain the homeostasis of the microenvironment (36, 37). Notably, the M1-like phenotype of microglia is usually associated with protection during the early acute stages of infection, but it can also be detrimental to the host in case of its persistence for a longer time. Changes in the expression of corresponding proteins also follow a similar course node (38–40). If homeostasis is destroyed or stimulation persists, inflammatory cascades can be induced, resulting in the massive release of inflammatory factors and neurotoxic substances, aggravating the inflammatory response, and inducing neuronal death (36, 37) (Table 1).

Polarization of the M2-like phenotype

According to the unique functions of microglia/macrophages and their gene expression profiles, there are four different types of polarized activation states of M2-like activated phenotype: M2a microglia, M2b microglia, M2c microglia, and M2d microglia (41). There are many markers in M2-like microglia, such as CD206, increased arginase 1 (Arg-1), TGF-β, insulin growth factor 1 (IGF-1), IL-10, and others, and they secrete anti-inflammatory cytokines and neurotrophic factors, such as IL-10β, brain- and glial cell-derived neurotrophic factors, and Arg-1, the expression of factors such as IGF-1, thereby inhibiting inflammation (42), involved in tissue repair, cell debris removal, tissue remodeling, the provision of nutritional factors, and the maintenance of tissue dynamics after infection or injury (43, 44). In general,

TABLE 1 Characteristics of M1 microglia and M2 microglia in ischemic stroke.

Phenotype	Markers	Mechanism	Effects
M1	CD16, CD32, CD86, IL-1β, IL-6, TNF-α, iNOS, MHCII, et al.	NF-κB is activated in microglia and transferred from cytoplasm to nucleus, activating the release of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. TNF-α increases endothelial necrosis and BBB leakage	Proinflammatory, phagocytosis, cytotoxicity, present antigens, and kill intracellular pathogens
M2	CD206, Arg-1, TGF-β, CD163, IGF-1, IL-10, et al.	PParγ was activated in microglia and moved from nucleus to cytoplasm, resulting in the release of anti-inflammatory cytokines from M2. The up-regulation of TGF-α expression promoted the proliferation and neuronal differentiation of nerve stem/progenitor cells in the inferior ipsilateral ventricle	Anti-inflammatory, nerve repair, and tissue remodeling

CD, Cluster of Differentiation; IL, interleukin; TNF-α, Tumor necrosis factor-α; iNOS, Inducible nitric oxide synthase; MHCII, Major histocompatibility complex class II; Arg-1, Arginase-1; TGF-β, Transforming growth factor-β; IGF-1, Insulin growth factor 1.

M2-like microglia can be identified by CD206 and Arg1, IGF-1, among other markers (45). M2a is produced by IL-4 and IL-13 stimulation and inhibits NF-κB signal transduction and the anti-inflammatory phenotype of activated B cells. Moreover, M2a is involved in parasite immunity, T helper 2 cell recruitment, tissue repair, and growth stimulation. M2b is produced by stimulating immune complexes and LPSs to secrete anti-inflammatory cytokines (such as IL-10, MHC II, and co-stimulatory CD86). This subset exhibits both pro- and anti-inflammatory features and is associated with adaptive immunity. M2c is activated by IL-10 and TGF- β. Arg-1, CD163, and CD206 are the markers of M2c cells, which mainly function in scavenging cell debris during the repair process and is related to immunosuppression and tissue remodeling (46–48). M2c is different from the M2-like subtypes described earlier and is produced by activating the activation state of adenosine A2a receptor (A2aR) in M1 pro-inflammatory cells. M2d is different from M2-like subtypes described earlier and is produced by activating the activation state of A2aR in M1 pro-inflammatory cells (49). Peroxisome proliferator-activated receptor γ (PPARγ), a transcription factor with anti-inflammatory properties, is activated in microglia and translocated from the nucleus to the cytoplasm under ischemia/hypoxia conditions (50). This leads to the activation of M2-like microglia, which release anti-inflammatory cytokines and improve stroke outcomes. In addition, Choi et al. (51) demonstrated that M2-like microglia promoted the proliferation and neuronal differentiation of nerve stem/progenitor cells in the ipsilateral subventricular region after ischemic stroke by upregulating the TGF-α expression level, which may provide an effective treatment for neurogenesis (Figure 2).

The transition between M1 and M2

A shift from M2 to M1 has been observed in models of traumatic brain injury and ischemic stroke; however, it re mains to be determined whether this transformation is caused by phenotypic transformation of individual microglia or by the migration and infiltration of M2-like microglia (52). Studies have shown that in ischemic stroke, activated microglia express M2-like microglia markers in the acute phase. However, within ∼1 week, a gradual transition to M1-like phenotypes occurs and persists for several weeks after injury, this phenotypic transition may be due to the recruitment of M1-like microglia to the injury site and the transformation of locally activated microglia from M2 to M1 cells (53). Therefore, selective neuro-immunomodulatory therapies, which largely focus on suppressing M1-like phenotypes and shifting microglia from the M1-like phenotype to the M2-like phenotype, have been proposed as neuroprotective strategies for stroke (54). Furthermore, an experiment showed that the silencing of NF-κB p65 downregulated the expression of M1-like biomarkers and promoted the expression of M2-like biomarkers in the *in vitro* and *in vivo* model of cerebral ischemia (55).

Recently, a mouse model of transient focal cerebral ischemia has been used to study the temporal dynamics of microglial/macrophage polarization after stroke. Research results suggest that microglia/macrophages respond dynamically to ischemic injury, experiencing an early "healthy" M2-like phenotype, followed by a transition to a "sick" M1-like phenotype (56). *In vivo* temporal distribution of increased iNOS and chitinase-like protein 3 (Chil3; Ym 1) promoter activity in the mouse brain (57). The relatively low iNOS signal in healthy brain increased ∼3-fold within 3 days of stroke induction, whereas Ym1 signal reached a maximum at 11–13 days after stroke induction and then declined over the following week. Hu et al. found an early increase of iNOS messenger RNA (mRNA) levels as well as of other pro-inflammatory markers, such as CD16, CD32, CD86, and CD11b beginning at 3 dps and continuing up to 14 dps, with the exception of CD86. They also reported that chitinase-3-like protein 3 (Ym1/2) mRNA levels peaked on day 3 and then declined up to 14 dps (56).

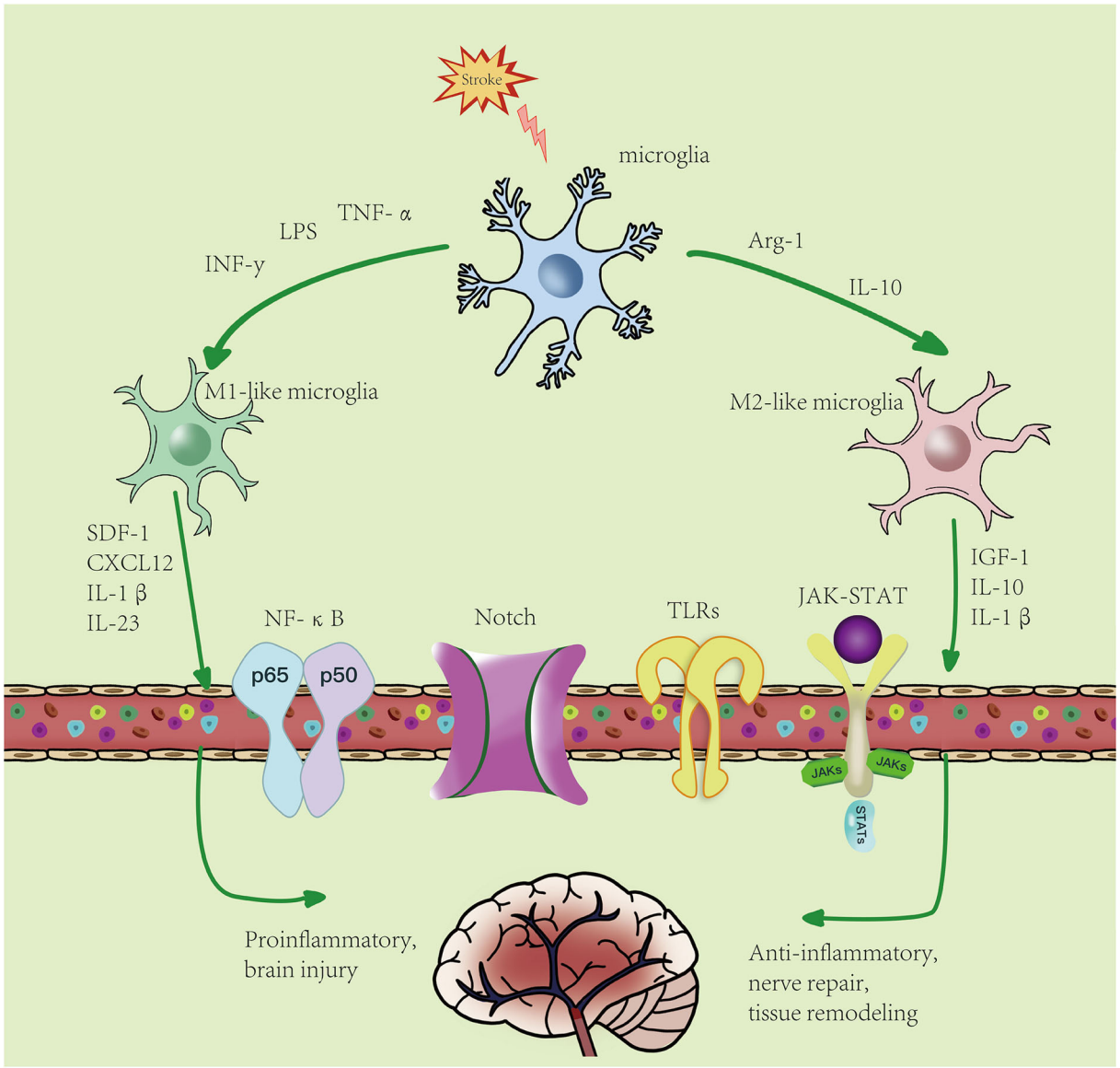

FIGURE 2
Microglial polarization after stroke: microglial activation is divided into two phenotypes: M1-like and M2-like microglia. M1 microglia can be induced by lipopolysaccharides (LPS), interferon-γ (IFN-γ), etc., resulting in an increase in pro-inflammatory factors. M2-like microglia can be induced by IL-4, IL-13, etc., resulting in an increase in anti-inflammatory factors. Activated microglia pass through NF-κB, JAK-STAT, Notch, TLRs, and other signaling pathways. M1-like type promotes the inflammatory response and kills intracellular substances, while M2-like type plays an anti-inflammatory, neuroprotective, and repairing role in tissues.

Mechanism of microglial polarization in ischemic stroke

Signaling pathways that regulate microglial polarization

NF-κB signaling pathways

Studies have shown that in mammals there are all five members of the NF-κB family including NF-κB1 (p105/p50), RelA (P65), and NF-κB2 (p100/p52), which are composed of homo- and heterodimers. In contrast to the c-NF-κB dimers, the p65/p50 heterodimer RelB is the most classical form of existence. It exists in most cell types and plays the most important role as an effective transcription factor. The activation of NF-κB is required for transcriptional induction of many pro-inflammatory mediators, such as IL-6, iNOS, intercellular adhesion molecule 1 (ICAM1), matrix metalloproteinase 9 (MMP-9), and cyclooxygenase-2 (COX-2), which are involved

in innate immunity. A previous study demonstrated that the NF-κB signaling pathway was overactivated in microglia after ischemic stroke. Therefore, the activation of NF-κB was responsible for the polarization of M1 and M2 in microglia (58, 59). In p50 KO mice, NF-κB activation exacerbated ischemic neuronal damage, especially in microglia. NF-κB p65 and p50 form heterodimers to initiate pro-inflammatory responses, thereby enhancing M1-like activation and attenuating microglial M2-like responses (60). The activation of the NF-κB signaling pathway promotes the conversion of microglia to M1-like type, and effective inhibition of the activation of the signaling pathway is more conducive to the conversion of microglia to M2-like type (61). Studies have shown that the inhibition of the NF-κB signaling pathway or the expression of NF-κBp65 and IκBα or interference with the nuclear metastasis of NF-κB can inhibit the activation of microglia and the expression of the M1-like phenotype, reduce the expression of inflammatory factors such as IL-1β, IL-6, TNF-α in microglia, and have neuroprotective effects (62, 63). Therefore, the suppression of neuroinflammation and the amelioration of brain injury by inhibiting the expression and activity of NF-κB in microglia after ischemic stroke has become a breakthrough target for therapeutic strategies (Table 2).

Janus kinase/signal transducer and activator of transcription pathway

Signal transducer and activator of transcription (STAT) is phosphorylated by Janus kinase (JAK), dimerized, and then transported to the nucleus through the nuclear membrane to regulate the expression of related genes. This pathway is termed the JAK/STAT signaling pathway (64). STAT plays a key role in signal activation and transcription. The STAT family in the cytoplasm is a downstream target of JAKs, which is one of the most crucial cytokine-activated transcription factors in the process of immune response. It is composed of seven members, namely STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6 (65). STAT1, STAT3, and STAT6 members of the STAT family are involved in the polarization of microglia. IFN-γ can induce microglia to polarize toward M1-like type through the STAT1/STAT3 pathway, and release inflammatory factors such as iNOS/nitrous oxide (NO) at a higher level than normal cells (66). STAT1 responds to M1-like microglial polarization signals (INF-γ and LPS), while STAT3 and STAT6 are selectively activated by M2-like microglial polarization cytokines (IL-10, IL-4, etc.). Thus, the release of inflammatory factors can be reduced and the injured nerve repair can be accelerated (67). Considering the data related to JAK/STAT and autoimmune diseases, this method is extremely attractive to the pharmaceutical industry, which is also one of its goals.

Notch signaling pathway

The extracellular domain of Notch is composed of epidermal growth factors (EGFs) like repeats, the number of which varies among species and different Notch receptors. Two functional domains are present in the extracellular region, the ligand-binding domain (EGF 11–12), which mediates the interaction with ligands, and the Abruptex domain (EGF 24–29), whose function remains unclear. The extracellular region is followed by the negative regulatory region (NRR), which masks a cleavage site (S2) important for Notch activation, the heterodimerization domain (HD), and the transmembrane spanning region of the receptor (64, 68). The Notch signal pathway is an important signal transduction pathway that begins with the binding of the Notch receptor and ligand and then forms a transcriptional activation complex through interactions with transcription factors, which activates the target genes of the transcriptional

TABLE 2 Overview of signaling pathways and their associated molecules.

Signaling pathways	Composition structure	Signal molecules	Activation paths	Clinical effects
NF-κB	NF-κB1, NF-κB2, NF-κB3, et al.	IL-6, iNOS, ICAM1, MMP-9, COX-2, et al.	Enhances M1 activation, attenuates M2 response	Inhibits inflammatory response and increases neuroprotection
JAK – STAT	JAKs and STAT1–6	INF-γ, LPS, IL-10, IL-4	Selectively activate M1 and M2	Reduce the release of inflammatory factors and accelerate the repair of damaged nerves
Notch	Four Notch receptors (Notch 1–4) and five Notch ligands (Delta type 1, 3, 4, sawtooth 1, 2)	unknow	Inhibit transition for M1–M2	Promote the release of inflammatory transmitters and aggravate nerve tissue damage
TLRs	a C-terminal TIR domain, a transmembrane region and an extracellular N-terminal	Pathogen-associated moleculars	Activation signaling pathways of NF-κB and MAPK	Increases pro-inflammatory factors and aggravates nerve damage

IL, interleukin; ICAM1, intercellular cell adhesion molecule-1; MMP-9, Matrix Metalloproteinase 9; COX-2, Cyclooxygenase-2; INF-γ, Interferon-γ; LPS, Lipopolysaccharide; MAPK, Mitogen-activated protein kinase.

suppressor family (e.g., HES, HEY, NERP, etc.) to play a transcriptional inhibitory role. In mammals, there are four Notch receptors (Notch 1–4) and five Notch ligands (Delta type 1, 3, 4, sawtooth 1, 2). Notch signaling can regulate the differentiation and development of cells, tissues, and associated cells. These cells include neurons, oligodendrocytes, astrocytes, and microglia. In the pathological state, the Notch pathway can promote the release of inflammatory transmitters and aggravate tissue damage by activating microglia and inhibiting the transformation of M1-like to M2-like microglia (69). It was confirmed in experiments of BV2 microglia-related cells that the release of inflammatory mediators from M1-like microglia decreased and converted to M2-like microglia after the use of a Notch signaling antagonist. Meanwhile, there is an increase in anti-inflammatory cytokines released by M2-like microglia. This confirms the involvement of the Notch pathway in the inflammatory response following microglial activation (70).

Toll-like receptor signaling pathway

Toll-like receptors, named after the Toll proteins in *Drosophila melanogaster* (13), are a class of inherent immune recognition receptors that detects microbial pathogens associated with molecular patterns to induce an immune response (71). TLR is expressed on neurons in glial cells (microglia, astrocytes, and oligodendrocytes), the CNS, and the peripheral nervous system (PNS) (72). TLRs are type I transmembrane proteins composed of a C-terminal TIR domain, a transmembrane region, and an extracellular N-terminal. An extracellular N-terminal mainly recognizes extracellular pathogens and tissue damage signals. TLR4 in human microglia can recognize pathogen-associated molecular models and activate nonspecific immunity in ischemic brain injury via a myeloid differentiation factor pathway, and both NF-κB and mitogen-activated protein kinase (MAPK) signaling pathways are activated and participate in the inflammatory response. TLR4 receptors can repeatedly recognize different pathogen-related molecular patterns through extracellular leucine (73), and ultimately lead to the production of NF-κB and an increase in pro-inflammatory factors, and the secretion of serotonin may aggravate nerve damage (74).

Regulatory mechanisms of microglial polarization

In addition to the influence of the abovementioned signaling pathways, there are also several regulatory mechanisms of microglial polarization: transcription factors, the regulation of gene expression, ion channels, and autophagy (75). Firstly, transcription factors, it was found that nuclear factor erythroid 2-related factor 2 (Nrf2) activation reduced the expression levels of reactive oxygen species (ROS), nucleotide-binding oligomerization domain- (NOD-) like receptor family Pyrin domain 3 (NLRP3), and IL-1 β in BV2 microglia, and played a protective role after ischemic stroke (12). In acute ischemic stroke, PPARγ is activated to directly reduce tissue damage by inhibiting the NF-κB pathway, reducing inflammation, and stimulating the Nrf2/ARE axis to reduce oxidative stress (76). IL-4 produced by neurons was determined to bind to IL-4 receptors expressed on microglia surfaces and activate M2-like microglia by modulating the PPARγ signaling pathway to reduce ischemic brain injury (77). Second, ion channel expression changes in response to voltage and pH gradients in the microenvironment, thereby inducing intracellular signal transduction. Currently, the two important ion channels Hv1 and Kv1.3 are closely related to microglial polarization. Studies have shown that Hvl can aggravate brain injury by increasing the expression levels of ROS and pro-inflammatory cytokines produced by M1-like microglia. However, it remains unclear whether Hv1 affects the polarization of M2-like microglia. The Kv1.3 inhibitor 5-(4-phenoxybutyl-psoralen) pSORalen (PAP-1) decreased the polarization of M1-like microglia and the expression level of pro-inflammatory cytokines. This also suggests that Kv1.3 may be one of the major mediators of the polarization of M1-like microglia (78, 79). Third, miRNA-155 and miRNA-124 in gene expression regulators are closely related to microglial polarization in ischemic stroke. The expression levels of miRNA-155 were significantly increased in LPS-activated microglia, which might target the inhibition of cytokine signaling to trigger M1-like microglia-mediated inflammation and aggravate brain injury (33). miRNA-124 induces neuroprotection and functional improvement by regulating M2-like microglial polarization in ischemic stroke (80). Finally, autophagy is a cellular metabolic pathway by which damaged organelles and misfolded proteins are degraded and recycled to maintain cellular homeostasis. Studies have shown that autophagy is activated in neurons, endothelial cells, microglia, and other brain cells in ischemic stroke and that interference with autophagy can aggravate brain injury. Studies have shown that autophagy may stimulate the transformation of microglia to the M1-like phenotype, thereby exacerbating cerebral ischemia. However, the role of autophagy in microglial polarization in ischemic stroke requires further investigation (81).

Treatment targets of microglial polarization in ischemic stroke

Currently, there are numerous studies on stroke treatment, including extensive research on small molecules. For example, the small molecule miRNA-124 can regulate the activation state of microglia/macrophages, thereby improving stroke recovery (82). Chemokine-like factor 1 (CKLF1) is an important mediator that skews microglia/macrophages toward the M1-like phenotype in the early stage of cerebral ischemic injury, and

targeting CKLF1 may also be a novel approach for IS treatment (83). Cytokine IL-4 may improve long-term neurological outcomes after stroke by inducing the M2-like phenotype in microglia/macrophages (84). In addition, the current treatment of SBI after stroke has become more promising. The inhibition of the inflammatory response promotes M2-like microglial polarization, reduces M1-like activation, and promotes the clearance of hematoma, thus playing a therapeutic role. The role of microglia-mediated inflammation in the undamaged CNS remains a hot spot of research. The development of multi-treatment targets is likely to become an important direction for the development of new therapeutic targets for ischemic stroke (Table 3).

Minocycline, an antibiotic of the tetracycline family, is known for its anti-inflammatory effects in neurological disorders, and has been reported to potentially improve functional recovery in ischemic stroke (85, 86). Minocycline can cross the BBB, accumulate in CNS cells, and inhibit microglia activation and proliferation, as well as MMP concentration and activity (85). Anti-inflammatory effects of minocycline have been demonstrated in neurological diseases in experimental models of ischemia, traumatic brain injury, and neuropathic pain as well as in Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease, amyotrophic lateral sclerosis, and several neurodegenerative diseases including spinal cord injury (87–93) (Table 4). Various experimental animal models and clinical trials have shown that minocycline can effectively cross the BBB, lead to the production of ROS and apoptosis by inhibiting the activation of microglia, and play a neuroprotective role against nervous system injury (94, 95). It has been reported that minocycline partially suppressed the production of inflammatory molecules (IL-6, TNF-A, and IL-1B) induced by LPS in peripheral monocytes by inhibiting nuclear translocation of NF-κB (96). In addition, experiments have shown that minocycline regulates M1/M2 microglial polarization through the STAT1/STAT6 pathway, reduces the production of M1-like polarization genes and enhances the expression of M2-like polarization genes by regulating STAT1 and STAT6 signaling, thus achieving the treatment of ischemia (97). Minocycline can effectively inhibit the diffusion of the neuroinflammatory cytokines IL-1β and NO, thereby reducing the brain water content and alleviating early brain edema and brain injury in the early stages of stroke by reducing the M1-like polarization of microglia (98).

Metformin, a well-known AMP-activated protein kinase (AMPK) activator, can be used in chronic post-stroke therapy to promote functional recovery after experimental stroke. Experimental evidence suggests that post-stroke metformin treatment results in a long-term elevation of M2-like signature gene expression and the suppression of M1-like signature gene expression. Metformin enhances the M2-like polarized function of microglia/macrophages involved in tissue repair and is beneficial in ischemic stroke, thereby improving post-stroke brain function recovery. Therefore, promoting the functional phenotype of microglia tilted toward M2-like polarization *via* AMPK activation after stroke emerges as a novel therapeutic strategy for stroke (99). Animal experiments have also shown that, in chronic ischemic stroke, metformin pretreatment inhibits the inflammatory pathway mediated by brain NF-κB, which is accompanied by a reduction in pro-inflammatory

TABLE 3 Therapeutic goals and related mechanisms of drugs in ischemic stroke.

Drugs	Mechanisms	Polarization pathway	Therapeutic effects	Clinical aspects
Minocycline	Inhibiting nuclear translocation of NF-KB, regulates STAT1/STAT6 pathway	Reduces the production of M1 and enhances the expression of M2	Inhibiting the activation and activation of microglia, the production of reactive oxygen species and cell apoptosis	Reducing the brain water content and brain edema, improve functional recovery in ischemic stroke
Metformin	Inhibits the inflammatory pathway mediated by brain NF-κB	M2	Reduces infarct volume and improves neurological deficits, promoting tissue repair	Chronic post-stroke therapy
rosiglitazone	Unknown	Promotes polarization of microglia toward the M2 phenotype	Educing oxidative stress, attenuating excitotoxicity	Improve white matter integrity after stroke, contributing to stroke long-term recovery
Dexmedetomidine	Unknown	Unknown	Diminish neuroinflammation in the mouse brain	Neuroprotective effect
Etifoxine	Unknown	Unknown	Reduce leukocyte infiltration, control the production of pro-inflammatory cells in microglia, improve the integrity of the blood-brain barrier	Reduce neurological deficits and infarct volume, limit brain inflammation, and provide protection against ischemia/reperfusion injury

TABLE 4 Summary of microglial polarization in neurological disorders.

Neurological disorders	Markers	Mechanism	Effects of microglial polarization
Alzheimer's disease (AD)	CD40, CD11c, CD33	Aβ clearance or Aβ clearance	neurodegeneration and cognitive impairment
Parkinson's disease (PD)	TNF-α, IL-6, CD36	May be similar to mechanism in AD	a double-edged sword
Multiple sclerosis (MS)	TGF-α	Microglia release proteases, pro-inflammatory cytokines, ROS, and RNS, and recruit reactive T lymphocytes	M1 microglia have enhanced antigen-presenting capacity, leading to demyelination and neurodegeneration. While M2 microglia protect oligodendrocytes and neurons from damage and improve disease severity
Huntington's disease (HD)	IL-6, TNFmRNA73	Microglia express higher HTT mRNA	Exacerbate neurodegeneration
Amyotrophic lateral sclerosis (ALS)	TGF-α	mSOD1 expression in microglia	Elimination of apoptotic cells, production of growth factors, maintenance of synapse structure and function are the main function of microglia

CD, Cluster of Differentiation; TNF-α, Tumor necrosis factor-α; IL, interleukin; TGF-α, Transforming growth factor-α; TNFmRNA73, Tumor necrosis factor mRNA73; Aβ, Amyloid-β; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; HTT mRNA, Huntingtin mRNA; mSOD1, Mutant human superoxide dismutase1.

cytokines, such as TNF-α, IL-1β, IL-6, and others. This significantly reduces infarct volume and improves neurological deficits while also promoting tissue repair (100).

Peroxisome proliferator-activated receptor, a ligand-activated transcription factor belonging to the nuclear receptor superfamily, has been shown to orchestrate the macrophage phenotype switch, thus leading to the inhibition of inflammation and tissue repair. Its agonist, rosiglitazone, promotes the polarization of microglia toward M2-like phenotype with a direct and indirect effect on the white matter. It may improve white matter integrity after stroke. In addition, it can reduce cerebral infarct size and edema in different animal models of stroke through the nuclear receptor PPAR-γ, thereby protecting neurons, reducing oxidative stress, attenuating excitotoxicity, and contributing to long-term recovery from stroke (44, 101). Unfortunately, the mechanism by which rosiglitazone improves stroke prognosis is still unknown and needs to be further explored.

Dexmedetomidine (DEX) is an α-adrenergic receptor agonist with different properties, including sedative, anxiolytic, antisympathetic, and analgesic, widely used as an adjuvant in the perioperative period (102). In a model of LPS-induced inflammation, many previous studies have reported that DEX can diminish neuroinflammation in the mouse brain and to modulate cytokine-associated changes in sickness behavior (103). In addition, it has been experimentally confirmed that in microglia, LPS induces a pro-inflammatory response through activation of the MAPK and NF-κB pathways (104). However, it remains to be further explored whether microglial polarization after stroke exerts neuroprotective effects through the abovementioned pathways, and the effect of clinically relevant concentrations of DEX on microglial M1/M2 polarization remains to be further investigated.

Etifoxine, a benzoxazine-based anti-anxiety compound, is an exogenous ligand of the 18-kDa translocator protein (TSPO) with high affinity (105). TSPO principally affects microglia (106). Experiments have confirmed that etifoxine can reduce brain damage and inflammation after stroke, reduce leukocyte infiltration, control the production of pro-inflammatory cells in microglia, improve BBB integrity, and reduce nerve cell death during hemorrhagic stroke. Thus, the function of repairing damaged nerves is achieved. In addition to finding reduced brain inflammation and altered microglial responses following etifoxine treatment, this still has been confirmed in mouse experiments. Together, these results demonstrate the therapeutic potential of etifoxine to reduce neurological deficits and infarct volume, limit brain inflammation, and provide protection against ischemia/reperfusion (I/R) injury. However, the mechanism of its effect needs further investigation (107).

In recent years, some traditional Chinese medicine formulations have also greatly improved post-stroke symptoms by promoting M2-like polarization. A novel resveratrol oligomer, named malibatol A, can reduce infarct size after MCAO in ischemic stroke (108) and increases M2-like microglial polarization markers such as CD206 and YM-1, producing anti-inflammatory protection. Its neuroprotective effect is largely associated with PPARγ-dependent activation of M2-like microglial polarization (44). The results of another similar study showed that the pharmacologically active component (hyperforin) of the medicinal plant Hypericum

perforatum (St. John's wort) reduced infarct volume and induced microglia from M1-like to M2-like phenotype via the inhibition of IL-17A (109). Meisoindigo, a second-generation derivative of indierythroid (110), modulates microglial/macrophage polarization by inhibiting TLR4/NF-κB, reducing ischemic stroke-induced brain injury *in vivo* and *in vitro*. In addition, Meisoindigo has a neuroprotective effect in the ischemic brain. This protective effect is attributed to the inhibition of NOD-like receptor protein 3 (NLRP3) (111) inflammasome activation and the prevention of microglia/macrophages from the pro-inflammatory M1-like phenotype to the protective M2-like phenotype to relieve the inflammation in the brain (112, 113). If the mechanism of action of these drugs is understood accurately, ischemic stroke will be treated better.

In addition, recent studies have shown that inflammasome inhibitors have also been crucial treatment targets of microglial polarization in ischemic stroke, and NLRP3 inflammasomes have been proven to play a role in ischemic stroke. JLX001, a novel compound structurally similar to cycloviral flavonoid D (CVB-D), inhibits the expression of NLRP3 and proteins associated with the NLRP3 inflammasome axis *in vivo*, promoting a transition to a microglial M2 phenotype, suggesting that JLX001 is a promising treatment for ischemic stroke (114). Treatment with the LPR3 inflammasome inhibitor tranilast reduces the expression of M1 markers and pro-inflammatory cytokines, while stimulating the expression of M2-like microglia markers, thereby ameliorating ischemic stroke (115). Acute treatment with NLRP3-specific drugs, such as MCC950, reduces neuroinflammation in IS and improves neurological outcomes after stroke (116). These outcomes may also provide targeted therapeutic opportunities for stroke-related inflammation; however, research on the role of inflammasome inhibitors against ischemic stroke is still a long way off.

In one study, it was found that the body's circulating steroid, dehydroepiandrosterone (DHEA), can penetrate the BBB, and the inflammatory response of microglia is regulated by phosphorylation of tropomyosin-associated kinase A (TrkA) and subsequent activation of pathways involving protein kinase B 1/protein kinase B 2 (Akt1/Akt2) cAMP response element-binding proteins. The latter induces the expression of Histone 3 Lysine 27 (H3K27) demethylase Jumonji D3 (Jmjd3), which enhances the polarization of M2-like microglia and may contribute to phenotype conversion in microglia. Thus, the expression of inflammation-related genes and microglial polarization were controlled, thus providing a platform for future therapeutic interventions in neuroinflammatory pathology (117, 118). Recent studies on single-cell analysis suggest that microglia are spatially and developmentally heterogeneous, have time-specific and region-dependent subtypes (119), and exhibit distinct genetic characteristics associated with changes in the CNS microenvironment (120). Heterogeneous subsets of microglia may provide a new pathway for microglia to target neuroinflammation (121, 122).

As mentioned earlier, TLR2/4 on microglia are important regulators of inflammatory responses during cerebral I/R. TLR2 and TLR4 were found to be significantly elevated during reperfusion injury, which was associated with the degree of ischemic injury and inflammation (123). TLR can also interact with endogenous and exogenous molecules released during ischemia to increase tissue damage. In addition, TLR2 and TLR4 activate different downstream inflammatory signaling pathways. The relationship between neurosteroids and TLR after ischemic events may serve as a therapeutic target for stroke therapy (124). Meanwhile, inflammatory signaling of TLR2 in the ischemic brain requires the scavenger receptor CD36. It is possible to suppress inflammation by not having this receptor. These findings suggest that the TLR2-CD36 complex can act as a sensor for ischemia at the onset of death signals and is critical for inflammatory responses (72). Therefore, TLR2 inhibition may be considered as the future treatment for ischemic stroke. TLR2 and TLR4 signaling appears to be important in controlling pathogenic immune responses after stroke, and estrogen, progesterone, and vitamin D3 all regulate TLR2 and TLR4 signaling, making them therapeutic options for stroke treatment (72).

In addition, after stroke, the immune response induces inflammation, which is one of the main reasons for the progression of ischemic injury. Microglia are involved in the inflammation of the brain and have a bone marrow source (125). A focus of current research is the trigger receptor 2 (TREM2) expressed on myeloid cells. TREM2 is a cell-surface receptor, a unidirectional transmembrane receptor, belonging to the immunoglobulin-like receptor superfamily. In the CNS, it is mainly expressed on microglia (126). The activation of trigger receptors expressed on TREM2 stimulates microglial phagocytic activity and downregulates the expression of TNF-α and inducible iNOS (8). TREM2 overexpression has been shown to have the opposite effect, while TREM2 deficiency attenuates microglial phagocytic activity and exacerbates ischemic damage in experimental stroke (127). TREM2 overexpression significantly inhibits the inflammatory response and neuronal apoptosis in cerebral I/R injury (125). Docosahexaenoic acid (DHA) treatment enhances mesencephalic astrocyte-derived neurotrophic factor (MANF), reduces the expression of TREM2 and ischemic brain damage, activates neurogenesis, and promotes functional recovery after experimental ischemic stroke (128). These findings suggest that TREM2 is an attractive target for microglia regulation in the treatment of ischemic stroke, which may be a promising therapeutic strategy (129).

Conclusion

After ischemic stroke, microglia polarize toward the classical pro-inflammatory type (M1-like) or the alternative protective

type (M2-like) for a certain period of time and under different conditions, respectively, to promote intracranial inflammation, exert an anti-inflammatory and nerve-repairing effect, and repair damaged nerve functions. Microglia play a dual role in the deleterious effects of ischemic stroke, by both protecting and controlling polarization through multiple signaling pathways. With the deepening of research, research hot spots of targeted drugs for microglial polarization are increasing year by year, providing a new therapeutic strategy for the treatment of ischemic stroke. We are looking forward to more drugs that will benefit patients.

Limitation

Although the two microglial polarization states are well studied, some researchers in the field have questioned this and even suggested discontinuing the M1/M2 classification. The idea is that the current nomenclature derived from the study of peripheral macrophages is applied to microglia, they argue that M1/M2 class macrophage activation is useless to organize our thinking about microglia, frankly said to be destructive (130). With the deepening of research, it was found that because microglia and macrophages are homologous, many markers of these two types of cells are the same (131), so research continues to use microglia/macrophages. In addition, from 14 May 2016 to 30 May 2022, a PubMed search for "M1 M2 microglia" retrieved 1,121 articles, and the number is increasing year by year. After that, in addition to using the original M1/M2-like microglia classification, some scholars proposed additional refined phenotypes (M1 microglia, M2a microglia, M2b microglia, and M2c microglia). If the M1/M2-like microglia classification had some flaws in the research at the time, then with more research, the M1-like, M2a-like, M2b-like, and M2c-like classifications would be more of the morphology and function of microglia, which would be exactly what this review reflected.

As mentioned earlier, there are many therapeutic targets for ischemic stroke in the microglial polarization process, but there are still many problems to be studied and solved. Firstly, experimental models and basic experiments of stroke are needed, and more experimental model data must be collected and organized to confirm the authenticity of relevant views. Secondly, the transition factors between M1-like and M2-like microglia and their processes require further studies. Finally, the homeostatic regulatory mechanisms of microglial polarization are discussed in this review, and the range of potential therapy targets needs to be further explored. Then, in the future, numerous studies on microglial polarization must be conducted.

Author contributions

YM and WX were in charge of the literature search and manuscript writing. The content of this article was made by consensus of all the authors. All the listed authors contributed substantially, directly, and intellectually to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Kuramatsu JB, Huttner HB. Management of oral anticoagulation after intracerebral hemorrhage. *Int J Stroke.* (2019) 14:238–46. doi: 10.1177/1747493019828555

2. Ding Q, Liu S, Yao Y, Liu H, Cai T, Han L. Global, regional, and national burden of ischemic stroke, 1990-2019. *Neurology.* (2022) 98:e279–90. doi: 10.1212/WNL.0000000000013115

3. Liu Y, Deng S, Song Z, Zhang Q, Guo Y, Yu Y, et al. MLIF modulates microglia polarization in ischemic stroke by targeting eEF1A1. *Front Pharmacol.* (2021) 12:725268. doi: 10.3389/fphar.2021.725268

4. Chen S, Yang Q, Chen G, Zhang JH. An update on inflammation in the acute phase of intracerebral hemorrhage. *Transl Stroke Res.* (2015) 6:4–8. doi: 10.1007/s12975-014-0384-4

5. Xu Y, Hu Y, Xu S, Liu F, Gao Y. Exosomal microRNAs as potential biomarkers and therapeutic agents for acute ischemic stroke: new expectations. *Front Neurol.* (2021) 12:747380. doi: 10.3389/fneur.2021.747380

6. Bernardo-Castro S, Sousa JA, Bras A, Cecilia C, Rodrigues B, Almendra L, et al. Pathophysiology of blood-brain barrier permeability throughout the different stages of ischemic stroke and its implication on hemorrhagic transformation and recovery. *Front Neurol.* (2020) 11:594672. doi: 10.3389/fneur.2020.594672

7. Mao JH, Xu Y, Li BW, Yang YL, Peng Y, Zhi F. Microglia polarization in ischemic stroke: complex mechanisms and therapeutic interventions. *Chin Med J.* (2021) 134:2415–7. doi: 10.1097/CM9.0000000000001711

8. Kanazawa M, Ninomiya I, Hatakeyama M, Takahashi T, Shimohata T. Microglia and monocytes/macrophages polarization reveal novel therapeutic mechanism against stroke. *Int J Mol Sci.* (2017) 18:2135. doi: 10.3390/ijms18102135

9. Ma Y, Wang J, Wang Y, Yang G-Y. The biphasic function of microglia in ischemic stroke. *Prog Neurobiol.* (2017) 157:247–72. doi: 10.1016/j.pneurobio.2016.01.005

10. Ozaki T, Nakamura H, Kishima H. Therapeutic strategy against ischemic stroke with the concept of neurovascular unit. *Neurochem Int.* (2019) 126:246–51. doi: 10.1016/j.neuint.2019.03.022

11. Thiebaut AM, Gauberti M, Ali C, Martinez De Lizarrondo S, Vivien D, Yepes M, et al. The role of plasminogen activators in stroke treatment: fibrinolysis and beyond. *Lancet Neurol.* (2018) 17:1121–32. doi: 10.1016/S1474-4422(18)30323-5

12. Xu X, Zhang L, Ye X, Hao Q, Zhang T, Cui G, et al. Nrf2/ARE pathway inhibits ROS-induced NLRP3 inflammasome activation in BV2 cells after cerebral ischemia reperfusion. *Inflamm Res.* (2018) 67:57–65. doi: 10.1007/s00011-017-1095-6

13. Azedi F, Mehrpour M, Talebi S, Zendedel A, Kazemnejad S, Mousavizadeh K, et al. Melatonin regulates neuroinflammation ischemic stroke damage through interactions with microglia in reperfusion phase. *Brain Res.* (2019) 1723:146401. doi: 10.1016/j.brainres.2019.146401

14. Zhuang P, Wan Y, Geng S, He Y, Feng B, Ye Z, et al. Salvianolic acids for injection (SAFI) suppresses inflammatory responses in activated microglia to attenuate brain damage in focal cerebral ischemia. *J Ethnopharmacol.* (2017) 198:194–204. doi: 10.1016/j.jep.2016.11.052

15. Chamorro Á, Dirnagl U, Urra X, Planas AM. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *Lancet Neurol.* (2016) 15:869–81. doi: 10.1016/S1474-4422(16)00114-9

16. Zhou S, Zhu W, Zhang Y, Pan S, Bao J. S100B promotes microglia M1 polarization and migration to aggravate cerebral ischemia. *Inflamm Res.* (2018) 67:937–49. doi: 10.1007/s00011-018-1187-y

17. Zhao H, Wan L, Chen Y, Zhang H, Xu Y, Qiu S. FasL incapacitation alleviates CD4(+) T cells-induced brain injury through remodeling of microglia polarization in mouse ischemic stroke. *J Neuroimmunol.* (2018) 318:36–44. doi: 10.1016/j.jneuroim.2018.01.017

18. Savage JC, Carrier M, Tremblay M-È. Morphology of microglia across contexts of health and disease. *Methods Mol Biol.* (2019) 2034:13–26. doi: 10.1007/978-1-4939-9658-2_2

19. Wyatt-Johnson SK, Herr SA, Brewster AL. Status epilepticus triggers time-dependent alterations in microglia abundance and morphological phenotypes in the hippocampus. *Front Neurol.* (2017) 8:700. doi: 10.3389/fneur.2017.00700

20. Xu J, Duan Z, Qi X, Ou Y, Guo X, Zi L, et al. Injectable gelatin hydrogel suppresses inflammation and enhances functional recovery in a mouse model of intracerebral hemorrhage. *Front Bioeng Biotechnol.* (2020) 8:785. doi: 10.3389/fbioe.2020.00785

21. Chen S, Dong Z, Cheng M, Zhao Y, Wang M, Sai N, et al. Homocysteine exaggerates microglia activation and neuroinflammation through microglia localized STAT3 overactivation following ischemic stroke. *J Neuroinflammation.* (2017) 14:187. doi: 10.1186/s12974-017-0963-x

22. Plastira I, Bernhart E, Goeritzer M, Reicher H, Kumble VB, Kogelnik N, et al. 1-Oleyl-lysophosphatidic acid (LPA) promotes polarization of BV-2 and primary murine microglia towards an M1-like phenotype. *J Neuroinflammation.* (2016) 13:205. doi: 10.1186/s12974-016-0701-9

23. Nikodemova M, Kimyon RS De I, Small AL, Collier LS, Watters JJ. Microglial numbers attain adult levels after undergoing a rapid decrease in cell number in the third postnatal week. *J Neuroimmunol.* (2015) 278:280–8. doi: 10.1016/j.jneuroim.2014.11.018

24. Tay TL, Carrier M, Tremblay M. Physiology of microglia. *Adv Exp Med Biol.* (2019) 1175:129–48. doi: 10.1007/978-981-13-9913-8_6

25. Ginhoux F, Garel S. The mysterious origins of microglia. *Nat Neurosci.* (2018) 21:897–9. doi: 10.1038/s41593-018-0176-3

26. Bilimoria PM, Stevens B. Microglia function during brain development: new insights from animal models. *Brain Res.* (2015) 1617:7–17. doi: 10.1016/j.brainres.2014.11.032

27. Casella G, Garzetti L, Gatta AT, Finardi A, Maiorino C, Ruffini F, et al. IL4 induces IL6-producing M2 macrophages associated to inhibition of neuroinflammation *in vitro* and *in vivo*. *J Neuroinflammation.* (2016) 13:139. doi: 10.1186/s12974-016-0596-5

28. Schneider UC, Davids AM, Brandenburg S, Müller A, Elke A, Magrini S, et al. Microglia inflict delayed brain injury after subarachnoid hemorrhage. *Acta Neuropathol.* (2015) 130:215–31. doi: 10.1007/s00401-015-1440-1

29. You W, Wang Z, Li H, Shen H, Xu X, Jia G, et al. Inhibition of mammalian target of rapamycin attenuates early brain injury through modulating microglial polarization after experimental subarachnoid hemorrhage in rats. *J Neurol Sci.* (2016) 367:224–31. doi: 10.1016/j.jns.2016.06.021

30. Wang G, Shi Y, Jiang X, Leak RK, Hu X, Wu Y, et al. HDAC inhibition prevents white matter injury by modulating microglia/macrophage polarization through the GSK3β/PTEN/Akt axis. *Proc Natl Acad Sci USA.* (2015) 112:2853–8. doi: 10.1073/pnas.1501441112

31. Yang S, Wang H, Yang Y, Wang R, Wang Y, Wu C, et al. Baicalein administered in the subacute phase ameliorates ischemia-reperfusion-induced brain injury by reducing neuroinflammation and neuronal damage. *Biomed Pharmacother.* (2019) 117:109102. doi: 10.1016/j.biopha.2019.109102

32. Weng L, Wu Z, Zheng W, Meng H, Han L, Wang S, et al. Malibatol A enhances alternative activation of microglia by inhibiting phosphorylation of Mammalian Ste20-like kinase1 in OGD-BV-2 cells. *Neurol Res.* (2016) 38:342–8. doi: 10.1080/01616412.2016.1174423

33. Zheng X, Huang H, Liu J, Li M, Liu M, Luo T. Propofol attenuates inflammatory response in LPS-activated microglia by regulating the miR-155/SOCS1 pathway. *Inflammation.* (2018) 41:11–9. doi: 10.1007/s10753-017-0658-6

34. Ji J, Xiang P, Li T, Lan L, Xu X, Lu G, et al. NOSH-NBP, a novel nitric oxide and hydrogen sulfide- releasing hybrid, attenuates ischemic stroke-induced neuroinflammatory injury by modulating microglia polarization. *Front Cell Neurosci.* (2017) 11:154. doi: 10.3389/fncel.2017.00154

35. Chen A-Q, Fang Z, Chen X-L, Yang S, Zhou Y-F, Mao L, et al. Microglia-derived TNF-α mediates endothelial necroptosis aggravating blood brain–barrier disruption after ischemic stroke. *Cell Death Dis.* (2019) 10:487. doi: 10.1038/s41419-019-1716-9

36. Zhang L, Zhang J, You Z. Switching of the microglial activation phenotype is a possible treatment for depression disorder. *Front Cell Neurosci.* (2018) 12:306. doi: 10.3389/fncel.2018.00306

37. Zheng J, Sun Z, Liang F, Xu W, Lu J, Shi L, et al. AdipoRon attenuates neuroinflammation after intracerebral hemorrhage through adipoR1-AMPK pathway. *Neuroscience.* (2019) 412:116–30. doi: 10.1016/j.neuroscience.2019.05.060

38. Lei B, Dawson HN, Roulhac-Wilson B, Wang H, Laskowitz DT, James ML. Tumor necrosis factor α antagonism improves neurological recovery in murine intracerebral hemorrhage. *J Neuroinflammation.* (2013) 10:103. doi: 10.1186/1742-2094-10-103

39. Liew HK, Pang CY, Hsu CW, Wang MJ Li TY, Peng HF, et al. Systemic administration of urocortin after intracerebral hemorrhage reduces neurological deficits and neuroinflammation in rats. *J Neuroinflammation.* (2012) 9:13. doi: 10.1186/1742-2094-9-13

40. Liu DL, Zhao LX, Zhang S, Du JR. Peroxiredoxin 1-mediated activation of TLR4/NF-κB pathway contributes to neuroinflammatory injury in intracerebral hemorrhage. *Int Immunopharmacol.* (2016) 41:82–9. doi: 10.1016/j.intimp.2016.10.025

41. Kolosowska N, Keuters MH, Wojciechowski S, Keksa-Goldsteine V, Laine M, Malm T, et al. Peripheral administration of IL-13 induces anti-inflammatory microglial/macrophage responses and provides neuroprotection in ischemic stroke. *Neurotherapeutics.* (2019) 16:1304–19. doi: 10.1007/s13311-019-00761-0

42. McGeer PL, McGeer EG. Targeting microglia for the treatment of Alzheimer's disease. *Expert Opin Ther Targets.* (2015) 19:497–506. doi: 10.1517/14728222.2014.988707

43. Qin C, Zhou LQ, Ma XT, Hu ZW, Yang S, Chen M, et al. Dual functions of microglia in ischemic stroke. *Neurosci Bull.* (2019) 35:921–33. doi: 10.1007/s12264-019-00388-3

44. Wang J, Xing H, Wan L, Jiang X, Wang C, Wu Y. Treatment targets for M2 microglia polarization in ischemic stroke. *Biomed Pharmacother.* (2018) 105:518–25. doi: 10.1016/j.biopha.2018.05.143

45. Jurga AM, Paleczna M, Kuter KZ. Overview of general and discriminating markers of differential microglia phenotypes. *Front Cell Neurosci.* (2020) 14:198. doi: 10.3389/fncel.2020.00198

46. Michell-Robinson MA, Touil H, Healy LM, Owen DR, Durafourt BA, Bar-Or A, et al. Roles of microglia in brain development, tissue maintenance and repair. *Brain.* (2015) 138:1138–59. doi: 10.1093/brain/awv066

47. Fumagalli S, Perego C, Pischiutta F, Zanier ER, De Simoni MG. The ischemic environment drives microglia and macrophage function. *Front Neurol.* (2015) 6:81. doi: 10.3389/fneur.2015.00081

48. Kim E, Cho S. Microglia and monocyte-derived macrophages in stroke. *Neurotherapeutics.* (2016) 13:702–18. doi: 10.1007/s13311-016-0463-1

49. Wang L, Zhang Z, Liang L, Wu Y, Zhong J, Sun X. Anti-high mobility group box-1 antibody attenuated vascular smooth muscle cell phenotypic switching and vascular remodelling after subarachnoid haemorrhage in rats. *Neurosci Lett.* (2019) 708:134338. doi: 10.1016/j.neulet.2019.134338

50. Liu R, Diao J, He S, Li B, Fei Y, Li Y, et al. XQ-1H protects against ischemic stroke by regulating microglia polarization through PPARgamma pathway in mice. *Int Immunopharmacol.* (2018) 57:72–81. doi: 10.1016/j.intimp.2018.02.014

51. Choi JY, Kim JY, Kim JY, Park J, Lee WT, Lee JE. M2 phenotype microglia-derived cytokine stimulates proliferation and neuronal differentiation of endogenous stem cells in ischemic brain. *Exp Neurobiol.* (2017) 26:33–41. doi: 10.5607/en.2017.26.1.33

52. Provencio JJ, Swank V, Lu H, Brunet S, Baltan S, Khapre RV, et al. Neutrophil depletion after subarachnoid hemorrhage improves memory *via* NMDA receptors. *Brain Behav Immun.* (2016) 54:233–42. doi: 10.1016/j.bbi.2016.02.007

53. Lyu J, Xie D, Bhatia TN, Leak RK, Hu X, Jiang X. Microglial/macrophage polarization and function in brain injury and repair after stroke. *CNS Neurosci Ther.* (2021) 27:515–27. doi: 10.1111/cns.13620

54. Ma DC, Zhang NN, Zhang YN, Chen HS. Salvianolic acids for injection alleviates cerebral ischemia/reperfusion injury by switching M1/M2 phenotypes and inhibiting NLRP3 inflammasome/pyroptosis axis in microglia in vivo and in vitro. *J Ethnopharmacol.* (2021) 270:113776. doi: 10.1016/j.jep.2021.113776

55. Ganbold T, Bao Q, Zandan J, Hasi A, Baigude H. Modulation of microglia polarization through silencing of NF-kappaB p65 by functionalized curdlan nanoparticle-mediated RNAi. *ACS Appl Mater Interfaces.* (2020) 12:11363–74. doi: 10.1021/acsami.9b23004

56. Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S, et al. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke.* (2012) 43:3063–70. doi: 10.1161/STROKEAHA.112.659656

57. Collmann FM, Pijnenburg R, Hamzei-Taj S, Minassian A, Folz-Donahue K, Kukat C, et al. Individual *in vivo* profiles of microglia polarization after stroke, represented by the genes iNOS and Ym1. *Front Immunol.* (2019) 10:1236. doi: 10.3389/fimmu.2019.01236

58. Xia CY, Zhang S, Chu SF, Wang ZZ, Song XY, Zuo W, et al. Autophagic flux regulates microglial phenotype according to the time of oxygen-glucose deprivation/reperfusion. *Int Immunopharmacol.* (2016) 39:140–8. doi: 10.1016/j.intimp.2016.06.030

59. Zhang X, Zhu XL Ji BY, Cao X, Yu LJ, Zhang Y, et al. LncRNA-1810034E14Rik reduces microglia activation in experimental ischemic stroke. *J Neuroinflammation.* (2019) 16:75. doi: 10.1186/s12974-019-1464-x

60. Taetzsch T, Levesque S, McGraw C, Brookins S, Luqa R, Bonini MG, et al. Redox regulation of NF-kappaB p50 and M1 polarization in microglia. *Glia.* (2015) 63:423–40. doi: 10.1002/glia.22762

61. Hou L, Che Y, Sun F, Wang Q. Taurine protects noradrenergic locus coeruleus neurons in a mouse Parkinson's disease model by inhibiting microglial M1 polarization. *Amino Acids.* (2018) 50:547–56. doi: 10.1007/s00726-018-2547-1

62. Su D, Cheng Y, Li S, Dai D, Zhang W, Lv M. Sphk1 mediates neuroinflammation and neuronal injury *via* TRAF2/NF-κB pathways in activated microglia in cerebral ischemia reperfusion. *J Neuroimmunol.* (2017) 305:35–41. doi: 10.1016/j.jneuroim.2017.01.015

63. Xiang B, Zhong P, Fang L, Wu X, Song Y, Yuan H. miR-183 inhibits microglia activation and expression of inflammatory factors in rats with cerebral ischemia reperfusion via NF-κB signaling pathway. *Exp Ther Med.* (2019) 18:2540–6. doi: 10.3892/etm.2019.7827

64. Xin P, Xu X, Deng C, Liu S, Wang Y, Zhou X, et al. The role of JAK/STAT signaling pathway and its inhibitors in diseases. *Int Immunopharmacol.* (2020) 80:106210. doi: 10.1016/j.intimp.2020.106210

65. Owen KL, Brockwell NK, Parker BS. JAK-STAT signaling: a double-edged sword of immune regulation and cancer progression. *Cancers.* (2019) 11:2002. doi: 10.3390/cancers11122002

66. Yang X, Xu S, Qian Y, Xiao Q. Resveratrol regulates microglia M1/M2 polarization via PGC-1α in conditions of neuroinflammatory injury. *Brain Behav Immun.* (2017) 64:162–72. doi: 10.1016/j.bbi.2017.03.003

67. Qu Z, Zheng N, Wei Y, Chen Y, Zhang Y, Zhang M, et al. Effect of cornel iridoid glycoside on microglia activation through suppression of the JAK/STAT signalling pathway. *J Neuroimmunol.* (2019) 330:96–107. doi: 10.1016/j.jneuroim.2019.01.014

68. Wang H, Zang C, Liu XS, Aster JC. The role of Notch receptors in transcriptional regulation. *J Cell Physiol.* (2015) 230:982–8. doi: 10.1002/jcp.24872

69. Yao L, Cao Q, Wu C, Kaur C, Hao A, Ling EA. Notch signaling in the central nervous system with special reference to its expression in microglia. *CNS Neurol Disord Drug Targets.* (2013) 12:807–14. doi: 10.2174/18715273113126660172

70. Wu F, Luo T, Mei Y, Liu H, Dong J, Fang Y, et al. Simvastatin alters M1/M2 polarization of murine BV2 microglia *via* Notch signaling. *J Neuroimmunol.* (2018) 316:56–64. doi: 10.1016/j.jneuroim.2017.12.010

71. Zhang Y, Liu J, Wang C, Liu J, Lu W. Toll-like receptors gene polymorphisms in autoimmune disease. *Front Immunol.* (2021) 12:672346. doi: 10.3389/fimmu.2021.672346

72. Sakate R, Nishiyama M, Fukuda Y, Kitaoka S, Furuyashiki T. The transcription factor Hhex regulates inflammation-related genes in microglia. *J Pharmacol Sci.* (2022) 149:166–71. doi: 10.1016/j.jphs.2022.04.006

73. Lindner S, Dahlke K, Sontheimer K, Hagn M, Kaltenmeier C, Barth TFE, et al. Interleukin 21–induced granzyme B–expressing B cells infiltrate tumors and regulate T cells. *Cancer Res.* (2013) 73:2468–79. doi: 10.1158/0008-5472.CAN-12-3450

74. Anttila JE, Whitaker KW, Wires ES, Harvey BK, Airavaara M. Role of microglia in ischemic focal stroke and recovery: focus on Toll-like receptors. *Prog Neuropsychopharmacol Biol Psychiatry.* (2017) 79:3–14. doi: 10.1016/j.pnpbp.2016.07.003

75. Jiang CT, Wu WF, Deng YH, Ge JW. Modulators of microglia activation and polarization in ischemic stroke (Review). *Mol Med Rep.* (2020) 21:2006–18. doi: 10.3892/mmr.2020.11003

76. Cai W, Yang T, Liu H, Han L, Zhang K, Hu X, et al. Peroxisome proliferator-activated receptor gamma (PPARgamma): a master gatekeeper in CNS injury and repair. *Prog Neurobiol.* (2018) 163–164:27–58. doi: 10.1016/j.pneurobio.2017.10.002

77. Zhao X, Wang H, Sun G, Zhang J, Edwards NJ, Aronowski J. Neuronal interleukin-4 as a modulator of microglial pathways and ischemic brain damage. *J Neurosci.* (2015) 35:11281–91. doi: 10.1523/JNEUROSCI.1685-15.2015

78. Di Lucente J, Nguyen HM, Wulff H, Jin LW, Maezawa I. The voltage-gated potassium channel Kv13 is required for microglial pro-inflammatory activation *in vivo*. *Glia.* (2018) 66:1881–95. doi: 10.1002/glia.23457

79. Tian DS Li CY, Qin C, Murugan M, Wu LJ, Liu JL. Deficiency in the voltage-gated proton channel Hv1 increases M2 polarization of microglia and attenuates brain damage from photothrombotic ischemic stroke. *J Neurochem.* (2016) 139:96–105. doi: 10.1111/jnc.13751

80. Hamzei Taj S, Kho W, Riou A, Wiedermann D, Hoehn M. MiRNA-124 induces neuroprotection and functional improvement after focal cerebral ischemia. *Biomaterials.* (2016) 91:151–65. doi: 10.1016/j.biomaterials.2016.03.025

81. Jiang M, Wang H, Jin M, Yang X, Ji H, Jiang Y, et al. Exosomes from MiR-30d-5p-ADSCs reverse acute ischemic stroke-induced, autophagy-mediated brain injury by promoting M2 microglial/macrophage polarization. *Cell Physiol Biochem.* (2018) 47:864–78. doi: 10.1159/000490078

82. Hamzei Taj S, Kho W, Aswendt M, Collmann FM, Green C, Adamczak J, et al. Dynamic modulation of microglia/macrophage polarization by miR-124 after focal cerebral ischemia. *J Neuroimmune Pharmacol.* (2016) 11:733–48. doi: 10.1007/s11481-016-9700-y

83. Chen C, Chu S-F, Ai Q-D, Zhang Z, Guan F-F, Wang S-S, et al. CKLF1 Aggravates focal cerebral ischemia injury at early stage partly by modulating microglia/macrophage toward M1 polarization through CCR4. *Cell Mol Neurobiol.* (2019) 39:651–69. doi: 10.1007/s10571-019-00669-5

84. Liu X, Liu J, Zhao S, Zhang H, Cai W, Cai M, et al. Interleukin-4 is essential for microglia/macrophage M2 polarization and long-term recovery after cerebral ischemia. *Stroke.* (2016) 47:498–504. doi: 10.1161/STROKEAHA.115.012079

85. Romero-Miguel D, Lamanna-Rama N, Casquero-Veiga M, Gomez-Rangel V, Desco M, Soto-Montenegro ML. Minocycline in neurodegenerative and psychiatric diseases: an update. *Eur J Neurol.* (2021) 28:1056–81. doi: 10.1111/ene.14642

86. Kohler E, Prentice DA, Bates TR, Hankey GJ, Claxton A, van Heerden J, et al. Intravenous minocycline in acute stroke: a randomized, controlled pilot study and meta-analysis. *Stroke.* (2013) 44:2493–9. doi: 10.1161/STROKEAHA.113.000780

87. Garrido-Mesa N, Zarzuelo A, Galvez J. Minocycline: far beyond an antibiotic. *Br J Pharmacol.* (2013) 169:337–52. doi: 10.1111/bph.12139

88. Hickman S, Izzy S, Sen P, Morsett L, El Khoury J. Microglia in neurodegeneration. *Nat Neurosci.* (2018) 21:1359–69. doi: 10.1038/s41593-018-0242-x

89. Liao B, Zhao W, Beers DR, Henkel JS, Appel SH. Transformation from a neuroprotective to a neurotoxic microglial phenotype in a mouse model of ALS. *Exp Neurol.* (2012) 237:147–52. doi: 10.1016/j.expneurol.2012.06.011

90. Ghosh R, Tabrizi SJ. Huntington disease. *Handb Clin Neurol.* (2018) 147:255–78. doi: 10.1016/B978-0-444-63233-3.00017-8

91. Yamasaki R, Lu H, Butovsky O, Ohno N, Rietsch AM, Cialic R, et al. Differential roles of microglia and monocytes in the inflamed central nervous system. *J Exp Med.* (2014) 211:1533–49. doi: 10.1084/jem.20132477

92. Kuhlmann T, Ludwin S, Prat A, Antel J, Brück W, Lassmann H. An updated histological classification system for multiple sclerosis lesions. *Acta Neuropathol.* (2016) 133:13–24. doi: 10.1007/s00401-016-1653-y

93. Bemiller SM, McCray TJ, Allan K, Formica SV, Xu G, Wilson G, et al. TREM2 deficiency exacerbates tau pathology through dysregulated kinase signaling in a mouse model of tauopathy. *Mol Neurodegener.* (2017) 12:74. doi: 10.1186/s13024-017-0216-6

94. Kim HS, Suh YH. Minocycline and neurodegenerative diseases. *Behav Brain Res.* (2009) 196:168–79. doi: 10.1016/j.bbr.2008.09.040

95. Plane JM, Shen Y, Pleasure DE, Deng W. Prospects for minocycline neuroprotection. *Arch Neurol.* (2010) 67:1442–8. doi: 10.1001/archneurol.2010.191

96. Kobayashi K, Imagama S, Ohgomori T, Hirano K, Uchimura K, Sakamoto K, et al. Minocycline selectively inhibits M1 polarization of microglia. *Cell Death Dis.* (2013) 4:e525. doi: 10.1038/cddis.2013.54

97. Lu Y, Zhou M, Li Y, Li Y, Hua Y, Fan Y. Minocycline promotes functional recovery in ischemic stroke by modulating microglia polarization through STAT1/STAT6 pathways. *Biochem Pharmacol.* (2021) 186:114464. doi: 10.1016/j.bcp.2021.114464

98. Zhao F, Hua Y, He Y, Keep RF Xi G. Minocycline-induced attenuation of iron overload and brain injury after experimental intracerebral hemorrhage. *Stroke.* (2011) 42:3587–93. doi: 10.1161/STROKEAHA.111.623926

99. Jin Q, Cheng J, Liu Y, Wu J, Wang X, Wei S, et al. Improvement of functional recovery by chronic metformin treatment is associated with enhanced alternative activation of microglia/macrophages and increased angiogenesis and neurogenesis following experimental stroke. *Brain Behav Immun.* (2014) 40:131–42. doi: 10.1016/j.bbi.2014.03.003

100. Zhu XC, Jiang T, Zhang QQ, Cao L, Tan MS, Wang HF, et al. Chronic Metformin preconditioning provides neuroprotection *via* suppression of NF-kappaB-mediated inflammatory pathway in rats with permanent cerebral ischemia. *Mol Neurobiol.* (2015) 52:375–85. doi: 10.1007/s12035-014-8866-7

101. Han L, Cai W, Mao L, Liu J, Li P, Leak RK, et al. Rosiglitazone promotes white matter integrity and long-term functional recovery after focal cerebral ischemia. *Stroke.* (2015) 46:2628–36. doi: 10.1161/STROKEAHA.115.010091

102. Cai Y, Xu H, Yan J, Zhang L, Lu Y. Molecular targets and mechanism of action of dexmedetomidine in treatment of ischemia/reperfusion injury. *Mol Med Rep.* (2014) 9:1542–50. doi: 10.3892/mmr.2014.2034

103. Yeh CH, Hsieh LP, Lin MC, Wei TS, Lin HC, Chang CC, et al. Dexmedetomidine reduces lipopolysaccharide induced neuroinflammation, sickness behavior, and anhedonia. *PLoS ONE.* (2018) 13:e0191070. doi: 10.1371/journal.pone.0191070

104. Park J, Min JS, Kim B, Chae UB, Yun JW, Choi MS, et al. Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-kappaB pathways. *Neurosci Lett.* (2015) 584:191–6. doi: 10.1016/j.neulet.2014.10.016

105. Simon-O'Brien E, Gauthier D, Riban V, Verleye M. Etifoxine improves sensorimotor deficits and reduces glial activation, neuronal degeneration, and neuroinflammation in a rat model of traumatic brain injury. *J Neuroinflammation.* (2016) 13:203. doi: 10.1186/s12974-016-0687-3

106. Bonsack FT, Alleyne CH Jr., Sukumari-Ramesh S. Augmented expression of TSPO after intracerebral hemorrhage: a role in inflammation? *J Neuroinflammation.* (2016) 13:151. doi: 10.1186/s12974-016-0619-2

107. Li HD Li M, Shi E, Jin WN, Wood K, Gonzales R, et al. A translocator protein 18 kDa agonist protects against cerebral ischemia/reperfusion injury. *J Neuroinflammation.* (2017) 14:151. doi: 10.1186/s12974-017-0921-7

108. Pan J, Jin JL, Ge HM, Yin KL, Chen X, Han LJ, et al. Malibatol A regulates microglia M1/M2 polarization in experimental stroke in a PPARgamma-dependent manner. *J Neuroinflammation.* (2015) 12:51. doi: 10.1186/s12974-015-0270-3

109. Ma L, Pan X, Zhou F, Liu K, Wang L. Hyperforin protects against acute cerebral ischemic injury through inhibition of interleukin-17A-mediated microglial activation. *Brain Res.* (2018) 1678:254–61. doi: 10.1016/j.brainres.2017.08.023

110. Zhao Y, Han P, Liu L, Wang X, Xu P, Wang H, et al. Indirubin modulates CD4(+) T-cell homeostasis via PD1/PTEN/AKT signalling pathway in immune thrombocytopenia. *J Cell Mol Med.* (2019) 23:1885–98. doi: 10.1111/jcmm.14089

111. Walsh JG, Muruve DA, Power C. Inflammasomes in the CNS. *Nat Rev Neurosci.* (2014) 15:84–97. doi: 10.1038/nrn3638

112. Lepore F, D'Alessandro G, Antonangeli F, Santoro A, Esposito V, Limatola C, et al. CXCL16/CXCR6 axis drives microglia/macrophages phenotype in physiological conditions and plays a crucial role in glioma. *Front Immunol.* (2018) 9:2750. doi: 10.3389/fimmu.2018.02750

113. Ye Y, Jin T, Zhang X, Zeng Z, Ye B, Wang J, et al. Meisoindigo protects against focal cerebral ischemia-reperfusion injury by inhibiting NLRP3 inflammasome activation and regulating microglia/macrophage polarization via TLR4/NF-kappaB Signaling pathway. *Front Cell Neurosci.* (2019) 13:553. doi: 10.3389/fncel.2019.00553

114. Bian HJ, Xu SY Li HQ, Jia JQ, Ye L, Shu S, et al. JLX001 ameliorates cerebral ischemia injury by modulating microglial polarization and compromising NLRP3 inflammasome activation via the NF-kappaB signaling pathway. *Int Immunopharmacol.* (2021) 101:108325. doi: 10.1016/j.intimp.2021.108325

115. Jia H, Qi X, Fu L, Wu H, Shang J, Qu M, et al. NLRP3 inflammasome inhibitor ameliorates ischemic stroke by reprogramming the phenotype of microglia/macrophage in a murine model of distal middle cerebral artery occlusion. *Neuropathology.* (2022) 42:181–9. doi: 10.1111/neup.12802

116. Bellut M, Papp L, Bieber M, Kraft P, Stoll G, Schuhmann MK. NLPR3 inflammasome inhibition alleviates hypoxic endothelial cell death in vitro and protects blood-brain barrier integrity in murine stroke. *Cell Death Dis.* (2021) 13:20. doi: 10.1038/s41419-021-04379-z

117. Alexaki VI, Fodelianaki G, Neuwirth A, Mund C, Kourgiantaki A, Ieronimaki E, et al. DHEA inhibits acute microglia-mediated inflammation through activation of the TrkA-Akt1/2-CREB-Jmjd3 pathway. *Mol Psychiatry.* (2017) 23:1410–20. doi: 10.1038/mp.2017.167

118. Tang Y, Li T, Li J, Yang J, Liu H, Zhang XJ, et al. Jmjd3 is essential for the epigenetic modulation of microglia phenotypes in the immune pathogenesis of Parkinson's disease. *Cell Death Differ.* (2014) 21:369–80. doi: 10.1038/cdd.2013.159

119. Bottcher C, Schlickeiser S, Sneeboer MAM, Kunkel D, Knop A, Paza E, et al. Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. *Nat Neurosci.* (2019) 22:78–90. doi: 10.1038/s41593-018-0290-2

120. Galatro TF, Holtman IR, Lerario AM, Vainchtein ID, Brouwer N, Sola PR, et al. Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. *Nat Neurosci.* (2017) 20:1162–71. doi: 10.1038/nn.4597

121. Masuda T, Sankowski R, Staszewski O, Prinz M. Microglia heterogeneity in the single-cell era. *Cell Rep.* (2020) 30:1271–81. doi: 10.1016/j.celrep.2020.01.010

122. Masuda T, Sankowski R, Staszewski O, Bottcher C, Amann L. Sagar, et al. Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature.* (2019) 566:388–92. doi: 10.1038/s41586-019-0924-x

123. Ling Y, Jin L, Ma Q, Huang Y, Yang Q, Chen M, et al. Salvianolic acid A alleviated inflammatory response mediated by microglia through inhibiting the activation of TLR2/4 in acute cerebral ischemia-reperfusion. *Phytomedicine.* (2021) 87:153569. doi: 10.1016/j.phymed.2021.153569

124. Deng W, Mandeville E, Terasaki Y, Li W, Holder J, Chuang AT, et al. Transcriptomic characterization of microglia activation in a rat model of ischemic stroke. *J Cereb Blood Flow Metab.* (2020) 40:S34–48. doi: 10.1177/0271678X20932870

125. Wu R, Li X, Xu P, Huang L, Cheng J, Huang X, et al. TREM2 protects against cerebral ischemia/reperfusion injury. *Mol Brain.* (2017) 10:20. doi: 10.1186/s13041-017-0296-9

126. Backes FN, de Souza A, Bianchin MM. Biomarkers in the prognostic evaluation of ischemic stroke: is there benefit in the measurements of TREM-1 and TREM-2 in the acute phase? *Clin Biochem.* (2021) 98:10–6. doi: 10.1016/j.clinbiochem.2021.07.016

127. Kawabori M, Kacimi R, Kauppinen T, Calosing C, Kim JY, Hsieh CL, et al. Triggering receptor expressed on myeloid cells 2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke. *J Neurosci.* (2015) 35:3384–96. doi: 10.1523/JNEUROSCI.2620-14.2015

128. Belayev L, Hong SH, Freitas RS, Menghani H, Marcell SJ, Khoutorova L, et al. DHA modulates MANF and TREM2 abundance, enhances neurogenesis, reduces infarct size, and improves neurological function after experimental ischemic stroke. *CNS Neurosci Ther*. (2020) 26:1155–67. doi: 10.1111/cns.13444

129. Ma WY, Wang SS, Wu QL, Zhou X, Chu SF, Chen NH. The versatile role of TREM2 in regulating of microglia fate in the ischemic stroke. *Int Immunopharmacol.* (2022) 109:108733. doi: 10.1016/j.intimp.2022.108733

130. Ransohoff RM. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci.* (2016) 19:987–91. doi: 10.1038/nn.4338

131. Grassivaro F, Martino G, Farina C. The phenotypic convergence between microglia and peripheral macrophages during development and neuroinflammation paves the way for new therapeutic perspectives. *Neural Regen Res.* (2021) 16:635–7. doi: 10.4103/1673-5374.295272

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Blood-based protein biomarkers for the diagnosis of acute stroke: A discovery-based SWATH-MS proteomic approach

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Background and purposes: Recent developments in high-throughput proteomic approach have shown the potential to discover biomarkers for diagnosing acute stroke and to elucidate the pathomechanisms specific to different stroke subtypes. We aimed to determine blood-based protein biomarkers to diagnose total stroke (IS+ICH) from healthy controls, ischemic stroke (IS) from healthy controls, and intracerebral hemorrhage (ICH) from healthy control subjects within 24 h using a discovery-based SWATH-MS proteomic approach.

Methods: In this discovery phase study, serum samples were collected within 24 h from acute stroke (IS & ICH) patients and healthy controls and were subjected to SWATH-MS-based untargeted proteomics. For protein identification, a high-pH fractionated peptide library for human serum proteins (obtained from SCIEX) comprising of 465 proteins was used. Significantly differentially expressed (SDE) proteins were selected using the following criteria: >1.5-fold change for upregulated, <0.67 for downregulated, p-value<0.05, and confirmed/tentative selection using Boruta random forest. Protein–protein interaction network analysis and the functional enrichment analysis were conducted using STRING 11 online tool, g:Profiler tool and Cytoscape 3.9.0 software. The statistical analyses were conducted in R version 3.6.2.

Results: Our study included 40 stroke cases (20 IS, 20 ICH) within 24 h and 40 age-, sex-, hypertension-, and diabetes-matched healthy controls. We quantified 375 proteins between the stroke cases and control groups through SWATH-MS analysis. We observed 31 SDE proteins between total stroke and controls, 16 SDE proteins between IS and controls, and 41 SDE proteins between ICH and controls within 24 h. Four proteins [ceruloplasmin, alpha-1-antitrypsin (SERPINA1), von Willebrand factor (vWF), and coagulation factor XIII B chain (F13B)] commonly differentiated total stroke, IS, and ICH from healthy

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control subjects. The most common significant pathways in stroke cases involved complement and coagulation cascades, platelet degranulation, immune-related processes, acute phase response, lipid-related processes, and pathways related to extracellular space and matrix.

Conclusion: Our discovery phase study identified potential protein biomarker candidates for the diagnosis of acute stroke and highlighted significant pathways associated with different stroke subtypes. These potential biomarker candidates warrant further validation in future studies with a large cohort of stroke patients to investigate their diagnostic performance.

KEYWORDS

stroke, ischemic stroke, intracerebral hemorrhage, proteomics, blood biomarkers, SWATH-MS

Introduction

Stroke is a medical emergency in which brain cells die rapidly post its onset. It is broadly classified based on its etiology into two types: (1) ischemic stroke (IS)—occlusion of the artery supplying oxygen-rich blood to the brain resulting in brain cell or tissue death within minutes; and (2) intracerebral hemorrhage (ICH)—rupturing of the blood vessel that bleeds into the surrounding brain leading to further brain damage (1). Despite the two stroke subtypes sharing a similar risk profile (2), they exhibit distinct molecular mechanisms in the acute phase (3–6). Thus, an efficient and rapid diagnosis of stroke is warranted within the first few hours of symptom onset for the effective treatment strategies to be implemented to prevent adverse outcomes. Due to the unavailability of neuroimaging facilities in most developing nations and time-sensitive nature of revascularization therapies, blood biomarkers are needed to aid clinical decision-making. Biomarkers detected in the blood may also help in elucidating the molecular mechanisms underlying the two stroke subtypes.

Recent developments in high-throughput proteomic approaches have shown the potential to discover biomarkers for diagnosing acute stroke and to elucidate the pathomechanisms specific to different stroke subtypes. The label-free approach using data-independent acquisition (DIA) method acquires superior peptide peaks compared to conventional proteomic data-dependent acquisition (DDA) methods and allows screening of a broad range of protein biomarkers with high reproducibility and efficiency.

Few studies in the past have utilized the high-throughput proteomic approaches for blood biomarker identification in stroke (7–11). However, these studies were conducted beyond the 24-h time window and failed to identify the expression pattern of proteins in the acute phase of stroke. Majority of these studies pooled their samples for proteomic analysis, which might lead to false-positive or false-negative results as pooled samples do not reflect the diseased/non-diseased state of a single person (10, 11). Therefore, our exploratory study aimed to determine blood-based protein biomarkers related to the pathogenesis of stroke in the acute phase of onset. Our goal was to provide a list of candidate protein markers that can diagnose and differentiate total stroke (IS + ICH) from healthy controls, IS from healthy controls, and ICH from healthy control subjects within 24 h of symptom onset using a discovery-based SWATH-MS proteomic approach without pooling any sample. We used an age-, sex-, and risk factor- (hypertension and diabetes) matched healthy control group, to identify biomarker expression pattern specific to stroke pathophysiology.

Methods

The study was conducted at the Department of Neurology, All India Institute of Medical Sciences, New Delhi, India, from August 2016 to August 2021 in collaboration with Institute of Genomics and Integrative Biology (IGIB), New Delhi, India. Stroke patients aged 18 years and above, ischemic or hemorrhagic confirmed by neuroimaging and clinical diagnosis admitted within 24 h of symptom onset to the neurology wards and/or emergency department of AIIMS, New Delhi, were included in the study. All included patients had clinical signs consistent with the definition of stroke given by the Stroke Council of American Heart Association (AHA)/ American Stroke Association (ASA) (12). A control group comprising of age- (±2 years), sex-, hypertension-, and diabetes-matched individuals was taken from subjects in the general outpatient department (OPD) with no prior history of any neurological disorder and was evaluated by questionnaire for verifying stroke-free status (QVSFS) (13). A written informed consent was taken from all the subjects included in the study prior to collecting blood samples and clinical history.

Sample size

The literature suggests a sample size of 10 to 30 to be adequate for conducting an exploratory/discovery phase study (14, 15). Therefore, based on the feasibility, budget, and time frame of the study, the sample size for the discovery phase was kept as 40 per group consisting of 40 stroke (20 IS and 20 ICH) and 40 control subjects.

Blood sample collection

After the written informed consent was obtained, 5 ml of peripheral blood samples was taken in serum vacutainer tubes from 20 IS and 20 ICH patients admitted within 24-h onset of stroke. Blood samples were also taken from 40 healthy individuals who served as controls for the study. For serum collection, it was left standing at room temperature for 30 min until clotted. It then underwent centrifugation at 3,000 rpm for 10 min, after which the serum was separated into serum-containing vials. Five aliquots of each sample (100 µl) were prepared and stored at −80°C until further analysis.

Sample preparation

Ten µl of serum samples was used for protein precipitation. To 90µl of 1X phosphate buffer saline (PBS), 10 µl serum was added and vortex mixed. Protein precipitation was performed using pre-chilled acetone. Briefly, to 100 µl protein extract, four times volume of pre-chilled acetone was added, vortex mixed, and centrifuged at 15,000 g for 10 min at 4°C. The supernatant was discarded, and the protein pellets were air-dried at room temperature and suspended in 0.1 M Tris-HCl with 8 M urea and pH 8.5. Protein quantitation was performed using the Bradford assay.

Reduction, alkylation, and trypsin digestion

A total of 20 µg of protein from each sample were reduced with 25 mM of dithiothreitol (DTT) for 30 min at 60°C, followed by alkylation using 55 mM of iodoacetamide (IAA) at room temperature (in the dark) for 30 min. These samples were then subjected to trypsin digestion in an enzyme to substrate ratio of 1:10 (trypsin: protein) for 16–18 h at 37°C. Finally, the tryptic peptides were vacuum-dried in vacuum concentrator.

Sequential window acquisition of all theoretical fragment ion spectra-mass spectrometry (SWATH-MS) data acquisition

Peptides from each sample were cleaned up using C18 ZipTip (Merck) using the manufacturer's protocol. SWATH-MS analysis (16) for the samples was performed on a quadrupole-TOF hybrid mass spectrometer (TripleTOF 6600, SCIEX) coupled to an Eksigent NanoLC-425 system. Optimized source parameters were used, and curtain gas and nebulizer gas were maintained at 25 psi and 30 psi, respectively. The ion spray voltage was set to 5.5 kV, and the temperature was set to 250°C. About 4 µg of peptides was loaded on a trap column (ChromXP C18CL 5 µm 120 Å, Eksigent, SCIEX), and online desalting was performed with a flow rate of 10 µl per min for 10 min. Next, the peptides were separated on a reverse-phase C18 analytical column (ChromXP C18, 3 µm 120 Å, Eksigent, SCIEX) in 57 min long gradient with a flow rate of 5 µl/min using water with 0.1% formic acid and acetonitrile with 0.1% formic acid.

SWATH method was created with 95 precursor isolation windows, defined based on precursor m/z frequencies in DDA run using the SWATH Variable Window Calculator (SCIEX), with a minimum window of 5 m/z. Data were acquired using Analyst TF 1.7.1 Software (SCIEX). Accumulation time was set to 250 msec for the MS scan (400–1,250 m/z) and 25 msec for the MS/MS scans (100–1,500 m/z). Rolling collision energies were applied for each window based on the m/z range of each SWATH and a charge 2+ ion, with a collision energy spread of five. The total cycle time was 3.37 s.

Bioinformatic and statistical analyses

For identification of the proteins using SWATH analysis, a high-pH fractionated peptide library for human serum proteins (obtained from SCIEX) comprising of 465 proteins was used. SWATH peaks were extracted using this library in SWATH 2.0 microapp in PeakView 2.2 software (SCIEX), excluding shared peptides. SWATH run files were added, and retention time calibration was performed using peptides from abundant proteins. The peptide query parameters (PQPs) for peak extraction were as follows: maximum of 10 peptides per protein, five transitions per peptide, >95% peptide confidence threshold, and 1% peptide false discovery rate (FDR). XIC extraction window was set to 55 min with 75 ppm XIC Width. These PQPs were derived from the high-pH fractionated peptide library for peptide identification. All information was exported in the form of MarkerView (mrkw) files. In MarkerView 1.2.1 (SCIEX), data normalization was performed using total area sum normalization for internal correction and exported to excel.

The data were $\log_2$ transformed to account for naturally skewed intensity values.

Batch correction for removing the non-biological experimental variations including the sample batches run at different timepoints was performed using the "ComBat" function inside the "sva" package (17) in R version 3.6.2. The principal component analysis (PCA) plots for the batch uncorrected and batch corrected data were plotted using the "prcomp" function inside the "factoextra" package (18) in R version 3.6.2. Significant differences between the means of the two groups were calculated using a *t*-test.

Significantly differentially expressed proteins were selected using two criteria: (i) *p*-value <0.05 and $\pm$ 1.5-fold change (>1.5 for upregulated and <0.67 for downregulated proteins) cutoffs wherein significantly upregulated/downregulated proteins were visualized using the volcano plot created in R version 3.6.2; or

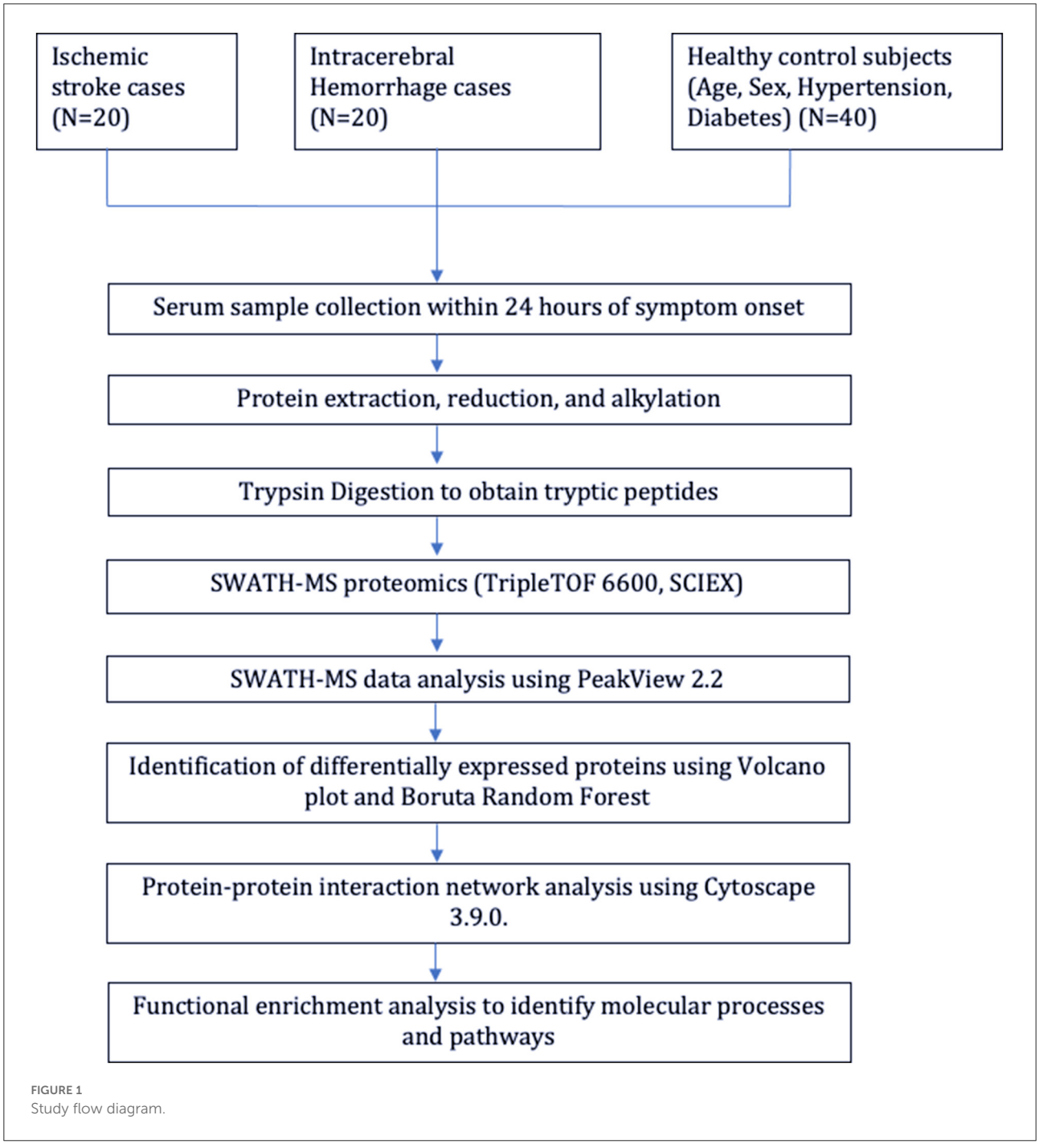

FIGURE 1
Study flow diagram.

(ii) confirmed/tentative selection in the Boruta random forest feature selection method using the "Boruta" package (19) in R version 3.6.2.

The STRING 11 online tool (Search Tool for the Retrieval of Interacting Genes/Proteins 11) (20) was used to create the protein network of the significantly differentially expressed proteins between various conditions. Furthermore, protein–protein interaction network analysis was conducted using Cytoscape 3.9.0 software (21). Centrality analysis was conducted to identify the most important node with a high degree of interaction in the network. The functional enrichment analysis was conducted using the g:Profiler tool.

Results

Our study included 80 subjects; 40 stroke cases (20 IS and 20 ICH) were recruited within 24 h of symptom onset and age- (±2), sex-, hypertension-, and diabetes-matched 40 healthy control subjects. The mean age of IS, ICH, and control subjects was 52.85 ± 10.86, 47.60 ± 9.76, and 50.20 ± 10.64 years, respectively. Both stroke cases and healthy controls consisted of 25 (62.5%) males and 15 (37.5%) females, respectively. The mean blood sampling time (in h) from the symptom onset was 12.11 ± 6.23 in IS cases and 12.46 ± 6.68 in ICH cases (p=0.86). The study flow diagram is given in Figure 1. The baseline characteristics of the subjects included in our study are given in Table 1, and blood investigations are given in Supplementary Table 1.

SWATH-MS to identify differential proteome in stroke cases and controls

Serum proteomic profiles were compared between 40 stroke (20 IS and 20 ICH) and 40 healthy controls using the SWATH-MS approach. From the high-pH fractionated peptide library for human serum proteins (obtained from SCIEX) comprising of 465 proteins, we could quantify 375 proteins at 1% peptide FDR between the stroke cases and control groups through SWATH-MS analysis. The total ion chromatogram (TIC) of all the 80 serum samples analyzed using the discovery-based SWATH-MS proteomics is given in Supplementary Figure 1. The batch variation observed in our samples due to the different run times was removed as depicted in the PCA plots in Supplementary Figure 2.

Differentially expressed proteins between total stroke and healthy controls

Between 40 stroke and 40 control subjects, 119 proteins were upregulated with a fold change of >1.5, and 72 were downregulated with a fold change of <0.67 in total stroke cases compared to healthy controls. Using the fold change and *p*-value cutoffs, 22 proteins were significantly differentially expressed between total stroke and healthy controls. Seventeen proteins were significantly upregulated, while five were significantly downregulated in total stroke compared to healthy controls (Figure 2A). Using the Boruta random forest method, 19 proteins were identified as confirmed/tentative features (Figure 2B; Supplementary Table 2). Ten proteins (UniProt IDs: P00450, P01009, P04275, P05160, P05155, P02750, P02786, Q15848, P06318, and P06331) were common in both the fold change with *p*-value and the Boruta random forest criteria. Thus, after combining the distinctly expressed proteins using both approaches, 31 significantly differentially expressed proteins were identified between total stroke and control subjects within 24 h (Table 2). A heatmap of 31 significantly differentially expressed proteins showing the $\log_2$ fold change expression pattern between total stroke and controls is given in Figure 3A.

Out of 31 proteins, 26 were successfully matched to proteins within the STRING database. The interaction network consisted of 26 nodes and 115 edges. Twenty-five proteins formed a highly connected network except for the GGH protein. Centrality analysis identified that APOB had the highest degree of interaction (DoI)= 19 with other proteins followed by haptoglobin (HP) (DoI = 18), APOB (DoI = 15), and SERPINA1 (DoI= 15). Eight protein–protein interactions in our network had an interaction score of more than 0.90, with the highest interaction score of 0.97 for HPX-HP followed by 0.944 for LBP-SAA1, 0.940 for SERPING1-C1QB, 0.937 for MMP2-A2M, and 0.92 for MMP2-SAA1 (Figure 3B).

Using Gene Ontology (GO) database, the top 10 cellular components, molecular interactions, or biological processes involved are as follows: serine-type endopeptidase inhibitor activity, acute phase response, acute inflammatory response, extracellular space, extracellular region, blood microparticle, extracellular exosome, extracellular vesicle, extracellular membrane-bounded organelle, and extracellular organelle. One Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, namely complement and coagulation cascades, was identified to be significantly involved. Using the Reactome database, the top five pathways involved are as follows: platelet degranulation, response to elevated platelet cytosolic Ca2+, hemostasis, binding and uptake of ligands by Scavenger Receptors, and innate immune system (Figure 4).

Differentially expressed proteins between ischemic stroke and healthy controls

Between 20 IS and 20 controls, 118 proteins were upregulated, and 72 were downregulated in IS cases compared to control subjects. Using the fold change and *p*-value criteria, 13 proteins were significantly differentially expressed,

TABLE 1 Baseline characteristics of acute stroke patients and healthy control subjects.

S. No	Characteristics	IS patients ($N = 20$)	ICH patients ($N = 20$)	*p*-value	Total stroke ($N = 40$)	No. of obs. (Controls)	Control subjects ($N = 40$)	*P*-value
1.	Age (years), Mean ± SD and Median (IQR)	52.85 ± 10.86, 53.5 (45.5–61.5)	47.60 ± 9.76, 48 (43–55.5)	0.12	50.22 ± 10.53, 49 (45–59.5)	40	50.20 ± 10.69, 48.5 (44.5–60)	Matched
2.	Male, *n* (%)	11 (55)	14 (70)	0.33	25 (62.5)	40	25 (62.5)	
3.	Female, *n* (%)	9 (45)	6 (30)		15 (37.5)	40	15 (37.5)	
4.	Blood sampling time from onset (in h.), Mean ± SD & Median (IQR)	12.11 ± 6.23, 11.5 (7.12–17)	12.46 ± 6.68, 12.58 (6.25–18.62)	0.86	12.28 ± 6.38, 12.58 (6.5–17.75)	-	-	-
5.	Time taken to reach hospital (in hrs.), Mean ± SD & Median (IQR)	4.21 ± 2.98, 3.87 (2–5)	6.41 ± 6.27, 3.75 (2.08–10.12)	0.16	5.31 ± 4.97, 3.87 (2–5.75)	-	-	-
6.	Ambulance as a mode of transport, *n* (%)	6 (30)	4 (20)	0.53	10 (25)	-	-	-
7.	Any surgical procedure, *n* (%)	2 (10)	5 (25)	0.21	7 (17.5)	-	-	-
Risk factors for stroke								
8.	Hypertension, *n* (%)	8 (40)	14 (70)	0.06	22 (55)	40	22 (55)	Matched
9.	Diabetes, *n* (%)	4 (20)	1 (5)	0.15	5 (12.5)	40	5 (12.5)	
10.	Dyslipidemia, n (%)	4 (20)	0 (0)	**0.03**	4 (10)	40	6 (15)	0.50
11.	Myocardial Infarction, *n* (%)	0	0	–	0	40	1 (2.5)	0.31
12.	Atrial Fibrillation, *n* (%)	0	0	–	0	40	1 (2.5)	0.31
13.	Angina Pectoris, *n* (%)	1 (5%)	0	0.31	1 (2.5)	30	1 (3.33)	0.83
14.	Migraine, *n* (%)	0	0	–	0	40	3 (7.50)	0.08
15.	Current Smoking, *n* (%)	9 (45)	10 (50)	0.75	19 (47.5)	40	7 (17.50)	**0.004**
17.	Alcohol Intake, *n* (%)	2 (10)	6 (30)	0.11	8 (20)	40	11 (27.5)	0.43
17.	No exercise, *n* (%)	18 (90)	17 (85)	0.63	35 (87.5)	39	11 (28.21)	**<0.001**
18.	Sedentary lifestyle, *n* (%)	7 (35)	7 (35)	1.00	14 (35)	37	6 (16.22)	0.06
19.	Low Education, *n* (%)	13 (65)	15 (75)	0.49	28 (70)	39	12 (30.77)	**0.0005**
20.	Low socio-economic status, *n* (%)	9 (45)	11 (55)	0.53	20 (50)	40	0	**<0.001**
21.	Obesity, *n* (%)	7 (35)	10 (50)	0.34	17 (42.5)	38	24 (63.16)	0.07
22.	Family history of stroke, *n* (%)	3 (15)	1 (5)	0.29	4 (10)	40	2 (5)	0.39
23.	Family history of hypertension, *n* (%)	10 (50)	6 (30)	0.20	16 (40)	40	12 (30)	0.44
24.	Family history of diabetes, *n* (%)	7 (35)	3 (15)	0.14	10 (25)	40	16 (40)	0.15
25.	Family history of heart attack, *n* (%)	4 (20)	3 (15)	0.68	7 (17.5)	40	12 (30)	0.19
Vitals at admission								
26.	SBP (mmHg), Mean ± SD & Median (IQR)	152.7 ± 35.71, 147 (127.5–175)	178 ± 35.29, 176 (150–214)	**0.03**	165.35 ± 37.31, 163 (137–190)	39	141.46 ± 24.55, 134 (121–155)	**0.001**
27.	DBP (mmHg), Mean ± SD & Median (IQR)	87.5 ± 17.27, 87 (80–95)	100.10 ± 17.00, 100 (90–110)	**0.02**	93.8 ± 18.08, 90 (82–104)	39	89.95 ± 15.90, 90 (78–98)	0.32

obs, observations; IS, ischemic stroke; ICH, intracerebral hemorrhage; SD, standard deviation; IQR, interquartile range; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Bold values, $p < 0.05$.

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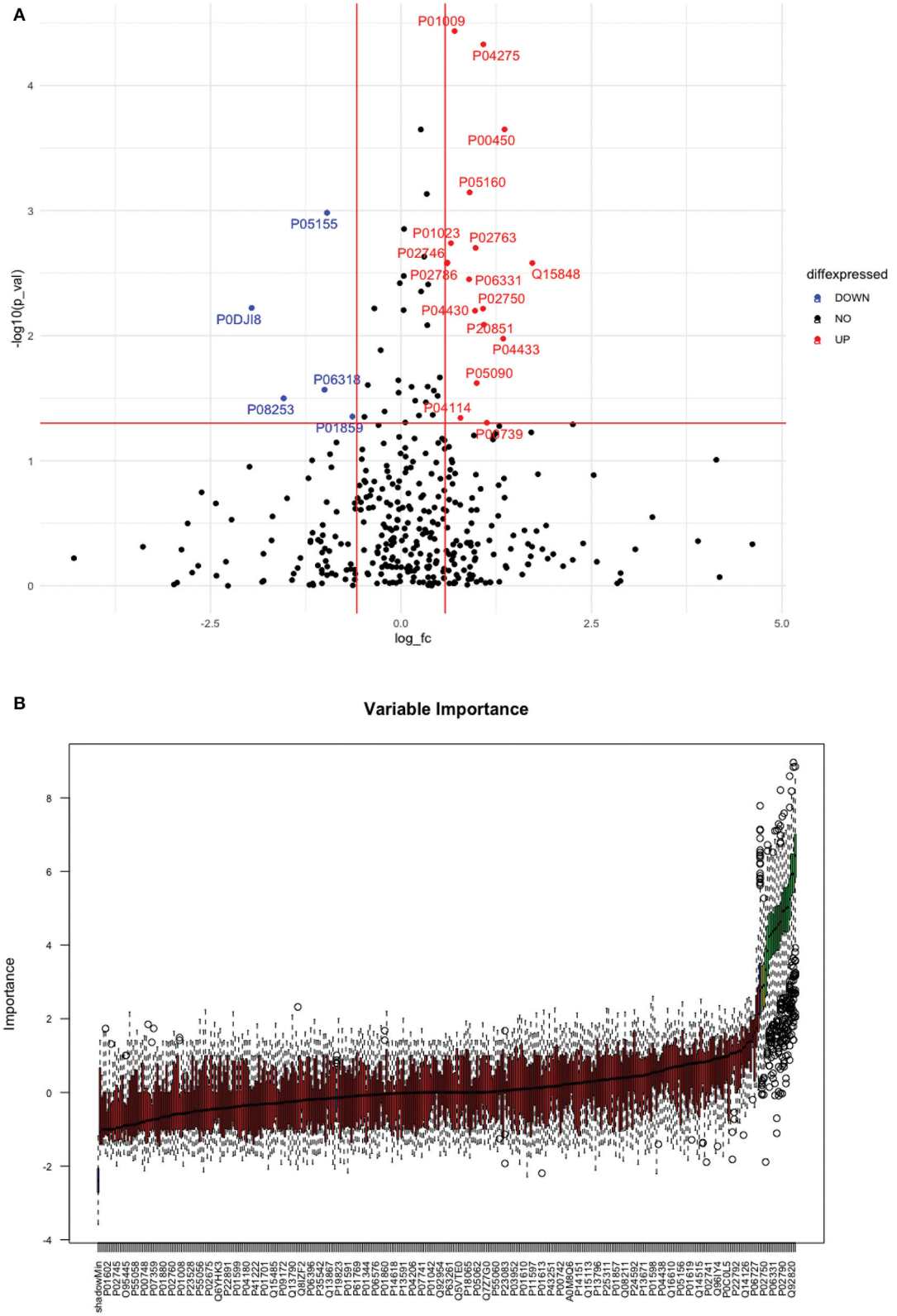

FIGURE 2
(A) Volcano plot depicting the $\log_2$ fold change on the x-axis and -log10 *p*-value on the y-axis for the upregulated and downregulated proteins in 40 total stroke cases compared to 40 healthy controls. The two vertical lines represent the threshold for log2 fold change (>0.58 and < −0.58), and the horizontal line represents the threshold for log10 *p*-value (>1.3). The red dots on the graph indicate the proteins which are

(Continued)

FIGURE 2 (Continued)
significantly upregulated (log2 fold change>0.58; log10 p-value>1.3), and blue dots indicate the proteins which are significantly downregulated (log2 fold change< −0.58; log10 p-value>1.3). **(B)** Feature selection using the Boruta random forest depicting important features/proteins to differentiate 40 total stroke and 40 healthy controls. The blue bars on the graph indicate shadow features for minimum, average, and maximum shadow feature values. The red bars on the graph indicate the proteins which were rejected as irrelevant features, yellow bars indicate the proteins which were marked tentative as uncertain features, and green bars indicate the proteins which were marked confirmed as valid features and identified as important proteins after the Boruta random forest feature selection analysis.

wherein 11 were significantly upregulated while two were significantly downregulated in IS compared to controls (Figure 5A). Using the Boruta random forest method, nine more proteins were identified as confirmed/tentative features (Figure 5B; Supplementary Table 3). Six proteins (UniProt IDs: P04114, P01023, P01009, P02786, P05090, and Q99972) were common using both approaches. Finally, 16 distinct proteins were identified using the above two criteria which were significantly differentially expressed between IS and healthy controls within 24 h (Table 3). A heatmap of 16 significantly differentially expressed proteins showing the $\log_2$ fold change expression pattern between IS and healthy controls is given in Figure 6A.

All the 16 proteins were successfully matched within the STRING database. The interaction network analysis identified 16 nodes and 29 edges. Thirteen out of 16 proteins formed a highly connected network with other proteins, whereas three proteins (TBC1D30, MINPP1, and MYOC) remained disconnected from the network. Centrality analysis identified that APOB and SERPINA1 had the highest DoI of 9 with other proteins, followed by vWF (DoI = 6), APOD (DoI = 5), and TFRC (DoI = 5). Seven protein–protein interactions in our network had an interaction score of more than 0.70 with the highest protein–protein interaction score of 0.950 for APOB-APOF followed by 0.888 for A2M-SERPINA1, 0.857 for CP-SERPINA1, and 0.806 for CP-A2M (Figure 6B).

For conducting the functional enrichment analysis of significantly differentially expressed proteins identified between IS and controls, the top 10 cellular components were selected in the GO database, including chaperone binding, acute phase response, negative regulation of smooth muscle cell proliferation, extracellular region, extracellular space, endoplasmic reticulum, endoplasmic reticulum lumen, blood microparticle, extracellular exosome, and extracellular vesicle. The only KEGG pathway that was found to be significantly associated was complement and coagulation cascades. When the enrichment analysis was done using the Reactome database, the top five pathways observed were formation of fibrin clot (clotting cascade), regulation of IGF transport and uptake by IGFBPs, post-translational protein phosphorylation, LDL remodeling, and plasma lipoprotein assembly, remodeling, and clearance (Figure 7).

Differentially expressed proteins between intracerebral hemorrhage and healthy controls

Between 20 ICH and 20 controls, 102 proteins were upregulated while 83 were downregulated in ICH cases compared to control subjects. Using the fold change and p-value criteria, 30 proteins were significantly differentially expressed; 23 significantly upregulated and seven significantly downregulated in ICH cases compared to healthy controls (Figure 8A). Using the Boruta random forest method, 21 more proteins were further identified as confirmed/tentative features (Figure 8B; Supplementary Table 4). Ten proteins (UniProt IDs: P00450, P04275, Q06033, P04217, P36955, B9A064, P02750, P01833, P35443, and P24592) were common using both the above-mentioned criteria for protein selection. Thus, after combining the distinct proteins using both the criteria, we identified 41 proteins that significantly differentially expressed ICH from healthy controls within 24 h (Table 4). A heatmap of 41 significantly differentially expressed proteins showing the $\log_2$ fold change expression pattern between ICH and healthy controls is given in Figure 9A.

Out of 41 proteins, 34 successfully matched within the STRING database. The interaction network analysis identified 34 nodes and 125 edges. Except for three proteins (TUBA1A, IGFBP6, and LCP1), the interaction network of the remaining 31 proteins was highly connected with each other. SERPINA1 had the highest DoI of 18 with other proteins followed by APOA1 (DoI = 17), CLU (DoI =14), PLG (DoI = 14), and ORM1 (DoI = 14) after conducting the centrality analysis. Twelve protein–protein interactions in our network had an interaction score of more than 0.90 with the highest protein–protein interaction score of 0.999 for APOA1-APOA2 and SERPING1-C1S followed by 0.998 for PLG-SERPINF2, 0.997 for APOA1-APOE, and 0.995 for APOA1-CLU and APOE-APOC1 (Figure 9B).

The top 10 cellular components and biological processes when analyzed using the GO database involved are as follows: extracellular space, extracellular exosome, extracellular region, extracellular vesicle, extracellular membrane-bounded organelle, extracellular organelle, blood microparticle, collagen-containing extracellular matrix, vesicle, and extracellular matrix. Using the KEGG database, we observed that two pathways were significantly involved: complement and coagulation cascades and cholesterol metabolism. The top five

TABLE 2 List of significantly differentially expressed proteins between total stroke and healthy controls within 24 h of symptom onset using fold change with *p*-value and Boruta random forest feature selection criteria.

S. No	UniProt ID	Protein name (Gene annotation)	Fold change*	*P*-value	Boruta decision
1	Q15848	Adiponectin (GN = ADIPOQ)	**3.30**	**0.003**	**Confirmed**
2	P00450	Ceruloplasmin (GN = CP)	**2.57**	**0.0002**	**Confirmed**
3	P04433	Ig kappa chain V-III region VG (Fragment)	**2.54**	**0.01**	Rejected
4	P00739	Haptoglobin-related protein (GN = HPR)	**2.18**	**0.04**	Rejected
5	P20851	C4b-binding protein beta chain (GN = C4BPB)	**2.13**	**0.008**	Rejected
6	P04275	von Willebrand factor (GN = VWF)	**2.12**	**<0.001**	**Confirmed**
7	P02750	Leucine-rich alpha-2-glycoprotein (GN = LRG1)	**2.11**	**0.006**	**Tentative**
8	P05090	Apolipoprotein D (GN = APOD)	**1.99**	**0.02**	Rejected
9	P02763	Alpha-1-acid glycoprotein 1 (GN = ORM1)	**1.97**	**0.002**	Rejected
10	P04430	Ig kappa chain V-I region BAN	**1.96**	**0.006**	Rejected
11	P05160	Coagulation factor XIII B chain (GN = F13B)	**1.87**	**0.0007**	**Confirmed**
12	P06331	Ig heavy chain V-II region ARH-77	**1.86**	**0.0035**	**Confirmed**
13	P04114	Apolipoprotein B-100 (GN = APOB)	**1.72**	**0.045**	Rejected
14	P01009	Alpha-1-antitrypsin (GN = SERPINA1)	**1.63**	**<0.001**	**Confirmed**
15	P01023	Alpha-2-macroglobulin (GN = A2M)	**1.58**	**0.002**	Rejected
16	P02746	Complement C1q subcomponent subunit B (GN = C1QB)	**1.53**	**0.003**	Rejected
17	P02786	Transferrin receptor protein 1 (GN = TFRC)	**1.52**	**0.003**	**Confirmed**
18	Q92820	Gamma-glutamyl hydrolase (GN = GGH)	1.28	**0.03**	**Confirmed**
19	Q06033	Inter-alpha-trypsin inhibitor heavy chain H3 (GN = ITIH3)	1.26	**0.0007**	**Confirmed**
20	P02790	Hemopexin (GN = HPX)	1.20	**0.004**	**Confirmed**
21	P18428	Lipopolysaccharide-binding protein (GN = LBP)	1.20	**0.0002**	**Confirmed**
22	P01011	Alpha-1-antichymotrypsin (GN = SERPINA3)	1.03	**0.001**	**Confirmed**
23	P08185	Corticosteroid-binding globulin (GN = SERPINA6)	0.99	**0.004**	**Confirmed**
24	P19827	Inter-alpha-trypsin inhibitor heavy chain H1 (GN = ITIH1)	0.98	0.06	**Confirmed**
25	Q9UK55	Protein Z-dependent protease inhibitor (GN = SERPINA10)	0.85	0.073	**Confirmed**
26	P08697	Alpha-2-antiplasmin (GN = SERPINF2)	0.78	**0.006**	**Confirmed**
27	P01859	Ig gamma-2 chain C region (GN = IGHG2)	**0.64**	**0.04**	Rejected
28	P05155	Plasma protease C1 inhibitor (GN = SERPING1)	**0.51**	**0.001**	**Confirmed**
29	P06318	Ig lambda chain V-VI region WLT	**0.50**	**0.03**	**Tentative**
30	P08253	72 kDa type IV collagenase (GN = MMP2)	**0.34**	**0.03**	Rejected
31	P0DJI8	Serum amyloid A-1 protein (GN = SAA1)	**0.26**	**0.006**	Rejected

*Fold change is a ratio representing the change of protein concentration between total stroke cases and healthy control subjects.
Bold values, Fold change >1.5 or <0.67, p-value<0.05 and confirmed/tentative in Boruta random forest.

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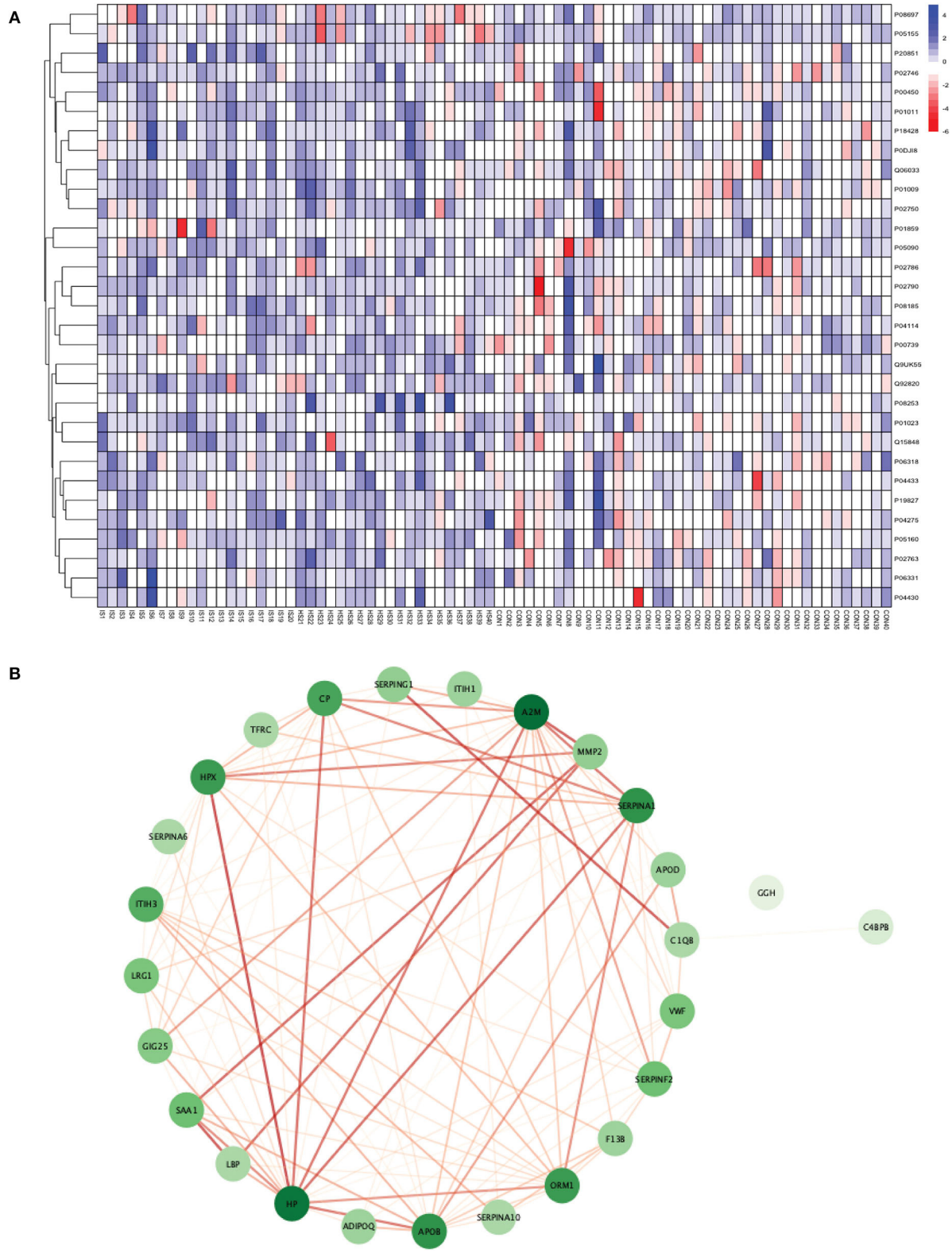

FIGURE 3
(A) Heatmap showing the distribution and expression pattern of 31 significantly differentially expressed proteins between total stroke and healthy controls. Each column represents a different subject, while each row represents the UniProt ID of different proteins. The blue squares represent upregulated, and the red squares represent downregulated proteins in total stroke compared to healthy controls. White squares represent no change in protein expression. **(B)** Protein–protein interaction network analysis of differentially expressed proteins between total

(Continued)

FIGURE 3 (Continued)
stroke and healthy controls. The color of the nodes represents the level of degree of interaction between the proteins ranging from 0 to 19, with dark green representing a high degree of interaction (toward 19) and light green representing a low degree of interaction (toward zero). The color of edges represents the interaction score ranging from zero to one, with dark red edges representing an interaction score with high confidence (toward one) and light red edges representing an interaction score with low confidence (toward zero).

FIGURE 4
Functional enrichment analysis of differentially expressed proteins between total stroke and healthy controls.

pathways identified using the Reactome database were platelet degranulation, response to elevated platelet cytosolic Ca2+, complement cascade, hemostasis, platelet activation, signaling, and aggregation. Complement cascade was a common pathway identified in KEGG and Reactome databases (Figure 10).

Discussion

A stroke, if left untreated, results in the loss of 1.9 million neurons per min after its onset (22). Therefore, rapid diagnosis of stroke is critical to initiate stroke type-specific treatment and prevent large-scale brain damage. In this discovery phase study, we identified several differentially expressed proteins in stroke and its subtypes that elucidated key pathological processes involved in the acute phase of IS and ICH. Our study identified four proteins (ceruloplasmin, SERPINA1, vWF, and F13B) that commonly differentiated total stroke, IS, and ICH from healthy control subjects. To the best of our knowledge, this is the first label-free proteomic study that identified blood biomarkers for the diagnosis of stroke within 24 h of symptom onset. A list of proteomic studies conducted till now for the identification of diagnostic biomarkers in stroke is given in the Supplementary Table 5.

Protein biomarkers identified between total stroke and healthy controls

We identified 31 significantly differentially expressed proteins between total stroke (IS + ICH) and healthy controls within 24 h. Twenty-five proteins formed a highly connected

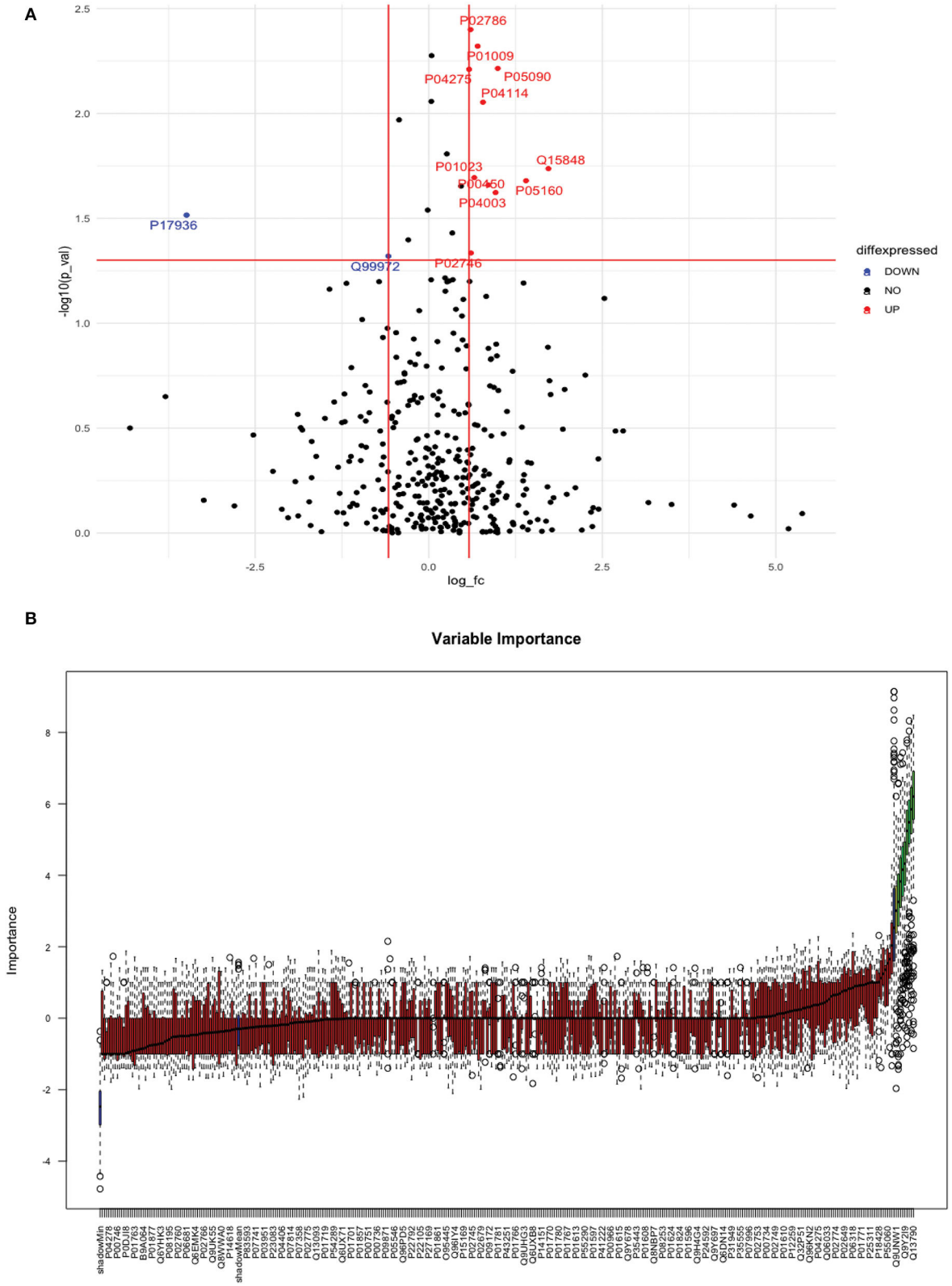

FIGURE 5
(A) Volcano plot depicting the log2 fold change on the x-axis and −log10 *p*-value on the y-axis for the upregulated and downregulated proteins in 20 IS cases compared to 20 healthy controls. **(B)** Feature selection using the Boruta random forest depicting important features/proteins to differentiate 20 IS and 20 healthy controls.

TABLE 3 List of significantly differentially expressed proteins between ischemic stroke and healthy controls using fold change with *p*-value and Boruta random forest feature selection criteria.

S. No	UniProt ID	Protein name (Gene annotation)	Fold change*	*P*-value	Boruta selection
1	Q15848	Adiponectin (GN = ADIPOQ)	**3.30**	**0.02**	Rejected
2	P05160	Coagulation factor XIII B chain (GN = F13B)	**2.64**	**0.02**	Rejected
3	P05090	Apolipoprotein D (GN = APOD)	**1.99**	**0.01**	**Confirmed**
4	P04003	C4b-binding protein alpha chain (GN = C4BPA)	**1.95**	**0.02**	Rejected
5	P00450	Ceruloplasmin (GN = CP)	**1.81**	**0.02**	Rejected
6	P04114	Apolipoprotein B-100 (GN = APOB)	**1.72**	**0.01**	**Confirmed**
7	P01009	Alpha-1-antitrypsin (GN = SERPINA1)	**1.63**	**0.005**	**Confirmed**
8	P01023	Alpha-2-macroglobulin (GN = A2M)	**1.58**	**0.02**	**Confirmed**
9	P02746	Complement C1q subcomponent subunit B (GN = C1QB)	**1.53**	**0.04**	Rejected
10	P02786	Transferrin receptor protein 1 (GN = TFRC)	**1.52**	**0.04**	**Confirmed**
11	P04275	von Willebrand factor (GN = VWF)	**1.50**	**0.01**	Rejected
12	Q13790	Apolipoprotein F (GN = APOF)	1.38	**0.02**	**Confirmed**
13	Q9UNW1	Multiple inositol polyphosphate phosphatase 1 (GN = MINPP1)	1.18	0.07	**Confirmed**
14	Q9Y2I9	TBC1 domain family member 30 (GN = TBC1D30)	0.74	**0.01**	**Confirmed**
15	Q99972	Myocilin (GN = MYOC)	**0.66**	**0.04**	**Confirmed**
16	P17936	Insulin-like growth factor-binding protein 3 (GN = IGFBP3)	**0.09**	**0.03**	Rejected

*Fold change is a ratio representing the change of protein concentration between IS cases and healthy control subjects.

Bold values: Fold change >1.5 or <0.67, p-value<0.05 and confirmed/tentative in Boruta random forest.

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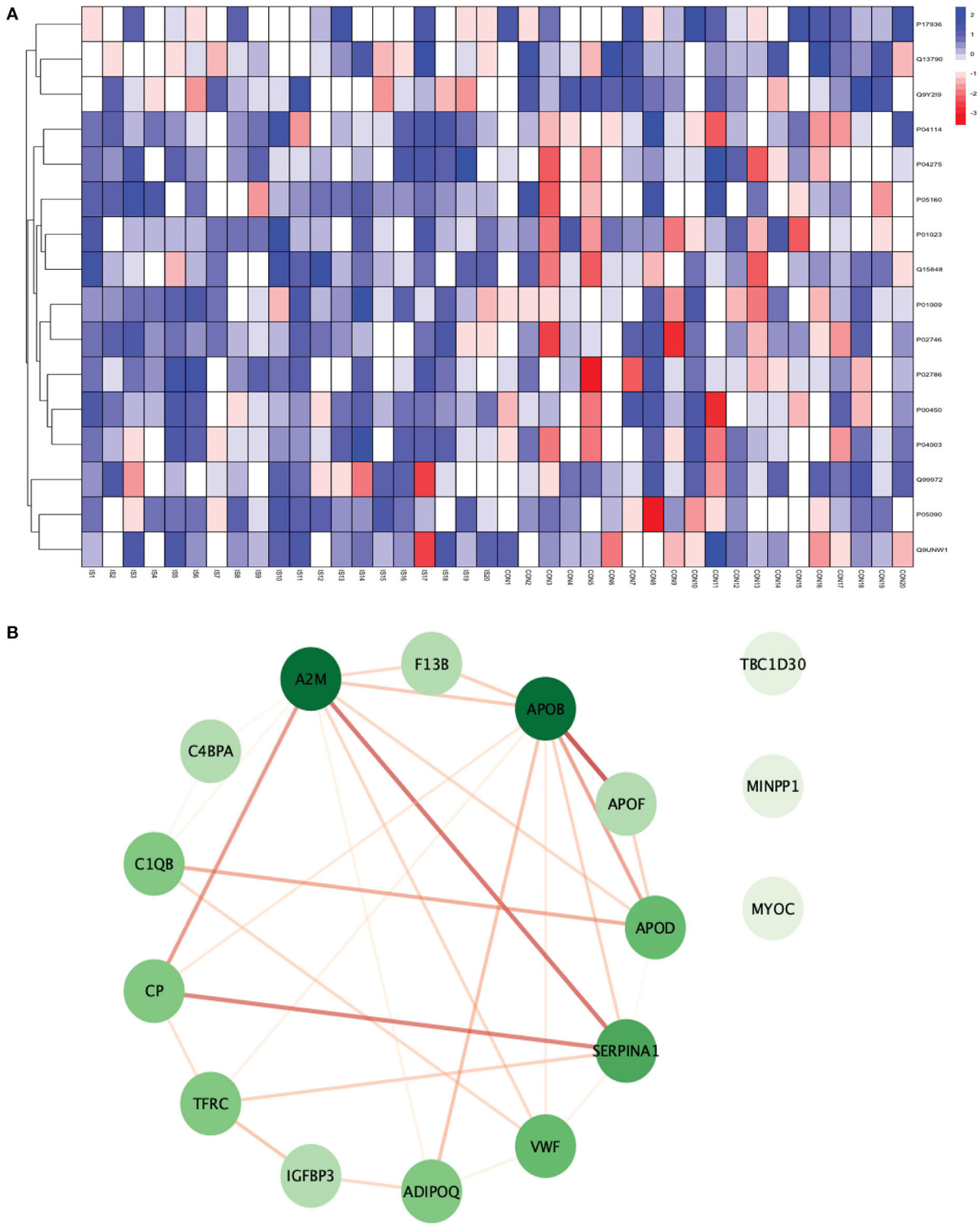

FIGURE 6
(A) Heatmap showing the distribution and expression pattern of 16 significantly differentially expressed proteins between IS and healthy controls. **(B)** Protein–protein interaction network analysis of differentially expressed proteins between IS and healthy controls.

FIGURE 7
Functional enrichment analysis of differentially expressed proteins between ischemic stroke and healthy controls.

network, with APOB having the highest DoI. The most common significant pathways involved complement and coagulation cascades, immune-related processes, acute phase response, acute inflammatory response, hemostasis, and pathways related to extracellular space and matrix. In the Malicek et al. (9) study, they identified 12 significantly differentially expressed proteins between seven stroke and two control subjects in plasma. The stroke subjects were recruited within an average of 7 days (1–15 days) of symptom onset. Only two proteins (ITIH3 and LBP) were commonly differentially expressed in our study when compared to Malicek et al. (9). Another proteomic study conducted by Allard et al. (23) in plasma samples utilized the SELDI approach and identified four differentially expressed proteins (Apo C-1, Apo C-III, serum amyloid A, and antithrombin-III fragment) between 21 total stroke (IS = 11, ICH = 10) and 21 healthy controls recruited within 72 h. Of these four proteins, serum amyloid A was also differentially expressed in our study and was significantly downregulated in total stroke (fold change= 0.26) compared to the control group.

Protein biomarkers identified between ischemic stroke and healthy controls

Between IS and healthy controls, our study identified 16 proteins within 24 h. The interaction network for 13 out of 16 proteins was highly connected. APOB and SERPINA1 had the highest DoI within the network. The most common significant pathways/processes associated with these proteins included

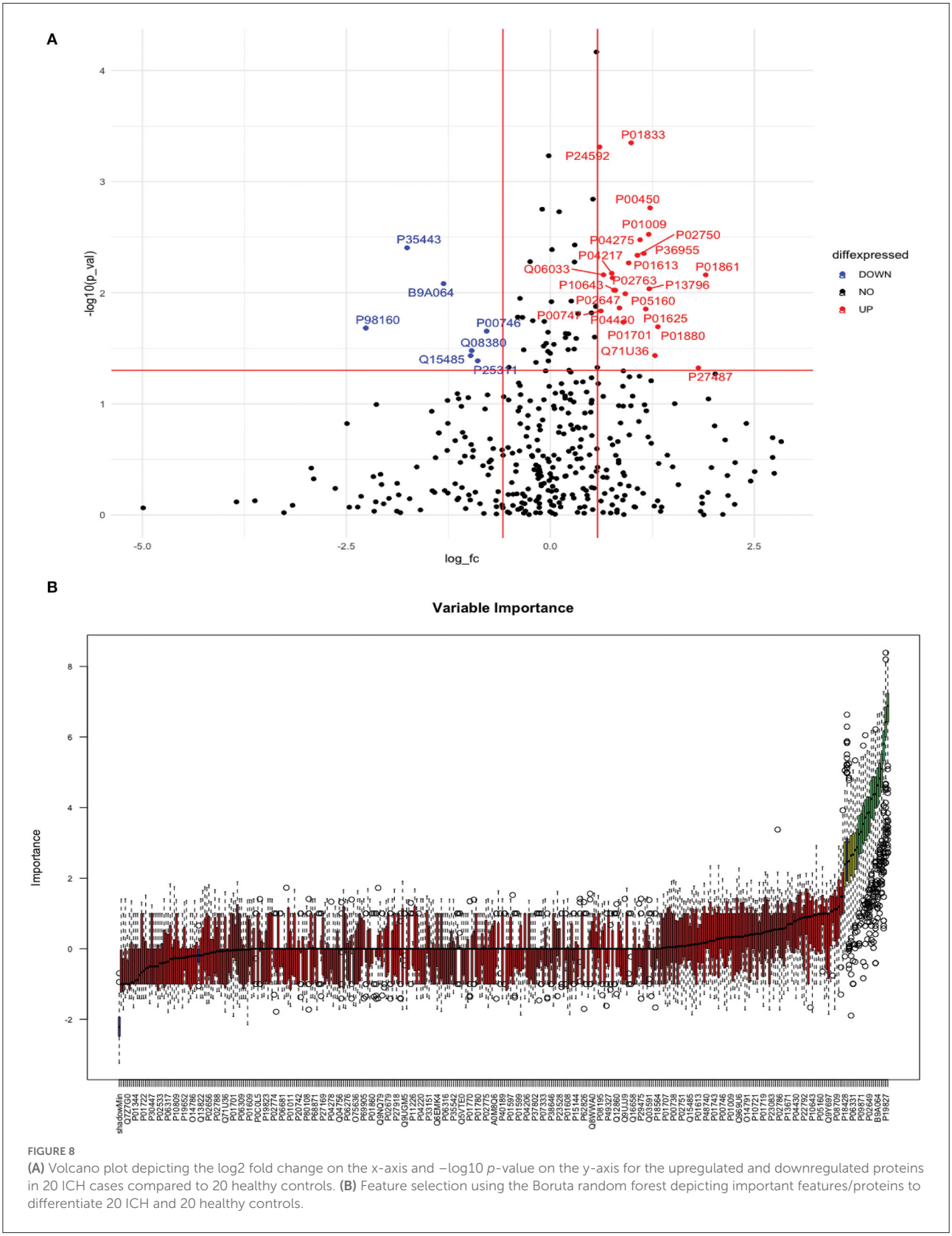

FIGURE 8
(A) Volcano plot depicting the log2 fold change on the x-axis and −log10 *p*-value on the y-axis for the upregulated and downregulated proteins in 20 ICH cases compared to 20 healthy controls. **(B)** Feature selection using the Boruta random forest depicting important features/proteins to differentiate 20 ICH and 20 healthy controls.

TABLE 4 List of significantly differentially expressed proteins between intracerebral hemorrhage and healthy controls using fold change with *p*-value and Boruta random forest feature selection criteria.

S. No	UniProt ID	Protein name (Gene annotation)	Fold change*	*P*-value	Boruta decision
1	P01861	Ig gamma-4 chain C region (GN = IGHG4)	**3.75**	**0.01**	Rejected
2	P27487	Dipeptidyl peptidase 4 (GN = DPP4)	**3.52**	**0.05**	Rejected
3	P01880	Ig delta chain C region (GN = IGHD)	**2.49**	**0.02**	Rejected
4	Q71U36	Tubulin alpha-1A chain (GN = TUBA1A)	**2.43**	**0.04**	Rejected
5	P00450	Ceruloplasmin (GN = CP)	**2.33**	**0.00**	**Confirmed**
6	P13796	Plastin-2 (GN = LCP1)	**2.32**	**0.01**	Rejected
7	P01009	Alpha-1-antitrypsin (GN = SERPINA1)	**2.31**	**0.00**	Rejected
8	P02649	Apolipoprotein E (GN = APOE)	**2.30**	**0.20**	**Confirmed**
9	P01625	Ig kappa chain V-IV region Len	**2.25**	**0.01**	Rejected
10	P36955	Pigment epithelium-derived factor (GN = SERPINF1)	**2.21**	**0.004**	**Confirmed**
11	P04275	von Willebrand factor (GN = VWF)	**2.15**	**0.003**	**Tentative**
12	P02750	Leucine-rich alpha-2-glycoprotein (GN = LRG1)	**2.10**	**0.005**	**Confirmed**
13	P01833	Polymeric immunoglobulin receptor (GN = PIGR)	**1.99**	**0.0004**	**Confirmed**
14	P09871	Complement C1s subcomponent (GN = C1S)	**1.96**	**0.06**	**Confirmed**
15	P01613	Ig kappa chain V-I region Ni	**1.95**	**0.01**	Rejected
16	P05160	Coagulation factor XIII B chain (GN = F13B)	**1.89**	**0.01**	Rejected
17	P01701	Ig lambda chain V-I region NEW	**1.86**	**0.02**	Rejected
18	P04430	Ig kappa chain V-I region BAN	**1.80**	**0.01**	Rejected
19	P10643	Complement component C7 (GN = C7)	**1.74**	**0.01**	Rejected
20	P02647	Apolipoprotein A-I (GN = APOA1)	**1.72**	**0.01**	Rejected
21	P02763	Alpha-1-acid glycoprotein 1 (GN = ORM1)	**1.70**	**0.01**	Rejected
22	P04217	Alpha-1B-glycoprotein (GN = A1BG)	**1.69**	**0.01**	**Tentative**
23	Q06033	Inter-alpha-trypsin inhibitor heavy chain H3 (GN = ITIH3)	**1.57**	**0.01**	**Tentative**
24	P00747	Plasminogen (GN = PLG)	**1.54**	**0.01**	Rejected
25	P24592	Insulin-like growth factor-binding protein 6 (GN = IGFBP6)	**1.52**	**0.0005**	**Confirmed**
26	P19827	Inter-alpha-trypsin inhibitor heavy chain H1 (GN = ITIH1)	1.48	**<0.001**	**Confirmed**
27	Q9UK55	Protein Z-dependent protease inhibitor (GN = SERPINA10)	1.44	**0.001**	**Confirmed**
28	P08697	Alpha-2-antiplasmin (GN = SERPINF2)	1.42	**0.02**	**Confirmed**
29	P10909	Clusterin (GN = CLU)	1.27	**0.02**	**Confirmed**
30	P06331	Ig heavy chain V-II region ARH-77	1.23	**0.004**	**Tentative**
31	P18428	Lipopolysaccharide-binding protein (GN = LBP)	1.23	**0.01**	**Tentative**
32	P05155	Plasma protease C1 inhibitor (GN = SERPING1)	1.01	**0.004**	**Confirmed**

(Continued)

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TABLE 4 (Continued).

S. No	UniProt ID	Protein name (Gene annotation)	Fold change*	*P*-value	Boruta decision
33	P02654	Apolipoprotein C-I (GN = APOC1)	0.99	**0.001**	**Confirmed**
34	P02652	Apolipoprotein A-II (GN = APOA2)	0.94	**0.03**	**Confirmed**
35	P00746	Complement factor D (GN = CFD)	**0.58**	**0.02**	Rejected
36	P25311	Zinc-alpha-2-glycoprotein (GN = AZGP1)	**0.54**	**0.04**	Rejected
37	Q08380	Galectin-3-binding protein (GN = LGALS3BP)	**0.51**	**0.03**	Rejected
38	Q15485	Ficolin-2 (GN = FCN2)	**0.51**	**0.04**	Rejected
39	B9A064	Immunoglobulin lambda-like polypeptide 5 (GN = IGLL5)	**0.40**	**0.01**	**Confirmed**
40	P35443	Thrombospondin-4 (GN = THBS4)	**0.30**	**0.004**	**Confirmed**
41	P98160	Basement membrane-specific heparan sulfate proteoglycan core protein (GN = HSPG2)	**0.21**	**0.02**	Rejected

*Fold change is a ratio representing the change of protein concentration between ICH cases and healthy control subjects.
Bold values: Fold change >1.5 or <0.67, p-value <0.05 and confirmed/tentative in Boruta random forest.

complement and coagulation cascade, acute phase response, blood microparticle, clot formation, and pathways including extracellular region. A few studies in the past have used the proteomic approach to identify diagnostic biomarkers in IS compared to healthy controls, but most of these studies recruited IS patients beyond the 24-h time window. In a study published last year by Malicek et al. (9) on plasma samples, four proteins were significantly differentially expressed between three IS and two controls using a label-free proteomic approach. The IS subjects in this exploratory study were recruited within an average duration of 7 days (1–15 days) from symptom onset. No protein was commonly expressed upon comparing their results with our study. The difference in the protein expression profile between our studies could be attributed to the small sample size and longer blood sample collection time in the Malicek et al. study (9). Another recent study by Lee et al. (7) on serum samples used a similar approach of discovery-based SWATH-MS proteomics and identified 163 differential proteins with more than 2-fold change in 20 IS patients recruited within 10 days of symptom onset compared to 20 healthy controls. After applying the FDR-corrected *p*-values, they identified 13 significant biomarker candidates. C4BPA was the only common protein that was differentially expressed (upregulated in IS in both studies) in our study and in Lee et al. (7). The same authors conducted another SWATH-MS proteomic study to identify serum biomarkers related to coagulation cascade between 18 IS cases recruited within 7 days and 16 healthy controls. (8). They identified 60 upregulated (fold change >1.5) and 50 downregulated (fold change <1/1.5) proteins in IS compared to controls out of which four proteins (prothrombin, plasminogen, fibrinogen alpha chain, and histidine-rich glycoprotein) related to coagulation cascade were finally selected, none of which were identified in our study. Another study by Qin et al. (10) on plasma samples recruited 40 IS patients with large vessel occlusion (LVO) within 7 days of symptom onset and 20 healthy controls. They identified seven differentially expressed proteins with a fold change of >1.2 or <0.83 between the two groups using the iTRAQ labeling-based proteomic approach. No protein was commonly expressed between our study and Qin et al. (10). Therefore, the differential proteins identified in our study within 24 h were vastly different from the ones identified in Lee et al. (7) within 10 days, Lee et al. (8) and Qin et al. (10) within 7 days of symptom onset. When comparing our results with the other three studies, these contrasting findings provide crucial insights into the differences in the expression level of protein markers in the acute phase of stroke (within 24 h) compared to 7–10 days after the stroke onset.

The only proteomic study conducted on stroke patients in the Indian population by Sharma et al. (11) quantified 389 proteins using the iTRAQ labeling approach between pooled serum samples of 20 IS and 20 healthy controls in their discovery phase and identified 60 proteins with a difference of 1.5-fold or greater between the two groups. They observed that 25 proteins

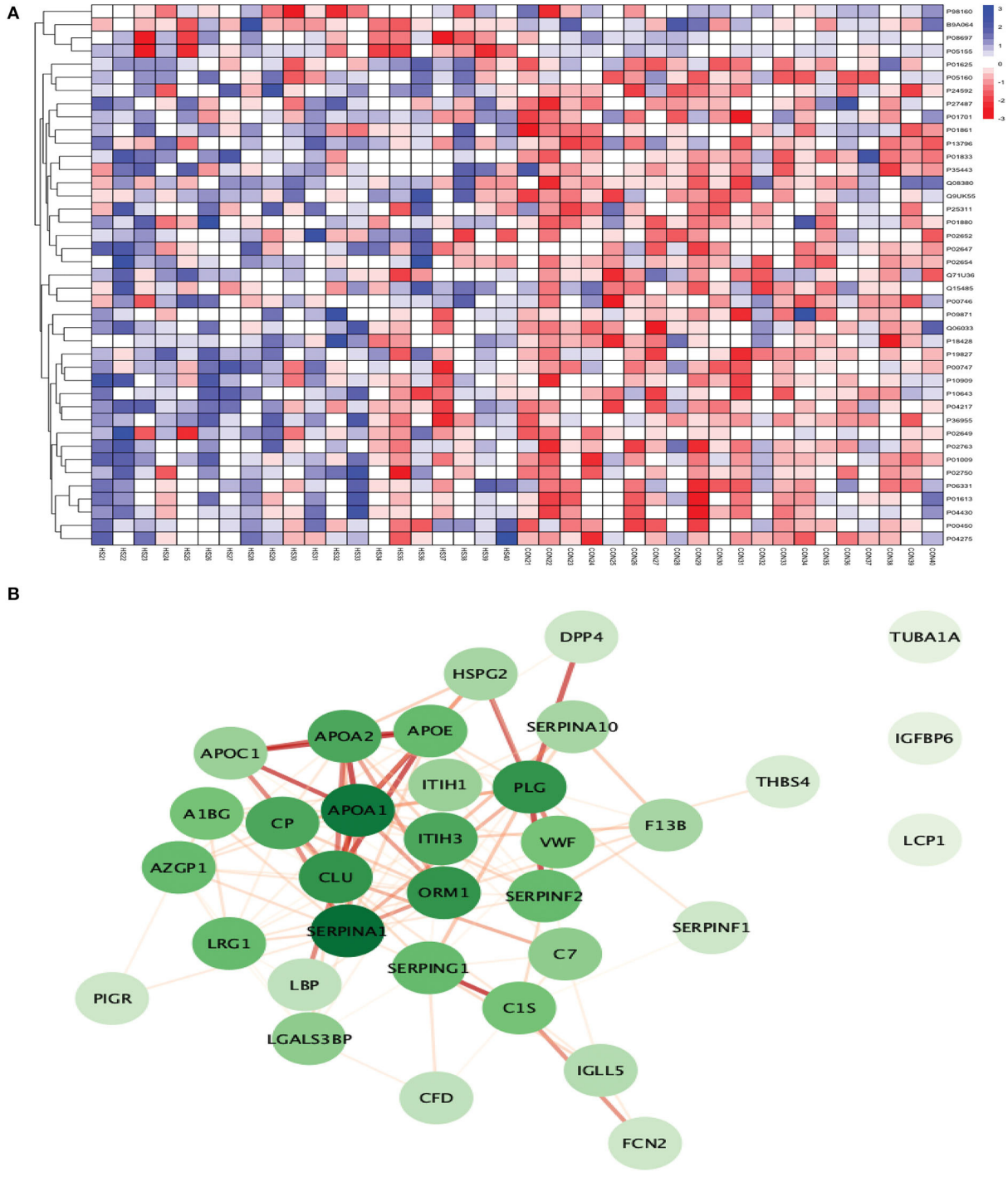

FIGURE 9
(A) Heatmap showing the distribution and expression pattern of 41 significantly differentially expressed proteins between ICH and healthy controls. **(B)** Protein–protein interaction network analysis of differentially expressed proteins between ICH and healthy controls.

were more abundant, while 35 were less abundant in IS cases compared to controls. Using the *p*-value cutoff, they observed 23 significantly differentially expressed proteins. Compared to their study, we obtained three times more (180 proteins) differential proteins in our study after applying the 1.5-fold cutoff criteria. Adiponectin and vWF were two proteins that were significantly differentially expressed in both the studies, and both were upregulated in IS patients compared to controls. However, the

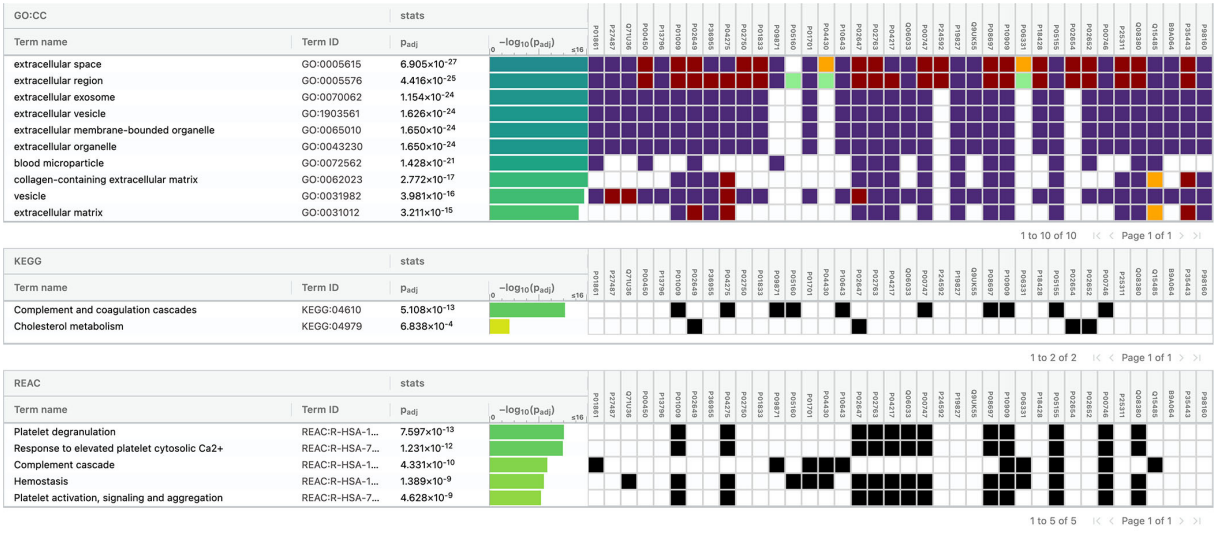

FIGURE 10
Functional enrichment analysis of differentially expressed proteins between intracerebral hemorrhage and healthy controls.

study by Sharma et al. (11) did not mention the time duration for blood sample collection from IS subjects.

Besides blood biomarkers, proteomic studies between IS and healthy controls have also been conducted on other biofluids. The platelet activation response was assessed in a study by Cevik et al. (24) in nine IS cases recruited within 24 h and equal number of control subjects. Using the UPLC-ESI-q-TOF-MS proteomic approach, they identified 83 statistically significant ($p < 0.05$) proteins in the platelets between the two groups. Two proteins (ceruloplasmin and SERPINA1) were commonly differentially expressed between our study and Cevik et al. (24); however, both were not statistically significant in the Cevik et al. study (24). Both proteins were upregulated in our study, while both were downregulated in Cevik et al. (24) in IS cases compared to control subjects. This difference between the expression pattern of the two proteins might be due to the different biofluids used to assess the biomarker levels in both studies. Future comparative studies between serum and platelet proteomic markers are required to validate these findings. Wang et al. (25) recently conducted a urinary proteomic study using the DIA approach between 35 carotid artery stenosis (CAS) patients and 18 healthy controls. They did not mention the timing of sample collection in CAS patients. They identified 194 significantly differentially expressed proteins in urine samples between the two groups (fold change >1.5 and <0.67 with $p < 0.05$), of which only myocilin was commonly expressed in our study. However, myocilin was downregulated in our study in contrast to Wang et al. (25), where it was upregulated. Since, Wang et al. recruited only IS patients with CAS, the difference in the expression pattern might be due to the different subtype of patient populations recruited in both the studies. Another urinary proteomic analysis was conducted by Dawson et al. (26) in a sample of 65 IS/TIA cases and 41 control subjects with urine samples collected within 24 h. Using the capillary electrophoresis-MS approach, they identified 35 statistically significant biomarkers between the two groups. Only ceruloplasmin was the statistically significant protein which was common between Dawson et al. and our study. A study conducted by Brea et al. (27) recruited 11 IS patients and an equal number of control subjects and isolated endothelial progenitor cell colonies within 7 days of symptom onset. They identified four differentially expressed proteins (endoplasmic reticulum protein-29, CdC-42, elongation factor-2, and peroxiredoxin-1) using the 2DE proteomic approach.

Protein biomarkers identified between intracerebral hemorrhage and healthy controls

We identified 41 significantly differentially expressed proteins between ICH and controls within 24 h. Thirty-four proteins formed a highly connected network, and the DoI was strongest for SERPINA1. The most common significant pathways underlying proteins that differentiated ICH from controls included pathways related to the extracellular region, platelet degranulation, complement and coagulation cascade, cholesterol metabolism, and hemostasis. The literature

on proteomic studies for the identification of diagnostic biomarkers for ICH is scarce. In the recent study by Malicek et al. (9) on plasma samples, 14 proteins were significantly differentially expressed between four ICH cases recruited within an average of 7 days (1–15 days) and two control subjects using a label-free proteomic approach. Plasminogen, inter-alpha-trypsin inhibitor heavy chain H3 (ITIH3), and lipopolysaccharide-binding protein (LBP) were three proteins that were commonly expressed in Malicek et al. (9) and our study. Lopez et al. (28) used the multiple reaction monitoring-based targeted proteomic approach on plasma samples and identified that Apo C-I individually and in combination with Apo A-II differentiated 26 ICH from 31 control subjects recruited within 7 days of symptom onset. Both the proteins were also confirmed in our study using the Boruta random forest method as important features for differentiating ICH from controls. Using the targeted metabolomic approach, Zhang et al. (29) used metabolites and recently identified two metabolic markers, i.e., 20-OH-LTB4 and arachidonic acid which differentiated 42 ICH cases from 65 control subjects recruited within 5 days. Our study identified novel protein biomarkers not discovered previously using a proteomic approach (apart from Apo C-I and Apo A-II) and provided crucial insights into the pathophysiology of ICH in acute stages.

Future directions

This discovery phase study provides crucial insights into the pathophysiology of stroke and its subtypes. It provides a potential list of candidate protein markers to explore and new methodological strategies, including the use of label-free high-throughput proteomics for conducting biomarker research in stroke. The label-free SWATH-MS proteomic approach used in this study provides relative protein expression with high sensitivity and selectivity. It also has the capacity to maintain a high throughput, allowing it to evaluate many samples in a short duration of time with minimal operator intervention. However, extensive work still needs to be done before these biomarkers can be implemented in the clinical settings. A point-of-care test needs to be developed for rapid assessment of these biomarkers in hospital settings. Future studies must validate our findings in a large cohort of stroke patients using either standard immunoassays or targeted proteomic approaches. They must identify the sensitivity, specificity, and positive and negative predictive values of these biomarkers for diagnosing stroke. Studies should further aim at collecting blood samples in the hyperacute phase of stroke within 3–4.5 h, which is the clinically acceptable time window for administering thrombolytic therapy. A temporal profile depicting the expression pattern of these biomarkers over the 24-h period is also urgently warranted in stroke patients.

Limitations

We conducted a pilot/discovery phase study; thus, the findings were only exploratory. We obtained relative quantification values for each protein. Therefore, our findings warrant validation in a large cohort using absolute quantification approaches. Since we collected serum samples in our study, the proteins highlighting the significant role of platelet granulation in stroke might account for some false positives due to the activation of platelets in the serum samples.

Conclusion

Our discovery phase exploratory study identified a list of potential protein biomarker candidates for the diagnosis of acute stroke and highlighted significant molecular pathways associated with different stroke subtypes. The results of our study could serve as a platform for conducting future validation studies. These potential biomarker candidates need to be validated in studies using either standard immunoassays or targeted proteomic approach in a large cohort of stroke patients to investigate their diagnostic performance.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ebi.ac.uk/pride/archive/projects/PXD032917.

Ethics statement

The studies involving human participants were reviewed and approved by the Local Institutional Ethics Committee of AIIMS, New Delhi (Ref. No. IECPG-395/28.09.2017). The patients/participants provided their written informed consent to participate in this study.

Author contributions

DV conceptualized the idea of this research topic, helped design the clinical methodology, and supervised each step of execution of this study. SM primarily conducted each step of this study ranging from blood sample collection, processing, proteomic experimentation, statistical and proteomic data analysis, results interpretation, and manuscript writing. SSG

supervised the proteomic experimentation and its data analysis. SM and PS conducted the proteomic experiments and data analysis. DB contributed in conducting the proteomic experimentations in the study. MN contributed in patient sample collection and processing. AK helped in statistical data analysis. DV, PA, AKS, AKP, DM, and KP aided in patient recruitment for this study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.989856/full#supplementary-material

References

1. Caplan LR. *Caplan's Stroke*. Cambridge: Cambridge University Press (2016). 651 p.

2. Michel P, Bogousslavsky J. Shared mechanisms of ischaemic and haemorrhagic stroke: still a lot to learn. *J Neurol Neurosurg Psychiatry*. (2006) 77:1. doi: 10.1136/jnnp.2005.078287

3. Kleinig TJ, Vink R. Suppression of inflammation in ischemic and hemorrhagic stroke: therapeutic options. *Curr Opin Neurol.* (2009) 22:294–301. doi: 10.1097/WCO.0b013e32832b4db3

4. Xi G, Keep RF, Hoff JT. Mechanisms of brain injury after intracerebral haemorrhage. *Lancet Neurol.* (2006) 5:53–63. doi: 10.1016/S1474-44220570283-0

5. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. *J Neuroimmunol.* (2007) 184:53–68. doi: 10.1016/j.jneuroim.2006.11.014

6. Mracsko E, Veltkamp R. Neuroinflammation after intracerebral hemorrhage. *Front Cell Neurosci.* (2014) 8:388. doi: 10.3389/fncel.2014.00388

7. Lee J, Park A, Mun S, Kim H-J, Son H, Choi H, et al. Proteomics-based identification of diagnostic biomarkers related to risk factors and pathogenesis of ischemic stroke. *Diagnostics.* (2020) 10:E340. doi: 10.3390/diagnostics10050340

8. Lee J, Mun S, Park A, Kim D, Lee Y-J, Kim H-J, et al. Proteomics reveals plasma biomarkers for ischemic stroke related to the coagulation cascade. *J Mol Neurosci.* (2020) 70:1321–31. doi: 10.1007/s12031-020-01545-4

9. Malicek D, Wittig I, Luger S, Foerch C. Proteomics-based approach to identify novel blood biomarker candidates for differentiating intracerebral hemorrhage from ischemic stroke-a pilot study. *Front Neurol.* (2021) 12:713124. doi: 10.3389/fneur.2021.713124

10. Qin C, Zhao X-L, Ma X-T, Zhou L-Q, Wu L-J, Shang K, et al. Proteomic profiling of plasma biomarkers in acute ischemic stroke due to large vessel occlusion. *J Transl Med.* (2019) 17:214. doi: 10.1186/s12967-019-1962-8

11. Sharma R, Gowda H, Chavan S, Advani J, Kelkar D, Kumar GSS, et al. Proteomic signature of endothelial dysfunction identified in the serum of acute ischemic stroke patients by the iTRAQ-based LC-MS approach. *J Proteome Res.* (2015) 14:2466–79. doi: 10.1021/pr501324n

12. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2013) 44:2064-89. doi: 10.1161/STR.0b013e318296aeca

13. Meschia JF, Brott TG, Chukwudelunzu FE, Hardy J, Brown RD, Meissner I, et al. Verifying the stroke-free phenotype by structured telephone interview. *Stroke.* (2000) 31:1076–80. doi: 10.1161/01.STR.31.5.1076

14. Wilmoth GH. Handbook in research and evaluation, second edition Stephen Isaac and William B. Michael. San Diego, CA: EDITS Pubs., 1981. *Group Organ Manag.* (1982) 7:124–126. doi: 10.1177/105960118200700111

15. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. *Pharm Stat.* (2005) 4:287–91. doi: 10.1002/pst.185

16. Gillet LC, Navarro P, Tate S, Röst H, Selevsek N, Reiter L, et al. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. *Mol Cell Proteomics.* (2012) 11:O111.016717. doi: 10.1074/mcp.O111.016717

17. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics.* (2012) 28:882–3. doi: 10.1093/bioinformatics/bts034

18. Package 'factoextra. *Extract and Visualize the Results of Multivariate Data Analyses.* Available online at: factoextra.pdf. https://cloud.r-project.org/web/packages/factoextra/factoextra.pdf (accessed November 18, 2021)

19. Kursa MB, Rudnicki WR. Feature Selection with the Boruta Package. *J Stat Softw.* (2010) 36:1–13. doi: 10.18637/jss.v036.i11

20. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* (2021) 49:D605–12. doi: 10.1093/nar/gkab835

21. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* (2003) 13:2498–504. doi: 10.1101/gr.1239303

22. Saver JL. Time is brain–quantified. *Stroke.* (2006) 37:263–6. doi: 10.1161/01.STR.0000196957.55928.ab

23. Allard L, Lescuyer P, Burgess J, Leung K-Y, Ward M, Walter N, et al. ApoC-I and ApoC-III as potential plasmatic markers to distinguish between ischemic and hemorrhagic stroke. *Proteomics.* (2004) 4:2242–51. doi: 10.1002/pmic.200300809

24. Cevik O, Baykal AT, Sener A. Platelets proteomic profiles of acute ischemic stroke patients. *PLoS ONE.* (2016) 11:e0158287. doi: 10.1371/journal.pone.0158287

25. Wang W, Wu J, Liu P, Tang X, Pang H, Xie T, et al. Urinary proteomics identifying novel biomarkers for the diagnosis and phenotyping of carotid

artery stenosis. *Front Mol Biosci.* (2021) 8:714706. doi: 10.3389/fmolb.2021.714706

26. Dawson J, Walters M, Delles C, Mischak H, Mullen W. Urinary proteomics to support diagnosis of stroke. *PLoS ONE.* (2012) 7:e35879. doi: 10.1371/journal.pone.0035879

27. Brea D, Rodríguez-González R, Sobrino T, Rodríguez-Yañez M, Blanco M, Castillo J. Proteomic analysis shows differential protein expression in endothelial progenitor cells between healthy subjects and ischemic stroke patients. *Neurol Res.* (2011) 33:1057–63. doi: 10.1179/1743132811Y.0000000038

28. Lopez MF, Sarracino DA, Prakash A, Athanas M, Krastins B, Rezai T, et al. Discrimination of ischemic and hemorrhagic strokes using a multiplexed, mass spectrometry-based assay for serum apolipoproteins coupled to multi-marker ROC algorithm. *Proteomics Clin Appl.* (2012) 6:190–200. doi: 10.1002/prca.201100041

29. Zhang J, Su X, Qi A, Liu L, Zhang L, Zhong Y, et al. Metabolomic profiling of fatty acid biomarkers for intracerebral hemorrhage stroke. *Talanta.* (2021) 222:121679. doi: 10.1016/j.talanta.2020.121679

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Effect of mean heart rate on 30-day mortality in ischemic stroke with atrial fibrillation: Data from the MIMIC-IV database

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Background: The relationship of mean heart rate (MHR) with 30-day mortality in ischemic stroke patients with atrial fibrillation in the intensive care unit (ICU) remains unknown. This study aimed to investigate the association between MHR within 24 h of admission to the ICU and 30-day mortality among patients with atrial fibrillation and ischemic stroke.

Methods: This retrospective cohort study used data on US adults from the Medical Information Mart for Intensive Care-IV (MIMIC-IV, version 1.0) database. Patients with ischemic stroke who had atrial fibrillation for and first time in ICU admission were identified from the MIMIC-IV database. We used multivariable Cox regression models, a restricted cubic spline model, and a two-piecewise Cox regression model to show the effect of the MHR within 24 h of ICU admission on 30-day mortality.

Results: A total of 1403 patients with ischemic stroke and atrial fibrillation (mean [SD] age, 75.9 [11.4] years; mean [SD] heart rate, 83.8[16.1] bpm; 743 [53.0%] females) were included. A total of 212 (15.1%) patients died within 30 days after ICU admission. When MHR was assessed in tertials according to the 25th and 50th percentiles, the risk of 30-day mortality was higher in participants in group 1 (<72 bpm; adjusted hazard ratio, 1.23; 95% CI, 0.79–1.91) and group 3 (≥82 bpm; adjusted hazard ratio, 1.77; 95% CI, 1.23–2.57) compared with those in group 2 (72–82 bpm). Consistently in the threshold analysis, for every 1-bpm increase in MHR, there was a 2.4% increase in 30-day mortality (adjusted HR, 1.024; 95% CI, 1.01–1.039) in those with MHR above 80 bpm. Based on these results, there was a J-shaped association between MHR and 30-day mortality in ischemic stroke patients with atrial fibrillation admitted to the ICU, with an inflection point at 80 bpm of MHR.

Conclusion: In this retrospective cohort study, MHR within 24 h of admission was associated with 30-day mortality (nonlinear, J-shaped association) in patients with ischemic stroke and atrial fibrillation in the ICU, with an inflection point at about 80 bpm and a minimal risk observed at 72 to 81 bpm of MHR.

This association was worthy of further investigation. If further confirmed, this association may provide a theoretical basis for formulating the target strategy of heart rate therapy for these patients.

KEYWORDS

heart rate, mortality, ischemic stroke, atrial fibrillation, intensive care unit

Introduction

Stroke is one of the major causes of death and disability in the world, which is characterized by a high incidence of morbidity, higher incidence of disability, high rate of mortality, high risk of recurrence, and high cost (1). Although new diagnostic and therapeutic techniques have emerged in the twenty-first century, such as functional brain imaging, cerebral perfusion imaging, intravenous thrombolysis, and mechanical thrombectomy, stroke is still a public health problem. Cardiogenic strokes, which make up 14% of all ischemic strokes (2), have quadrupled in the past few decades and, according to estimates from the United Kingdom, may triple once more by 2050 (3).

Previous studies have explored the relationship between heart rate and stroke outcomes, but each study used different heart rate parameters, such as baseline heart rate, mean heart rate, and heart rate variability (4–13). There is no consensus on which heart rate parameters or periods are best for the autonomic nervous system. Some studies employed heart rate parameters within a week after onset (14) and others within 24 h of admission (4, 8, 11, 15), while yet other studies used heart rate parameters at the time of the patient's first admission (6, 7, 16). Previous studies have reported controversial associations between heart rate and stroke outcomes. Studies have shown that high resting heart rates or low resting heart rates are associated with high mortality or future cardiovascular and cerebrovascular events (6, 7, 17). However, other studies have found that tachycardia and bradycardia do not independently predict the clinical course or outcome of stroke patients (18). The effect of heart rate variability on disease outcomes is also controversial (8–11, 13, 19).

However, most of the previous studies on the relationship between heart rate and prognosis of ischemic stroke have been conducted in patients with mild to moderate stroke, and the definition of heart rate parameters varies from study to study, and most of them focus on the relationship between heart rate parameters and medium- and long-term prognosis. As it has an erratic rhythm, atrial fibrillation (AF), which is the most frequent reason for heart thrombus development and is to blame for 45% of cardiogenic strokes (20), has often been excluded. The association between mean heart rate (MHR) and stroke prognosis in patients with atrial fibrillation is uncertain because most studies did not include patients with AF. Therefore, this study aimed to investigate the association between MHR and 30-day mortality in patients with ischemic stroke and AF admitted to the intensive care unit (ICU).

Abbreviations: MMIV-IV, Medical Information Mart for Intensive Care-IV; MHR, Mean Heart Rate; ICU, Intensive care unit; AF, Atrial Fibrillation; SOFA, Sequential Organ Failure Assessment score; SAPS II, Simplified Acute Physiology Score II; GCS, Glasgow Coma Scale; CVD, Cardiovascular disease; SD, Standard deviation; IQR, Interquartile range; HR, Hazard Ratio; NIHSS, National Institute of Health Stroke Scale; HRV, Heart rate variability.

Materials and methods

Study population

This retrospective cohort study used the Medical Information Mart for Intensive Care-IV (MIMIC-IV version 1. 0) database (21, 22). This is a longitudinal, single-center database that contains data from 2008 to 2019. The overall information was saved as a relational database, consisting of patient demographics, vital signs, laboratory tests, diagnostic information, treatment information, and in-hospital mortality. One author (Shaoli Yao, ID: 10808597) who has finished the Collaborative Institutional Training Initiative examination can access the database and was responsible for data extraction and analysis. The use of the MIMIC-IV database was approved by the review boards of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. The data are anonymous, and the requirement for informed consent was therefore waived. The code of data extraction is available on GitHub (23) (http://github.com/MIT-LCP/mimic-iv). All reporting followed the Strengthening the Reporting of Observational Studies in Epidemiology guidelines (24). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Patients with ischemic stroke who had AF for and first time in ICU admission were considered eligible for our study. The diagnosis of ischemic stroke and AF were based on the International Classification of Disease, the Ninth Version, and the Tenth Version (Supplementary Table 1). As ischemic stroke may not always be listed as the principal diagnosis, we also included records with ischemic stroke in any of the first five diagnostic positions according to the diagnostic sequence. The

inclusion criteria were as follows: (1) patients were aged ≥18 years; (2) patients were in ICU for more than 24 h; and (3) only the first ICU admission was considered. The exclusion criteria were as follows: (1) patients were aged <18 years; (2) patients had a minimum heart rate < 35 beats per minute; (3) heart rate data were not available; and (4) patients were in ICU for <24 h.

General data collection

The Structured Query Language was used for data extraction (23). All vital signs, Sequential Organ Failure Assessment (SOFA) score, and Simplified Acute Physiology Score II (SAPS II) were collected within 24 h of admission to ICU. We extracted the following variables: (1) basic demographics, including age, sex, weight, insurance, marital status, and ethnicity; (2) vital signs and the severity of illness, which was defined at ICU admission using the SOFA score, SAPS II, Glasgow Coma Scale (GCS), and Charlson comorbidity index; (3) treatment, including ventilation use, vasoactive drug use, and dialysis use; and (4) comorbidities, myocardial infarct, congestive heart failure, cerebrovascular disease, dementia, chronic pulmonary disease, renal disease, cancer, severe liver disease, and metastatic solid tumor. Height and body mass index were not included because more than 50% of the data were missing in this study.

Variable definition and outcomes

Tracheotomy, invasive ventilation, and noninvasive ventilation were all considered to be indications of ventilation use in patients. Vasoactive drugs included norepinephrine, epinephrine, phenylephrine, vasopressin, dopamine, dobutamine, isoprenaline, sodium nitroprusside, nicardipine, labetalol, esmolol, and diltiazem. The MHR was defined as the calculated average (by adding together all of the heart rate readings recorded and dividing by the total number of readings) of the heart rate measured within 24 h of admission to the ICU. The mean (SD) of the heart rate recordings was 28.9 (8.4). According to the 25th and 50th percentiles of MHR, participants were divided into three groups. Cardiovascular disease (CVD) is defined as a history of myocardial infarction or congestive heart failure. In this study, we regarded 30-day mortality as the outcome event, which was also extracted from the MIMIC-IV database. The outcome events were monitored up to 30 days after admission to ICU.

Statistical analysis

The distribution of the baseline data of the patients included in this study was presented for the different outcome groups. Categorical data were presented as a number (percentages), while continuous data were presented as the mean ± standard deviation or median (interquartile range), as appropriate. Differences in continuous variables were tested using the analysis of variance test or rank-sum test as appropriate. The chi-square test or Fisher's exact test for categorical variables was applied to compare the characteristics of the study subjects among the outcome groups.

We simply replaced the missing data with a median because 5% of the GCS score was missing. Because the percentage of missing data was small (missing rate varied from 0.5 to 0.7%) for mean glucose and weight, no imputation method was used. Multivariable Cox regression analyses were performed to assess the independent association between MHR and 30-day mortality. Mean heart rate was entered as a categorical variable (tertials) and as a continuous variable (with a hazard ratio (HR) calculated per 10 bpm MHR increase). We applied four models in the regression analysis. Multivariable models were adjusted as follows: model 1 was not adjusted; model 2 was adjusted for age, gender, mean blood oxygen saturation (spo2), mean glucose, weight, and Charlson's comorbidity index; model 3 was adjusted for model 2 plus SOFA score, SAPS II, and GCS; and model 4 was adjusted for model 3 plus ventilation use and vasoactive drug use. Survival curves were plotted by Kaplan–Meier and log-rank analyses.

We used restricted cubic spline models to examine the possible nonlinear association between the levels of MHR and the incidence of 30-day mortality (25). Analyses treating MHR levels of 80 bpm as the reference with adjustment of the aforementioned (model 4) covariates, and a knot was located at the 5th, 35th, 65th, and 95th percentiles of the MHR. Threshold analysis in the association of MHR with the study outcome was conducted with a likelihood ratio test comparing the model with only a linear term against the model with linear and cubic spline terms. We considered that the association between MHR level and 30-day mortality may be influenced by ventilation use, vasoactive drug use, history of cardiovascular diseases, etc. Therefore, heterogeneity across subgroups was assessed by Cox proportional hazards models, and interactions between subgroups were examined by likelihood ratio testing.

As we included patients with ischemic stroke in any of the first five diagnostic positions by the diagnostic sequence and could not rule out the possibility that some patients were admitted to the ICU for other illnesses, we included patients with ischemic stroke as the first diagnosis in the second dataset. The analysis described above was then carried out in the second dataset to ensure the reliability and validity of our findings.

A two-tailed test was performed, and a $P < 0.05$ was considered statistically significant in our study. All the analyses were performed with the statistical software packages R (http://www.R-project.org, The R Foundation) and Free Statistics software version 1.6 (26).

TABLE 1 Baseline characteristics of patients stratified by 30-day mortality.

Variables	Total ($n = 1403$)	Survivors ($n = 1191$)	Non-survivors ($n = 212$)	p value
ICU stay, day	8.7 (5.0, 15.1)	9.1 (5.3, 15.7)	6.2 (3.1, 11.7)	<0.001
Minimum HR, bpm	67.8 ± 14.5	67.3 ± 14.2	70.8 ± 15.8	0.001
Maximum HR, bpm	105.2 ± 23.4	103.8 ± 22.9	112.6 ± 24.9	<0.001
MHR, bpm	83.8 ± 16.1	83.0 ± 15.7	88.4 ± 17.9	<0.001
HRF, bpm	33.0 (24.0, 46.0)	31.0 (23.0, 44.0)	39.0 (27.0, 53.2)	<0.001
MBP, bpm	82.9 ± 12.0	82.9 ± 11.9	82.9 ± 12.5	0.950
Mean respiratory rate, times/min	19.5 ± 3.4	19.3 ± 3.2	20.8 ± 4.1	<0.001
Mean body temperature, °C	36.8 ± 0.5	36.8 ± 0.4	36.9 ± 0.7	0.194
Mean spo2, %	97.2 (95.8, 98.4)	97.1 (95.7, 98.3)	97.9 (96.1, 99.1)	<0.001
Mean blood glucose, mg/dl	140.8 ± 42.9	138.9 ± 41.4	152.0 ± 49.3	<0.001
Sofa score	4.0 (3.0, 7.0)	4.0 (2.0, 6.0)	6.0 (4.0, 8.0)	<0.001
Weight, kg	79.3 ± 21.3	80.2 ± 21.3	74.5 ± 20.7	<0.001
SAPSII	38.3 ± 11.6	37.0 ± 10.7	45.6 ± 13.6	<0.001
Age, years	75.9 ± 11.4	75.3 ± 11.6	79.3 ± 10.0	<0.001
Charlson's comorbidity index	7.5 ± 2.4	7.3 ± 2.3	8.3 ± 2.3	<0.001
GCS	13.0 (8.0, 14.0)	13.0 (9.0, 14.0)	7.0 (4.0, 10.0)	<0.001
Gender, *n* (%)				0.146
Female	743 (53.0)	621 (52.1)	122 (57.5)	
Male	660 (47.0)	570 (47.9)	90 (42.5)	
Myocardial infarct, *n* (%)				0.107
NO	1135 (80.9)	972 (81.6)	163 (76.9)	
Yes	268 (19.1)	219 (18.4)	49 (23.1)	
Congestive heart failure, *n* (%)				0.059
NO	856 (61.0)	739 (62)	117 (55.2)	
Yes	547 (39.0)	452 (38)	95 (44.8)	
Cerebrovascular disease, *n* (%)				<0.001
NO	356 (25.4)	340 (28.5)	16 (7.5)	
Yes	1047 (74.6)	851 (71.5)	196 (92.5)	
Dementia, *n* (%)				0.134
NO	1316 (93.8)	1122 (94.2)	194 (91.5)	
Yes	87 (6.2)	69 (5.8)	18 (8.5)	
Chronic pulmonary disease, *n* (%)				0.835
NO	1093 (77.9)	929 (78)	164 (77.4)	
Yes	310 (22.1)	262 (22)	48 (22.6)	
Renal disease, *n* (%)				0.323
NO	1057 (75.3)	903 (75.8)	154 (72.6)	
Yes	346 (24.7)	288 (24.2)	58 (27.4)	
Malignant cancer, *n* (%)				0.207
NO	1294 (92.2)	1103 (92.6)	191 (90.1)	
Yes	109 (7.8)	88 (7.4)	21 (9.9)	
Severe liver disease, *n* (%)				0.309
NO	1386 (98.8)	1178 (98.9)	208 (98.1)	
Yes	17 (1.2)	13 (1.1)	4 (1.9)	
Metastatic solid tumor, *n* (%)				0.063
NO	1359 (96.9)	1158 (97.2)	201 (94.8)	
Yes	44 (3.1)	33 (2.8)	11 (5.2)	

(Continued)

TABLE 1 Continued

Variables	Total ($n = 1403$)	Survivors ($n = 1191$)	Non-survivors ($n = 212$)	p value
Dialysis				0.101
No	1335 (95.2)	1138 (95.5)	197 (92.9)	
Yes	68 (4.8)	53 (4.5)	15 (7.1)	
Ventilation, *n* (%)				<0.001
No	907 (64.6)	813 (68.3)	94 (44.3)	
Yes	496 (35.4)	378 (31.7)	118 (55.7)	
Vasoactive drugs, *n* (%)				<0.001
No	646 (46.0)	572 (48)	74 (34.9)	
Yes	757 (54.0)	619 (52)	138 (65.1)	
Insurance				0.445
Medicaid	59 (4.2)	51 (4.3)	8 (3.8)	
Medicare	838 (59.7)	703 (59)	135 (63.7)	
Other	506 (36.1)	437 (36.7)	69 (32.5)	
Marital status, *n* (%)				0.148
Divorced	88 (7.1)	79 (7.3)	9 (5.3)	
Married	605 (48.6)	530 (49.2)	75 (44.4)	
Single	254 (20.4)	221 (20.5)	33 (19.5)	
Widowed	299 (24.0)	247 (22.9)	52 (30.8)	
Ethnicity, *n* (%)				<0.001
American Indian/Alaska Native	3 (0.2)	3 (0.3)	0 (0)	
Asian	54 (3.8)	47 (3.9)	7 (3.3)	
Black/African-American	137 (9.8)	116 (9.7)	21 (9.9)	
Hispanic/Latino	34 (2.4)	29 (2.4)	5 (2.4)	
Other	50 (3.6)	43 (3.6)	7 (3.3)	
Unable to obtain	12 (0.9)	8 (0.7)	4 (1.9)	
Unknown	180 (12.8)	126 (10.6)	54 (25.5)	
White	933 (66.5)	819 (68.8)	114 (53.8)	

For each variable, mean ± standard deviation, median (interquartile range), or number (percent) was reported (as appropriate).
ICU, Intensive care unit; MHR, mean heart rate; bpm, beats per minute; HRF, Heart rate fluctuation; MBP, mean blood pressure; SpO2, blood oxygen saturation; SOFA, sequential organ failure assessment; SPASII, simplified acute physiology score II; GCS, glasgow coma scale.

Results

Baseline characteristics of the study patients

Three thousand and nine hundred and eight individuals with ischemic stroke who were admitted to the ICU for the first time were identified according to the International Classification of Disease, the Ninth Version, and the Tenth Version. Among them, 3,715 individuals with ischemic stroke were listed as the first five diagnostic positions according to the diagnostic sequence, and 1,635 individuals had AF. After screening according to the exclusion criteria, the final cohort included 1,403 patients with ischemic stroke and AF. Of these patients, 212 (15.1%) died within 30 days after ICU admission. The detailed flowchart of participant recruitment is shown in Supplementary Figure 1. The interested reader can find them in Supplementary material online.

The mean age of the 1403 patients was 75.9 ± 11.4 years, and about half of them were female (53.0%). The baseline characteristics of the population included in the study are listed in Table 1. When compared with the survivors, the heart rate parameters, SOFA score, and SAPS II were higher in deceased patients. The deceased patients were more likely to be older and combined with many other diseases compared with the survivors.

Effects of MHR on 30-day mortality

Kaplan–Meier curve showed there was lower mortality by day 30 in patients with MHR<80 bpm (log-rank test: $p <$

0.0001, Figure 1). In the multivariable Cox models (Table 2), we observed that the risk of 30-day mortality was higher in participants in group 1 (<72 bpm; adjusted HR, 1.23; 95% CI, 0.79–1.91) and group 3 (≥82 bpm; adjusted HR, 1.77; 95% CI, 1.23–2.57) compared with those in group 2 (72–81 bpm). After adjustment for confounding factors, a 19% higher 30-day mortality could be shown in patients with MHR increased per 10 bpm. Multivariable-adjusted restricted cubic spline analyses suggested J-shaped associations of MHR with 30-day mortality (Figure 2, $p = 0.021$).

Using a two-piecewise Cox regression model, we found that the threshold of MHR was 80 bpm (Table 3). Above the threshold, for every 1-bpm increase in MHR, there was a 2.4% increase in 30-day mortality (adjusted HR, 1.024; 95% CI, 1.01–1.039) (Table 3).

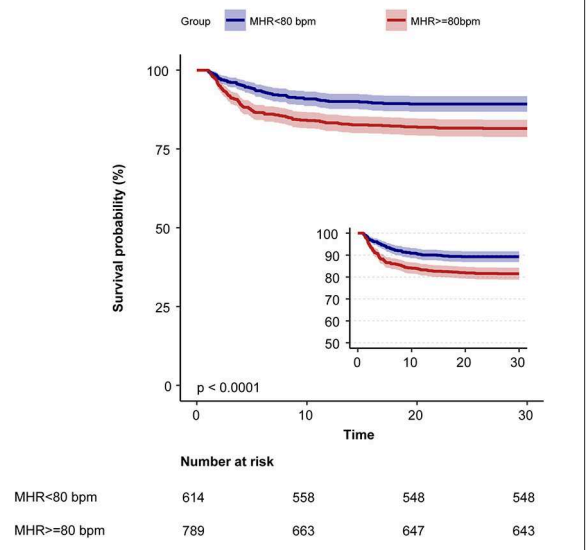

FIGURE 1
Kaplan–Meier survival curves for day 30 of ischemic stroke patients with atrial fibrillation. MHR, mean heart rate; bpm, beats per minute.

Sensitivity analysis

Much more sensitivity analyses were run than can be included in the article. The interested reader can find them in Supplementary material online. After subgroup analysis according to the confounders including age, gender, SOFA score, CVD disease, ventilation use, and vasoactive drug use (Supplementary Figure 2), the result remains robust, and we did not observe any significant interaction in the subgroups (all p-values for interaction > 0.05).

Because GCS data for 74 individuals were not available, they were excluded from the sensitivity analysis. The association between MHR and 30-day mortality has remained steady (Supplementary Table 2).

In patients with ischemic stroke as the first diagnosis, we also observed that the association between MHR and 30-day mortality was consistently significant in all models in the multivariable Cox models (Supplementary Table 3). Kaplan–Meier curve also showed there was lower mortality by day 30 in patients with MHR<80 bpm (log-rank test: $p < 0.001$, Supplementary Figure 3).

Discussion

The main result

This study aimed to analyze the relationship between MHR and short-term outcomes in patients with ischemic stroke and AF admitted to the ICU. MHR within 24 h after admission to ICU was found to be independently associated with 30-day

TABLE 2 Hazard ratio and 95% CI of mean heart rate for 30-day mortality.

Variable	Model 1		Model 2†		Model 3†		Model 4†	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*p* value
MHR per10, bpm	1.2 (1.11–1.29)	<0.001	1.21 (1.11–1.32)	<0.001	1.16 (1.07–1.27)	<0.001	1.19 (1.09–1.3)	<0.001
MHR tertials, bpm								
<72	1.21 (0.78–1.87)	0.403	1.22 (0.79–1.89)	0.378	1.2 (0.77–1.87)	0.416	1.23 (0.79–1.91)	0.363
72–81	1(Reference)		1(Reference)		1(Reference)		1(Reference)	
≥82	1.92 (1.34–2.76)	<0.001	1.91 (1.32–2.75)	0.001	1.71 (1.18–2.46)	0.004	1.77 (1.23–2.57)	0.002
P for trend		0.001		0.002		0.015		0.012

MHR, mean heart rate; bpm, beats per minute, HR, hazard ratio; CI, Confidence interval.
† There were 9 patients missing blood glucose data and 10 patients missing weight data, using sample size = 1384.
Model 1: no adjusted.
Model 2: adjusted for gender, mean SpO2, mean glucose, weight, Charlson's comorbidity index, and age.
Model 3: adjusted for model 2 plus SOFA score, SAPS II, and GCS.
Model 4: adjusted for model 3 plus ventilation use, and vasoactive drugs use.

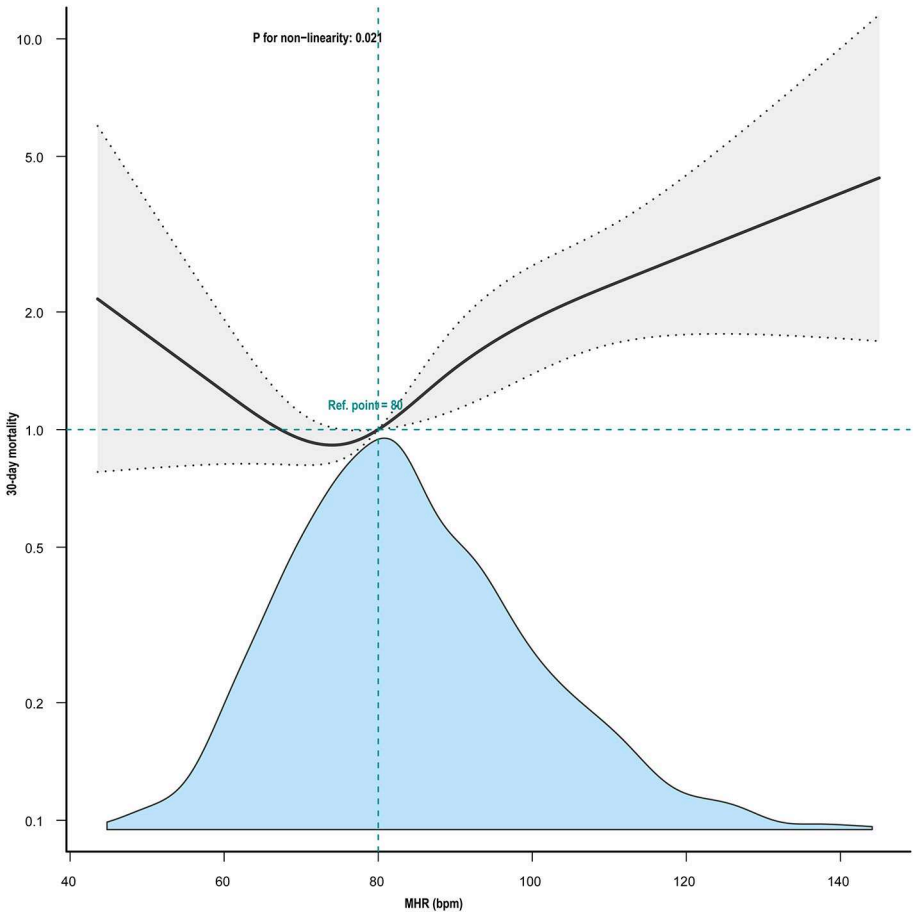

FIGURE 2
Nonlinear association between mean heart rate and 30-day mortality. Adjustment factors included gender, mean SpO2, mean glucose, weight, SOFA score, Charlson's comorbidity index, SAPS II, age, GCS, Ventilation use, and vasoactive drug use. The black line and gray area represent the estimated values and their corresponding 95% confidence intervals, respectively. MHR, mean heart rate; bpm, beats per minute.

TABLE 3 Threshold analyses of MHR on 30-day mortality using two-piecewise regression models.

Threshold of MHR, bpm	HR	95% CI	*P* value
<80	0.974	0.943–1.006	0.1117
≥80	1.024	1.01–1.039	<0.001
Likelihood ratio test	–	–	0.0070

MHR, mean heart rate; bpm, beats per minute; HR, hazard ratio; CI, confidence interval. Adjustment factors included gender, mean SpO2, mean glucose, weight, SOFA score, Charlson's comorbidity index, SAPS II, age, GCS, ventilation use, vasoactive drugs use.

mortality. Furthermore, a typical J-shaped curve was observed in restricted cubic splines for the association between MHR and 30-day mortality in our study population, indicating an inflection point at about 80 bpm and minimal risk observed at 72 to 81 bpm of MHR.

Effects of heart rate parameters on 30-day mortality

Numerous studies have found that heart rate was associated with the prognosis of many diseases, including coronary heart disease, myocardial infarction, heart failure, acute ischemic stroke, acute hemorrhagic stroke, and so on (7, 9, 11, 13, 27–30). In addition, heart rate variability is associated with the incidence and duration of poststroke depression (27). In patients with AF, the association between MHR and short-term prognosis in stroke patients has been limited. Lee et al. found a J-shaped association between MHR and 1-year mortality after stroke, with an optimal mean HR of about 80 bpm (13). This was in line with our findings. Our restricted cubic splines clearly showed a J-shaped curve for the association between MHR and the 30-day mortality in our study population. Kaplan–Meier curve showed there was lower mortality by day 30 in patients with mean HR<80 bpm. Interestingly, for the 30-day mortality of patients

with ischemic stroke and AF admitted to the ICU, the lowest risk of MHR was ∼80 bpm, which might be a candidate marker for decision making in HR control strategies. However, an observational study carried out by Steinberg Ba et al. showed a J-shaped relationship between heart rate and mortality in patients with permanent AF, and a heart rate around 65 bpm seems to be the optimal heart rate (5). Meanwhile, Böhm et al. found a nonlinear relationship between MHR and stroke incidence in patients with diabetes, with the lowest risk of stroke at an MHR of 65 bpm (31). This difference indicates that the optimum heart rate may differ among populations.

In patients with AF, the small number of studies on the relationship between heart rate and prognosis in stroke patients is controversial. However, Han et al. found a different result. In their study, no independent association between heart rate and in-hospital mortality was observed in patients with acute ischemic stroke who had AF (16). We speculate that the reasons for our inconsistent findings may be as follows: The study conducted by Han et al. used heart rate at admission; as a result of arrhythmia in patients with AF, only one heart rate measurement may not be representative; on the contrary, their study population was mainly concentrated in patients with minor stroke (median National Institutes of Health Stroke Scale (NIHSS) score was 4.0).

The pathophysiological mechanism of cardiovascular autonomic dysfunction in patients with ischemic stroke remains unclear, and we speculate that the following mechanisms may be involved. There was increasing evidence suggesting that the pathophysiological process of acute stroke is not an isolated brain process. Inflammatory, endocrine, and autonomic pathways are activated simultaneously with the ischemic cascade of systemic responses (32, 33). After a stroke, activation of the sympathetic nervous system is thought to be a trigger for systemic immunodepression and an increased risk of infection, which is also one of the major risk factors for mortality and disability (34). A fast heart rate may also indicate sympathetic nerve overactivity, which has been related to inflammatory processes and higher blood pressure at night, both of which are well-known indicators of stroke mortality (35–37). Previous studies on the relationship between heart rate variability and stroke outcomes have also found that patients with low heart rate variability have a worse prognosis, while low heart rate variability indicates high sympathetic nervous system activity (8, 10, 11). In addition to the increased sympathetic nervous system tone in the acute phase of stroke, Lee et al. found a stronger association between mean heart rate and mortality in the late acute stage (13). Autonomic nervous system dysfunction after stroke exacerbated subsequent brain damage *via* changes in hemodynamics and non-hemodynamic variables (38). Higher or lower heart rate in the acute phase of a stroke may lead to a reduction in cardiac output resulting in insufficient perfusion of the ischemic area and ultimately adverse outcomes.

Strengths and limitations

Our study has some strengths. First, to the best of our knowledge, the association of heart rate with short-term prognosis has not been developed in patients with ischemic stroke and AF admitted to the ICU, and our study found a J-shaped association between MHR and 30-day mortality. The lowest risk of death was found when the MHR was about 80 bpm, which may provide a theoretical basis for formulating the target strategy of heart rate therapy for these patients. This finding extends conclusion to a wider range of clinical entities. Second, we adopted the MHR, which is easy to get and easy for clinicians to use. Third, we performed multiple sensitivity analyses: (1) the use of vasoactive drugs may have an effect on heart rate in ICU, acute stroke patients were exposed to varying degrees of artificial light, noise, and various organ support, which usually leads to dysrhythmias in sleep architecture, blood pressure, and HR (39), so we performed subgroup analyses by age, sex, SOFA score, vasoactive drugs use, ventilation use, and the results remained stable; (2) we used multi-model adjustment in Cox regression analyses to correct for the effect of confounders, which remained stable after full model adjustment (model 4); (3) the MHR was analyzed with continuous and categorical variables in the regression model and this method can reduce the chance of data analysis and improve the stability of the results; and (4) according to the diagnostic sequence, the patients with ischemic stroke ranked in the first five or the first were analyzed, and the results were still stable.

This study has some limitations. First, previous studies have found that heart rate variability (HRV) as an autonomous cardiac biomarker is associated with prognosis in a variety of diseases, and its calculation methods can be divided into linear and nonlinear methods with the high cost and low clinical availability, and we did not use this parameter because we could not get the corresponding data in MIMIC-IV database to calculate HRV in the time domain and frequency domain. However, our study is based on real-world clinical data, and heart rate parameters are measured at the bedside, which are simple and easy to obtain and use by a clinician at the bedside. Second, it is well known that the NIHSS score is widely used to assess the severity of the ischemic stroke, and previous studies have found that the NIHSS score is an independent predictor of stroke outcome (9), but because these data were unavailable in the MIMIC-IV database, we could not include this variable for analysis. However, we included the GCS score, another scoring system for assessing neurological function, and in the regression model, MHR remained positively associated with 30-day mortality in our study population after adjustment for the GCS score. Third, while caution should be used when extending the results because the study population was restricted to a single nation (the USA) and a single ICU institution, our sample size was sizable and relatively representative. Future multicenter prospective studies may be done to confirm our

findings. Fourth, many factors can affect the prognosis of stroke patients, such as the strategy of reperfusion therapy in the acute phase. We could not exclude the effects of this factor on our result as the data were not accessible in the MIMIC-IV database, but we attempted to adjust for the effect of available confounders. Our results are consistent with the conclusion of a multicenter prospective cohort study conducted by Lee et al., which included a reperfusion therapy strategy for adjustment (13). Fifth, selection bias is inevitable due to the design of retrospective cohort studies, and future randomized controlled trials would help confirm our findings. Sixth, the patient's condition at the moment of the heart rate measurement was not recorded in the MIMIC-IV1.0 database. And this may affect the real relationship between mean heart rate and 30-day mortality in ischemic stroke with atrial fibrillation. These associations were worthy of further investigation. Seventh, studies have indicated a connection between dysautonomia and certain arrhythmia patterns and different parts of the central nervous system (40–42). Unfortunately, the MIMIC-IV database does not have information on the location and sizes of the strokes. Future prospective studies may further investigate the impact of various lesion sites and sizes on heart rate parameters and in-hospital all-cause mortality.

Conclusion

In conclusion, this retrospective cohort study revealed a J-shaped association between MHR within 24 h of admission and 30-day mortality in patients with ischemic stroke and atrial fibrillation in the ICU, with increased 30-day mortality when MHR > 80 bpm. This association was worthy of further investigation. If further confirmed, this association may provide a theoretical basis for formulating the target strategy of heart rate therapy for these patients.

Data availability statement

The data analyzed in this study was obtained from the Medical Information Mart for Intensive Care IV (MIMIC-IV) Clinical Database, the following licenses/restrictions apply: To access the data you must be a credentialed user, complete the required training (CITI Data or Specimens Only Research) and sign the data use agreement for the project. Requests to access these datasets should be directed to PhysioNet, https://physionet.org/; https://doi.org/10.13026/s6n6-xd98.

Ethics statement

The studies involving human participants were reviewed and approved by the review boards of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

S-lY participated in the design of research schemes, extracted and analyzed the data, and wrote the main manuscript text. X-wC collated the data. YZ and X-rC participated in the design of research schemes. JL reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1017849/full#supplementary-material

References

1. Global R. National burden o f stroke and its risk factors, 1990–2019: a systematic analysis for the global burden of disease study 2019. *Lancet Neurol.* (2021) 20:795–820. doi: 10.1016/S1474-4422(21)00252-0

2. Murtagh B, Smalling RW. Cardioembolic stroke. *Curr Atheroscler Rep*. (2006) 8:310–6. doi: 10.1007/s11883-006-0009-9

3. Yiin GS, Howard DP, Paul NL Li L, Luengo-Fernandez R, Bull LM, et al. Age-specific incidence, outcome, cost, and projected future burden of atrial fibrillation-related embolic vascular events: a population-based study. *Circulation.* (2014) 130:1236–44. doi: 10.1161/CIRCULATIONAHA.114.010942

4. Nolte CH, Erdur H, Grittner U, Schneider A, Piper SK, Scheitz JF, et al. Impact of heart rate on admission on mortality and morbidity in acute ischaemic stroke patients - results from vista. *European journal of neurology.* (2016) 23:1750–6. doi: 10.1111/ene.13115

5. Steinberg BA, Kim S, Thomas L, Fonarow GC, Gersh BJ, Holmqvist F, et al. Increased heart rate is associated with higher mortality in patients with atrial fibrillation (af): results from the outcomes registry for better informed treatment of Af (Orbit-Af). *J Am Heart Assoc.* (2015) 4:e002031. doi: 10.1161/JAHA.115.002031

6. Erdur H, Scheitz JF, Grittner U, Laufs U, Endres M, Nolte CH. Heart Rate on admission independently predicts in-hospital mortality in acute ischemic stroke patients. *Int J Cardiol.* (2014) 176:206–10. doi: 10.1016/j.ijcard.2014.07.001

7. Qiu M, Sato S, Zheng D, Wang X, Carcel C, Hirakawa Y, et al. Admission heart rate predicts poor outcomes in acute intracerebral hemorrhage: the intensive blood pressure reduction in acute cerebral hemorrhage trial studies. *Stroke.* (2016) 47:1479–85. doi: 10.1161/STROKEAHA.115.012382

8. Chen CH, Huang PW, Tang SC, Shieh JS, Lai DM, Wu AY, et al. Complexity of heart rate variability can predict stroke-in-evolution in acute ischemic stroke patients. *Sci Rep.* (2015) 5:17552. doi: 10.1038/srep17552

9. Duckheim M, Gaebler M, Mizera L, Schreieck J, Poli S, Ziemann U, et al. Deceleration capacity for rapid risk stratification in patients suffering from acute ischemic stroke: a prospective exploratory pilot study. *Medicine.* (2021) 100:e25333. doi: 10.1097/MD.0000000000025333

10. Li C, Meng X, Pan Y, Li Z, Wang M, Wang Y. The association between heart rate variability and 90-day prognosis in patients with transient ischemic attack and minor stroke. *Front Neurol.* (2021) 12:636474. doi: 10.3389/fneur.2021.636474

11. Miwa K, Koga M, Fukuda-Doi M, Yamamoto H, Tanaka K, Yoshimura S, et al. Effect of heart rate variabilities on outcome after acute intracerebral hemorrhage: a post hoc analysis of atach-2. *J Am Heart Assoc.* (2021) 10:e020364. doi: 10.1161/JAHA.120.020364

12. Tang SC, Jen HI, Lin YH, Hung CS, Jou WJ, Huang PW. et al. Complexity of heart rate variability predicts outcome in intensive care unit admitted patients with acute stroke *J Neurol Neurosurg Psychiatry.* (2015) 86:95–100. doi: 10.1136/jnnp-2014-308389

13. Lee KJ, Kim BJ, Han MK, Kim JT, Choi KH, Shin DI, et al. Effect of heart rate on stroke recurrence and mortality in acute ischemic stroke with atrial fibrillation. *Stroke.* (2020) 51:162–9. doi: 10.1161/STROKEAHA.119.026847

14. Lee KJ, Kim BJ, Han MK, Kim JT, Choi KH, Shin DI, et al. Effect of heart rate on 1-year outcome for patients with acute ischemic stroke. *J Am Heart Assoc.* (2022) 11:e025861. doi: 10.1161/JAHA.122.025861

15. Nakanishi K, Jin Z, Homma S, Elkind MSV, Rundek T, Lee SC, et al. Association between heart rate and subclinical cerebrovascular disease in the elderly. *Stroke.* (2018) 49:319–24. doi: 10.1161/STROKEAHA.117.019355

16. Han Q, Zhang C, You S, Zheng D, Zhong C, Dong H, et al. Resting heart rate and in-hospital mortality in acute ischemic stroke patients with and without atrial fibrillation. *Circulat J Official J Jpn Circulat Soc.* (2020) 84:656–61. doi: 10.1253/circj.CJ-19-0946

17. Böhm M, Cotton D, Foster L, Custodis F, Laufs U, Sacco R, et al. Impact of resting heart rate on mortality, disability and cognitive decline in patients after ischaemic stroke. *Eur Heart J.* (2012) 33:2804–12. doi: 10.1093/eurheartj/ehs250

18. Ritter MA, Rohde A, Heuschmann PU, Dziewas R, Stypmann J, Nabavi DG, et al. Heart rate monitoring on the stroke unit. What does heart beat tell about prognosis? An observational study. *BMC Neurol.* (2011) 11:47. doi: 10.1186/1471-2377-11-47

19. Tang S, Xiong L, Fan Y, Mok VCT, Wong KS, Leung TW. Stroke outcome prediction by blood pressure variability, heart rate variability, and baroreflex sensitivity. *Stroke.* (2020) 51:1317–20. doi: 10.1161/STROKEAHA.119.027981

20. Malone F, McCarthy E, Delassus P, Buhk JH, Fiehler J, Morris L. An *in vitro* assessment of atrial fibrillation flow types on cardiogenic emboli trajectory paths. Proceedings of the institution of mechanical engineers part H. *J Eng Med.* (2020) 234:1421–31. doi: 10.1177/0954411920946873

21. Goldberger AL, Amaral L, Glass L, Hausdorff JM, Ivanov PC, Mark RG, et al. Physiobank, physiotoolkit, and physionet: components of a new research resource for complex physiologic signals. *Circulation.* (2000) 101:E215. doi: 10.1161/01.CIR.101.23.e215

22. Johnson A, Bulgarelli L, Pollard T, Horng S, Celi LA, Mark R. *Mimic-Iv (Version 1.0).Physionet*. (2021).

23. Johnson AE, Stone DJ, Celi LA, Pollard TJ. The mimic code repository: enabling reproducibility in critical care research. *J Am Med Inf Assoc JAMIA*. (2018) 25:32–9. doi: 10.1093/jamia/ocx084

24. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The strengthening the reporting of observational studies in epidemiology (strobe) statement: guidelines for reporting observational studies. *Lancet.* (2007) 370:1453–7. doi: 10.1016/S0140-6736(07)61602-X

25. Núñez E, Steyerberg EW, Núñez J. Regression modeling strategies. *Rev Esp Cardiol.* (2011) 64:501–7. doi: 10.1016/j.rec.2011.01.017

26. Yang Q, Zheng J, Chen W, Chen X, Wen D, Chen W, et al. Association between preadmission metformin use and outcomes in intensive care unit patients with sepsis and type 2 diabetes: a cohort study. *Front Med.* (2021) 8:640785. doi: 10.3389/fmed.2021.640785

27. He L, Wang J, Zhang L, Wang F, Dong W, Yang H. Admission heart rate variability is associated with poststroke depression in patients with acute mild-moderate ischemic stroke. *Front Psychiatry.* (2020) 11:696. doi: 10.3389/fpsyt.2020.00696

28. Wang J, Zhou L, Zhang Y, Zhang H, Xie Y, Chen Z, et al. Minimum heart rate and mortality in critically ill myocardial infarction patients: an analysis of the mimic-iii database. *Ann Translat Med.* (2021) 9:496. doi: 10.21037/atm-21-992

29. Guo Q, Li H, Ouyang H, Sun R, Wang J, Wu M, et al. Heart rate fluctuation and mortality in critically ill myocardial infarction patients: a retrospective cohort study. *Front Cardiovas Med.* (2021) 8:577742. doi: 10.3389/fcvm.2021.577742

30. Fox K, Borer JS, Camm AJ, Danchin N, Ferrari R, Lopez Sendon JL, et al. Resting heart rate in cardiovascular disease. *J Am Coll Cardiol.* (2007) 50:823–30. doi: 10.1016/j.jacc.2007.04.079

31. Böhm M, Schumacher H, Teo KK, Lonn EM, Mahfoud F, Ukena C, et al. Resting heart rate and cardiovascular outcomes in diabetic and non-diabetic individuals at high cardiovascular risk analysis from the ontarget/transcend trials. *Eur Heart J.* (2020) 41:231–8. doi: 10.1093/eurheartj/ehy808

32. El Husseini N, Laskowitz DT. The role of neuroendocrine pathways in prognosis after stroke. *Expert Rev Neurother.* (2014) 14:217–32. doi: 10.1586/14737175.2014.877841

33. Fu Y, Liu Q, Anrather J, Shi FD. Immune interventions in stroke. *Nat Rev Neurol.* (2015) 11:524–35. doi: 10.1038/nrneurol.2015.144

34. Dirnagl U, Klehmet J, Braun JS, Harms H, Meisel C, Ziemssen T, et al. Stroke-induced immunodepression: experimental evidence and clinical relevance. *Stroke.* (2007) 38:770–3. doi: 10.1161/01.STR.0000251441.89665.bc

35. Sander D, Winbeck K, Klingelhöfer J, Etgen T, Conrad B. Prognostic relevance of pathological sympathetic activation after acute thromboembolic stroke. *Neurology.* (2001) 57:833–8. doi: 10.1212/WNL.57.5.833

36. Winklewski PJ, Radkowski M, Demkow U. Cross-talk between the inflammatory response, sympathetic activation and pulmonary infection in the ischemic stroke. *J Neuroinflammation.* (2014) 11:213. doi: 10.1186/s12974-014-0213-4

37. den Hertog HM, van Rossum JA, van der Worp HB, van Gemert HM, de Jonge R, Koudstaal PJ, et al. C-reactive protein in the very early phase of acute ischemic stroke: association with poor outcome and death. *J Neurol.* (2009) 256:2003–8. doi: 10.1007/s00415-009-5228-x

38. Mortara A, La Rovere MT, Pinna GD, Prpa A, Maestri R, Febo O, et al. Arterial baroreflex modulation of heart rate in chronic heart failure: clinical and hemodynamic correlates and prognostic implications. *Circulation.* (1997) 96:3450–8. doi: 10.1161/01.CIR.96.10.3450

39. Dessap AM, Roche-Campo F, Launay JM, Charles-Nelson A, Katsahian S, Brun-Buisson C, et al. Delirium and circadian rhythm of melatonin during weaning from mechanical ventilation: an ancillary study of a weaning trial. *Chest.* (2015) 148:1231–41. doi: 10.1378/chest.15-0525

40. Alasfour A, Jiang X, Gonzalez-Martinez J, Gilja V, Halgren E. High Γ activity in cortex and hippocampus is correlated with autonomic tone during sleep. *eNeuro.* (2021) 19:8. doi: 10.1523/ENEURO.0194-21.2021

41. Gollion C, Lerebours F, Nemmi F, Arribarat G, Bonneville F, Larrue V, et al. Insular Functional Connectivity in Migraine with Aura. *J Headache Pain.* (2022) 23:106. doi: 10.1186/s10194-022-01473-1

42. Guyenet PG, Stornetta RL. Rostral ventrolateral medulla, retropontine region and autonomic regulations. *Autonomic Neurosci Basic Clin.* (2022) 237:102922. doi: 10.1016/j.autneu.2021.102922

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Neuroimaging biomarkers of cognitive recovery after ischemic stroke

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Post-stroke cognitive impairment affects more than one-third of patients after an ischemic stroke (IS). Identifying markers of potential cognitive recovery after ischemic stroke can guide patients' selection for treatments, enrollment in clinical trials, and cognitive rehabilitation methods to restore cognitive abilities in post-stroke patients. Despite the burden of post-stroke cognitive impairment, biomarkers of cognitive recovery are an understudied area of research. This narrative review summarizes and critically reviews the current literature on the use and utility of neuroimaging as a predictive biomarker of cognitive recovery after IS. Most studies included in this review utilized structural Magnetic Resonance Imaging (MRI) to predict cognitive recovery after IS; these studies highlighted baseline markers of cerebral small vessel disease and cortical atrophy as predictors of cognitive recovery. Functional Magnetic Resonance Imaging (fMRI) using resting-state functional connectivity and Diffusion Imaging are potential biomarkers of cognitive recovery after IS, although more precise predictive tools are needed. Comparison of these studies is limited by heterogeneity in cognitive assessments. For all modalities, current findings need replication in larger samples. Although no neuroimaging tool is ready for use as a biomarker at this stage, these studies suggest a clinically meaningful role for neuroimaging in predicting post-stroke cognitive recovery.

KEYWORDS

ischemic stroke, reperfusion, recovery, reorganization, neuroplasticity, neuroimaging, cognition

Introduction

The American Heart Association (AHA) and the American Stroke Association (ASA) define ischemic stroke (IS) as an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction (1). IS represents 80% of all stroke types (2) and is a major cause of disability (3). With increasing survival after stroke and population aging, the prevalence of stroke is projected to increase by 3.4 million in 2030 (4, 5).

Many patients who survive a stroke live with a significant long-term disability that affects multiple functions, including motor, sensory, language, and cognitive abilities. An increasing number of studies have attempted to determine potential factors that can influence recovery after stroke (6). In a pivotal report from the Stroke Rehabilitation Roundtable, the importance of using a biomarker approach to identify the potential for recovery after stroke was outlined (6). The consensus introduced the term Stroke Recovery Biomarker (SRB), defined as "indicators of disease state that can be used clinically as a measure reflecting underlying processes that may be difficult to measure directly in humans and could be used to predict recovery or treatment response" (6, 7). The report referred to recovery for several stroke-type deficits, including motor, sensory, language, and cognition. The SRB approach aims to guide patients' treatment selection, enrollment in clinical trials, and rehabilitation interventions (6, 7).

IS recovery studies have focused mostly on motor recovery (8–20). Cognition is another important domain frequently affected by IS, resulting in post-stroke cognitive impairment (PSCI). A recent systematic review and meta-analysis identified a pooled prevalence of PSCI of 39%, measured within the first year post-stroke (21). Others report a PSCI prevalence ranging from 20 to 80% depending on factors such as race and methodology (22). Cognitive recovery remains an understudied aspect of stroke, and no biomarkers are currently ready for use in clinical trials (6, 23). Some studies reported spontaneous restoration of cognitive function after the subacute phase of IS (24–26). However, many patients have cognitive impairment beyond the subacute phase of IS. A recent, large, population-based study of first-ever stroke patients from the South London Stroke Register between 1995 and 2018 (n = 6,504, mean age = 73 years) found that one-third of patients cognitively improved during the first 3 months post-stroke, one-third deteriorated, and the rest remained cognitively unchanged (27). The study further reported that PSCI was associated with a 5-year increase in the risk of mortality (RR = 30%), dependency (RR = 90%), depression (RR = 60%) and institutionalization (RR = 50%) (27).

Imaging is a potential biomarker for cognitive recovery after IS (6). A systematic review evaluating all biological and imaging markers found that global atrophy and medial temporal lobe atrophy were the most consistent predictors of cognitive impairment after stroke; however, this review did not link cognitive recovery over time with neuroimaging (28). Given the accessibility and the wide use of neuroimaging as part of stroke workup, neuroimaging is a promising tool to study the potential for cognitive recovery after stroke. Neuroimaging techniques are currently being used to understand higher cortical function and recovery among comatose patients with the eventual goal to identify potential early and tailored rehabilitative interventions and underlying patient-specific characteristics that are most responsive to these interventions (29, 30). Cognitive aging is another area where neuroimaging is increasingly used to comprehend brain cognitive processes (31). Thus, our goal for this review was to summarize literature within the last 10 years describing neuroimaging as a predictive marker of cognitive recovery in IS. We focus on IS, the most common type of stroke associated with PSCI. We highlight important findings and limitations in the studies and discuss some of the challenges for future studies to consider.

Search methodology and literature selection

PubMed was used as the primary database for studies published in the last 10 years through September 6th, 2022. We used the Medical Subject Headings (MeSH) term "stroke" with the MeSH subheadings "complications" or "psychology" or the term "ischemic stroke" paired with both of the following terms in the abstract/title of each article or as MeSH terms:

- A cognition term ("cognition," "cognitive," "cognitive decline," "cognition disorder," or "dementia," "neuropsychological," or "neuropsychological tests").
- A neuroimaging term ("neuroimaging," "magnetic resonance imaging/MRI," "functional magnetic resonance imaging/fMRI," "diffusion tensor imaging/DTI," "default mode network," or "connectivity").

Additional studies obtained through review of relevant article citations were included.

Studies examining other types of strokes—hemorrhagic stroke (HS), traumatic stroke, subarachnoid hemorrhage (SH), and transient ischemic attacks (TIA)—were excluded. HS were excluded due to their different recovery trajectories compared to IS (32, 33). Likewise, TIA were also excluded due to a lack of clear and persistent ischemic injury, which may result in a different recovery course (34, 35). Since cognitive recovery implies a change in cognitive performance over time, studies that reported only one cognitive assessment were excluded. Included studies associated a change in a cognitive assessment measure between at least two time points with baseline neuroimaging.

Finally, we required baseline imaging and cognitive assessments to be completed within 6 weeks of stroke. This is to ensure clinical relevance, as most imaging used to predict recovery would be completed during a hospital admission. A baseline cognitive assessment more than 6 weeks after an initial ischemic stroke may represent a different stage of stroke recovery and therefore not comparable to the other articles in this review.

Studies of potential relevance were selected, and 35 were excluded after careful full-text review based on the criteria detailed above (Figure 1, Supplementary Table 1). A total of 13 studies were included.

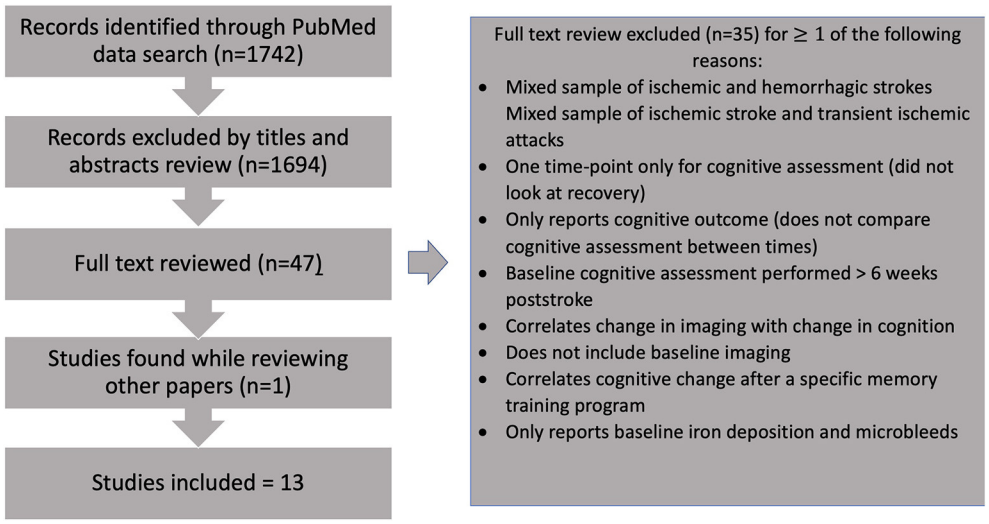

FIGURE 1
Flow diagram of study selection process.

Neuroimaging modalities used as biomarkers for cognitive recovery after ischemic stroke

MRI: T1-weighted MRI, T2-weighted, and fluid-attenuated inversion recovery (FLAIR)

MRI as a means of characterizing or predicting cognitive impairment through structural biomarkers is well-documented (22, 36). These biomarkers include cerebral small vessel disease (SVD) such as white matter hyperintensities (WMH) and microinfarcts, cortical volume, and size and location of IS lesions (36). Researchers have attempted to use these same biomarkers to predict cognitive recovery following IS (37–39). Studies utilizing structural MRI that fit the criteria for this review are summarized in Table 1.

Markers of cerebral small vessel disease

An important study by Sagnier et al. reported that first-time IS patients with pre-existing severe WMH on MRI had less improvement in verbal fluency tests at 3 months to 1 year after IS (38). Similarly, the presence of Cortical Superficial Siderosis (cSS) after first-time IS, another radiologic biomarker of cerebral SVD, was an indicator of worse cognitive recovery in tests of processing speed and attention, independent of IS volume/location, gray matter volume, other SVD biomarkers, and clinical severity, cardiovascular risk factors, and demographic confounders (38).

Similarly, Fruthwirth et al. found that deep WMH volume at baseline predicted recovery of set-shifting at 15 month follow up among patients with recent small subcortical infarcts: participants with no or mild deep WMH improved in set-shifting, while those with moderate to severe WMH showed no improvement (41). A similar pattern was seen for periventricular WMH (pWMH) at baseline and recovery of attention, although this interaction was no longer significant after controlling for age. For set-shifting, mild pWMH predicted improvement, whereas no pWMH and moderate-severe pWMH demonstrated no improvement. Of note, this study found that all patients, regardless of baseline WMH volume, improved in Montreal Cognitive Assessment (MoCA) scores, processing speed, and attention.

Another study examining cerebral small vessel disease (mCSVD) score—determined by MRI evaluation—and medial temporal atrophy (MTA) score—a measure of hippocampal atrophy—found that neither of these imaging markers predicted change in MoCA scores between baseline and 1 year follow up (44). Despite these imaging markers not being associated with a change in cognitive scores, this study did find that mCSVD and MTA scores could be used to predict low vs. high cognitive performance at 1 year, with higher mCSVD and MTA scores associated with increased likelihood of low MoCA scores at follow up.

Cortical volume

Sagnier found that total gray matter (GM) volume was the only radiographic factor predictive of cognitive improvement at 12 month follow up among IS patients. Specifically, GM

TABLE 1 Summary of key studies within the last 10 years on neuroimaging biomarkers of cognitive recovery after ischemic stroke.

References	Study population, *N*, type of stroke, age	Duration of study	Type of imaging and imaging outcomes examined	Cognitive measures	Findings
fMRI					
Vicentini et al. (40)	First subacute IS patients with no previous known neurologic disorder. Time 1 ($n = 37$, mean age $= 62.92 \pm 9.49$ years, stroke onset $= 24.32 \pm 7.44$ days, NIHSS: 2.66 $\pm$ 3.45). Time 2 after 6 months ($n = 20$, stroke onset: $182.05 \pm$ 8.17 days). Cognitively healthy controls ($n = 20$)	6 months: 2-time points. fMRI and cognitive assessments were done at time 1 (subacute phase, within the first month) and 2 (chronic phase, after 6 months).	rsFC Three networks were examined: DMN, SN, and CEN network.	MoCA	At time 1 Patients had weaker interhemispheric connectivity in the DMN than controls ($p = 0.028$, FDR-corrected). Better cognitive performance was associated with a stronger interhemispheric ($r = 0.409$, FDR $p = 0.058$) and ipsilesional DMN connectivity ($r = 449$, FDR $p = 0.068$) and weaker contralesional SN connectivity ($r = -0.426$, FDR $p = 0.049$) At time 2 No change in functional connectivity in patients compared to time 1. Better cognitive recovery at time 2 was associated with stronger DMN connectivity ($r = 0.511$, FDR $p = 0.090$) and weaker SN interhemispheric subacute connectivity ($r = -0.638$, FDR $p = 0.076$)
MRI: T1-weighted MRI, T2-weighted MRI, FLAIR					
Fruhwirth et al. (41)	Recent small subcortical infarct (RSSI) patients ($\leq$25 mm), no preexisting cognitive impairment ($N = 82$, mean age $= 61 \pm 10$ years, 23% female)	15 months: Baseline cognitive assessment at time 1 (mean 6 days post-stroke) and time 2 (15 months). MRI at Time 1.	WMH lesion volume.	MoCA, Symbol Digital Modalities Test, Comprehensive Trail Making Test	All patients improved on MoCA, SDMT (processing speed) after 15 months, regardless of WMH severity ($p = 0.011$ for MoCA, $p = 0.010$ for SDMT (processing speed); after age adjustment, no difference between WMH severity and MoCA ($p = 0.109$) or SDMT (processing speed) ($p = 0.414$). No differences between CTMT-2 (attention) scores and WMH.

(Continued)

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TABLE 1 (Continued)

References	Study population, *N*, type of stroke, age	Duration of study	Type of imaging and imaging outcomes examined	Cognitive measures	Findings
					CTMT-5 (set-shifting): no improvement for patients with mild ($p = 0.086$) or moderate-severe WMH ($p = 0.801$) at 15 months, compared to improvement in patients without WMH ($p = 0.001$). Same results even when corrected for age. WMH volume at baseline was only significant factor that predicted attention at 15 month follow up ($p = 0.002$) beyond demographics.
Scharf et al. (42)	Acute first-ever ischemic thalamic stroke, >18 yo Exclusion: previous psychiatric/neuro disorders ($N = 37$ case, 37 controls)	2 years: Neuropsychological assessment at time 1 (1 month), time 2 (6 month), time 3 (12 month) and time 4 (24 month) MRI at time 1, time 2, and time 4.	MRI—T1, T2, FLAIR, DWI	Digit span, rivermead behavioral memory test, Regensburg semantic and phonemic word fluency, TMA A/B, Stroop	Paramedian thalamic stroke patients demonstrated moderate language and executive deficits, with the best recovery of the three thalamic stroke topographies. Anterior thalamic stroke patients demonstrated the most severe deficits in verbal memory, language, and executive functions, which poorly recovered during follow-up. Inferolateral stroke patients also suffered from verbal memory, language, and executive deficits; the verbal memory and executive deficits recovered during follow-up, while the language deficits persisted.
Sagnier et al. (38)	Acute Ischemic supratentorial stroke patients with no previous neuropsychiatric disorder or dementia ($n = 199$, mean age = 65 ± 13, NIHSS median = 3 (4)	1 year: cognitive assessment was done at time 1 (baseline), time 2 (after 3 months), and time 3 (after 1 year). MRI was done at time 1 only (baseline between 24 and 72 h).	WMH, deep and lobar microbleeds, enlarged perivascular spaces in basal ganglia and centrum semiovale, previous small deep infarcts, and cSS.	MoCA ZCT processing speed and attention IST of verbal fluency for executive function.	- Cognitive performance improved more significantly in the first 3 months. - Severe WMH was identified in 34% of the patients, and cSS in 3.5%. Patients with severe WMH and focal cSS had overall worse cognitive performances.

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TABLE 1 (Continued)

References	Study population, *N*, type of stroke, age	Duration of study	Type of imaging and imaging outcomes examined	Cognitive measures	Findings
					- Patient with severe WMH had less improvement over time in IST of verbal fluency ($\beta = -0.16$, $p = 0.02$) and the number of errors to ZCT ($\beta = 0.19$, $p = 0.02$). Those with focal cSS had less improvement over time for ZCT completion time ($\beta = 0.14$, $p = 0.01$) and number of errors ($\beta = 0.17$, $p = 0.008$).
Sagnier et al. (39)	Acute Ischemic supratentorial stroke patients with no previous neuropsychiatric disorder or dementia ($n = 199$, mean age = 67 ± 14, NIHSS ≥1)	One year: 3-time points cognitive assessment was done at time 1 (baseline), time 2 (after 3 months), and time 3 (after 1 year). MRI was done at time 1 only (baseline between 24 and 72 h)	CMI.	MoCA The Zazzo's cancellation task (ZCT) for processing speed and attention Issac set a test for verbal fluency (IST)	The number of CMI was associated with increased time at the ZCT over 1 year regardless of the other MRI markers, stroke severity, and demographic factors (B = 3.84, $P = 0.01$).
Sagnier et al. (43)	Acute supratentorial ischemic stroke without prestroke disability related to neurological disorder, mRS > 1 at baseline ($N = 248$, mean age 65 ± SD 14 years old, 66% men)	12 months: Baseline MRI Cognitive assessment at time 1 (within 24–72 h), time 2 (after 3 months), and time 3 (after 12 months)	WMH + stroke volume, gray matter (GM), white matter, and CSF volume.	Cognitive assessment: MoCA, Isaacs Set Test (IST), Zazzo's cancellation test Cognitive improvement, stability, or decline calculated using all 3 time points	- Radiographic model only: total GM volume was the only variable predictive of changes in all cognitive scores over the year of follow-up ($P < 0.001$ for MoCA, $P = 0.03$ for IST, $P = 0.002$ for time to perform Zazzo's cancellation task and $P < 0.001$ for the number of errors) - In clinical/radiographic model: total GM volume independently associated with cognition [MoCA ($P = 0.04$) and Zazzo's cancellation task ($P = 0.04$)] - GM volume of left fronto-temporo-insular regions, right temporo-insular cortex, and basal ganglia was significantly associated with cognitive improvement.

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TABLE 1 (Continued)

References	Study population, *N*, type of stroke, age	Duration of study	Type of imaging and imaging outcomes examined	Cognitive measures	Findings
Sung et al. (44)	First ever ischemic stroke without specific etiologies predisposing to recurrence, cognitive impairment at baseline, neurodegenerative disease ($n = 112$, median age 64.5 (IQR 57.0–73.5) years, NIHSS at baseline 3.77 (IQR: 1.75–5).	1 year: MRI and cognitive assessment at time 1 (within 7 days), cognitive assessment again at time 2 (3 months) and time 3 (1 year)	Stroke location, SVD burden and hippocampal atrophy (HA) Modified cerebral small vessel disease (mCSVD) score calculated using lacunar infarction, microbleeds, moderate to severe perivascular space at the ganglionic level or a deep white matter Fazekas score ≥ 2. Medial temporal atrophy score used to determine hippocampal atrophy.	MoCA Weschler Adult Intelligence Scale III, Wechsler Memory Scale III the Semantic Association of Verbal Fluency Test for semantic verbal fluency, Wisconsin Card Sorting Test	No significant difference in change in MOCA scores between higher CSVD burden or abnormal HA. In the multivariate model, higher mCSVD score (adjusted odds ratio (aOR) 2.74, 95%CI 1.09–6.86, $p = 0.032$) independently predicted low cognitive performance at 1 year [but not an abnormal MTA score (aOR 1.53, 95%CI 0.56–4.21, $p = 0.405$)]. A combination of a higher mCSVD score and an abnormal MTA score resulted in the highest probability of classification in the LP group (aOR 4.18, 95%CI 1.05–16.66, $p = 0.043$).
Turunen et al. (45)	First ever supratentorial ischemic stroke, no baseline neurological or psychiatric disorder ($n = 132$, mean age 54 years, 68.2% male)	6 months: Baseline imaging Cognitive assessment at time 1 (~8 days post-stroke) and time 2 (6 month follow up)	Stroke location, categorized into two groups: infarction in cortical gray matter (including additional white matter) or infarction in subcortical gray and/or white matter.	Weschler memory scale, phonemic fluency task, TMA A/B, WMS-R, searching task On 6-month repeat, general intellect added	No differences in the recovery of cognitive profile amongst lesion location groups were found; after adjusting for baseline scores, the lesion location groups did not differ at follow-up.
Zhang et al. (46)	Imaging confirmed first time acute ischemic stroke, no history of cognitive or psychiatric disorder ($n = 865$, mean age 59.67 $\pm$ 10.92 years and 74.22% male)	12 months: Baseline imaging Baseline MOCA at time 1 (2 weeks/discharge) and time 2 (12 month follow-up)	Infarct location, small vessel disease features, WMHs, lacunes, microbleeds, enlarged perivascular spaces, cortical atrophy	MOCA Cognitive decline defined as reduction of 2+ points between time 1 and time 2, improvement defined as increase of 2+ between time points, cognitive stability defined as change of <2 points.	In cognitive decline group, statistically significantly higher incidence of thalamic (11.43 vs. 5.66%, $p = 0.023$) and right sided lesions (6.67 vs. 1.97%, $p = 0.004$). Thalamic infarction increased risk of cognitive decline (OR 2.152, 95% CI 1.095–4.227). Thalamic infarction quadrupled the risk of cognitive decline (OR 4.873, 95% CI 1.634–14.534) in fully adjusted model.

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TABLE 1 (Continued)

References	Study population, *N*, type of stroke, age	Duration of study	Type of imaging and imaging outcomes examined	Cognitive measures	Findings
MRI: DTI, DWI					
Aben et al. (47)	Ischemic stroke patients with no prior cognitive disorder [$n = 75$, mean age = 70 ±8.5, NIHSS =2 (2–4)]	1 year: 2- time points Cognitive assessments were done at time 1 (baseline 5 weeks ±1 week) and time 2 (after 1 year). MRI was done at time 1 only.	Lesion impact score, calculated by multiplying the percentage of node volume affected by the infarct with the node's corresponding hub-score.	4 cognitive domains: attention and processing speed, working memory and learning, and frontal executive function	- A higher lesion impact score, indicating an increasing infarct size in nodes with a higher hub-score, was related to lower global brain network efficiency [$\beta = -0.528$ (-0.776 to -0.277); $P < 0.001$]. - A lower lesion impact score was an independent predictor of cognitive recovery 1 year after stroke [OR = 0.434 (0.193–0.978); $P = 0.044$].
Aben et al. (48)	Ischemic stroke patients ($n = 217$, aged ≥50 years, and MoCA < 26 during hospitalization)	1 year: 2- time points Cognitive assessments were done at time 1 (baseline 5 weeks) and time 2 (after 1 year). MRI was done at time 1 only.	4 DWI-based measures of brain connectivity: global network efficiency and mean connectivity strength, both weighted for MD and FA.	MoCA	- Of 135 patients with PSCI at time 1, 41 (30%) showed cognitive recovery. three out of four DTI measures of brain connectivity: global efficiency FA weighted, mean connectivity strength FA weighted, and mean connectivity strength MD weighted predict cognitive recovery 1 year after IS. These measures, however, did not add a better predictive value over the multivariable model.
Kuceyeski et al. (49)	IS patients ($n = 40$, mean age = 68.1 ± 13.2 years, NIHSS: 6.8 ± 5.6)	6 months Cognitive assessment after discharge and at 6 months. Imaging was done at baseline (within 14 days)	Connectome disruption at three levels: whole brain, individual gray matter regions and between pairs of gray matter regions. Lesion volume model for comparison.	Computer adaptive version of the Activity Measure for Post-Acute Care.	- The regional disconnection model best predicted applied cognitive functioning ($R^2 = 0.56$) - The pairwise disconnection model best predicted the daily activity measure ($R^2 = 0.72$)

fMRI, Functional Magnetic Resonance Imaging; FLAIR, Fluid-Attenuated Inversion Recovery; DTI, Diffusion Tensor Imaging; DWI, Diffusion-Weighted Imaging; IS, Ischemic Stroke; rsFC, resting-state functional connectivity; DMN, Default Mode Network; SN, Salience Network; CEN, Central Executive network; MoCA, Montreal Cognitive Assessment; MMSE, Mini-Mental State Examination; PSCI, Post-Stroke Cognitive Impairment; PCC, Posterior Cingulate Cortex; PCu, Precuneus; MPFC, Medial Prefrontal Cortex; FIM, Functional-Independence Measures; WMH, White Matter Hyperintensities; NFL, Neurofilament Lights; ZCT, Zazzo's Cancellation Task; IST, Issac Set a Test; cSS, Cortical Superficial Siderosis; CMI, Cortical Cerebral Microinfarct; MD, Mean Diffusivity; FA, Fractional Anisotropy; GPT, Grooved Pegboard Test.

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volume of left fronto-temporo-insular regions, right temporo-insular cortex, and basal ganglia were significantly associated with cognitive improvement (43).

Infarct characteristic

Several studies examined the impact of infarct location on cognitive recovery using structural MRI. Turunen examined differences in cognitive recovery for cortical vs. subcortical lesions among patients with first-ever supratentorial ischemic stroke (45). While this study found that subcortical infarctions were associated with decreased verbal memory and psychomotor speed in the acute phase and persistent verbal memory differences at 6-month follow-up, there was no difference in recovery of cognition between the two lesion location groups. In contrast, a recent study by Zhang et al. found a significantly higher incidence of thalamic and right-sided lesions in the group that cognitively declined at follow up, determined by a difference in MoCA scores between baseline and 12-month exam (46). In their fully adjusted models, infarct in the thalamus more than quadrupled the risk of cognitive decline among these patients. A recent study further characterized cognitive recovery among patients with ischemic thalamic stroke: in this case control study with a 2-year follow-up period, patients with anterior and inferolateral thalamic strokes were found to have poorer recovery of language, memory, and executive function than those with paramedian strokes (42).

Together, these studies support the use of structural MRI sequences to predict changes in cognition after ischemic stroke. Baseline measures of cerebral SVD, including WMH, as well as baseline cortical volume may be important indicators of the potential for restoration of cognitive ability after stroke. In addition, these articles suggest that different infarct locations as seen on structural MRI may be used to predict differing cognitive recovery trajectories after stroke, although more studies are needed to fully elucidate this relationship.

Functional magnetic resonance imaging (fMRI)

Only one study reported post-IS cognitive recovery using resting-state fMRI (rs-fMRI) (Table 1) (40). Rs-fMRI utilizes Blood Oxygen Level Dependent (BOLD) to study spontaneous brain neural activity at rest in a specific functional brain region (rsfMRI activity) (50). In their study examining cognitive recovery after IS, Vincentini et al. found weaker interhemispheric spontaneous temporal correlations between different brain functional regions [rs-functional connectivity (rsFC)] within the Default Mode Network (DMN). Alterations of rsFc among IS patients have also been previously reported (51–53). Stroke injury has been shown to disrupt communications between hemispheres and results in both intra-and interhemispheric changes in rsFC (52). Vicentini et al. reported no change in rsFC from the subacute to the chronic phase in IS patients (40). This study did find, however, that better cognitive recovery at 6 months was correlated with rsFC in two networks: DMN and Executive Network (40).

This study argues in favor of the potential of rsFC to predict the course of cognitive changes post-IS and the involvement of DMN in the recovery process, although more studies are required to confirm these findings and further understand brain functional networks involved in the acute to chronic phases of cognitive recovery (20). Furthermore, there are still practical challenges to using rsFMRI in clinical settings, such as availability, time, and the need for interpretation expertise. The lack of technique standardization, including variability in rsfMRI data acquisition, preprocessing, and analytical methods, presents another challenge for clinical applicability (50, 54).

Diffusion tensor imaging (DTI) and diffusion-weighted imaging (DWI)

While fMRI examines the brain's functional integrity, DTI looks at the brain's white matter structural integrity. Fractional anisotropy (FA) is the most common parameter derived from DTI to assess brain structural connectivity. Other DTI measures are mean diffusivity (MD), radial diffusivity (RD), axial diffusivity (AD), independent of direction, and relative anisotropy.

Important studies using DTI/DWI to study cognitive recovery after IS are summarized in Table 1. A notable study by Aben et al. (47) used diffusion-weighted data after IS to create a lesion impact score that reflected the impact of IS size on the brain's network hubs. The authors demonstrated that a lower lesion impact score was an independent predictor of cognitive recovery 1 year after IS while controlling for WMH and infarct volumes (47). The authors showed that this score could also be calculated using structural MRI sequences (T1, FLAIR), which are routinely ordered as part of a stroke workup and therefore easier to implement in a clinical setting to predict long-term recovery after IS (47). A subsequent study compared DTI measures with a multivariable model, including age, education, and infarct size, and found that three out of four DTI measures of brain connectivity (global efficiency FA weighted, mean connectivity strength FA weighted, and mean connectivity strength MD weighted) predicted cognitive recovery 1 year after IS. These measures, however, did not improve prediction over the multivariable model that included education level and infarct size as significant predictors of cognitive recovery (48).

In a different study using DWI, Kuceyeski et al. (49) compared models of connectome disruption to determine which model best predicted recovery after IS. The authors found that the regional disconnection model, which reflects changes

in structural connectivity of gray matter regions (WM tracts connecting brain regions) to the rest of the network, best predicted cognitive recovery. This regional disconnection model was found to be superior to models based on lesion volume and other disconnection models (whole brain and pairwise) (49).

The evidence for DTI and DWI as a means of predicting cognitive recovery after stroke is promising. More research is needed to determine the additional utility of this modality over structural MRI and to improve predictive value.

Study limitations and future directions

To the best of our knowledge, this review is among the first to focus on neuroimaging biomarkers of cognitive recovery among IS patients. This review is limited by inherent challenges in using keywords to search literature, including a lack of consistent use of terminology to characterize study subject matter. We carefully reviewed the literature to ensure our search was as robust as possible but may have inadvertently missed relevant studies. One key aspect of our study that necessitated the exclusion of multiple otherwise relevant articles was that we focused specifically on the association of neuroimaging with cognitive recovery after stroke; each included study reported on the association between baseline neuroimaging and a change in a cognitive measure over time. Articles that merely reported a cognitive outcome (e.g., post-stroke cognitive impairment vs. no post-stroke cognitive impairment) were therefore excluded from this review of neuroimaging biomarkers for cognitive recovery after IS.

In addition, there are several gaps in the current literature, as discussed below:

1. The best time to study cognitive recovery remains unclear and likely stems from uncertainty in the time frame of expected cognitive recovery post-stroke. In our review, we focused on baseline cognitive assessments done within 6 weeks of stroke to ensure clinical relevance and to adequately compare different articles. However, cognitive recovery studies among chronic stroke patients may help answer this question.
2. The ability to detect cognitive recovery depends largely on the sensitivity of the cognitive test used. The studies included in this review used a variety of different tests—all outlined in Table 1—which may limit our ability to compare them and to generalize their results. Screening tests such as the Montreal Cognitive Assessment (MoCA) and Mini-Mental State Exam (MMSE) are gross measures of cognition and may not capture subtle cognitive dysfunction nor subtypes of cognitive impairment (e.g., left neglect, aphasia). MoCA, for instance, is less sensitive to right-hemispheric lesion-based deficits (55). Adequate studies of cognitive recovery may require more detailed assessments tailored to the setting (e.g., brief baseline exams for inpatients, longer baseline exams for outpatients).
3. To be included in this review, we required at least one repeat measure of cognition. However, it should be noted that having longitudinal imaging is also important to show concordance between neuroimaging and cognitive testing parameters across the recovery course.
4. Some studies—which were excluded from this review—utilized cohorts of both ischemic (IS) and hemorrhagic strokes (HS) to study cognitive recovery. IS and IH have different pathophysiologies and different recovery processes (32, 33). Although the differences in recovery between the two-stroke types are not entirely known (56), future post-stroke cognitive recovery studies should analyze stroke types separately.
5. Only some of the reviewed studies explicitly assessed pre-stroke cognitive status. Pre-stroke cognitive status may influence the recovery process and should be taken into consideration (57). Similarly, not all studies mentioned if only first-time IS patients were included (49). In the future, it would be helpful to standardize screening of pre-stroke cognitive status to better allow comparison between studies of cognitive recovery.
6. Finally, many studies in this review combined neuroimaging with non-neuroimaging tools to improve the prediction of cognitive recovery after IS. For example, Sangier created models that incorporated demographic and clinical factors, including age, sex, education, cardiovascular risk factors, and modified Rankin score (38, 43). Such models are currently used to predict cognitive function after stroke, suggesting their utility in predicting cognitive recovery post-stroke; the SIGNAL2 score and the CHANGE score, both examining the risk of post-stroke cognitive impairment, incorporate age and education into their prediction tools, in addition to imaging variables (58, 59).

Conclusion

In summary, the current literature on cognitive recovery using neuroimaging as a predictive marker, although small, is promising. No imaging tool is ready for use as an established biomarker yet. Future studies should replicate current findings in larger samples using a consistent methodology.

Author contributions

MT and VK conducted the literature search and drafted the manuscript in equal contribution. MP reviewed the manuscript for intellectual content. IN conceptualized the project and reviewed the manuscript for intellectual content.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.923942/full#supplementary-material

References

1. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2013) 44:2064–89. doi: 10.1161/STR.0b013e318296aeca

2. Mehndiratta P, Chapman Smith S, Worrall BB. Etiologic stroke subtypes: updated definition and efficient workup strategies. *Curr Treat Options Cardiovasc Med.* (2015) 17:357. doi: 10.1007/s11936-014-0357-7

3. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. Stroke statistics, heart disease and stroke statistics-2021 update: a report from the American Heart Association. *Circulation.* (2021) 143:e254–743. doi: 10.1161/CIR.0000000000000950

4. Ovbiagele B, Goldstein LB, Higashida RT, Howard VJ, Johnston SC, Khavjou OA, et al. Forecasting the future of stroke in the United States: a policy statement from the American Heart Association and American Stroke Association. *Stroke.* (2013) 44:2361–75. doi: 10.1161/STR.0b013e31829734f2

5. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics-2019 update: a report from the American Heart Association. *Circulation.* (2019) 139:e56–528. doi: 10.1161/CIR.0000000000000659

6. Boyd LA, Hayward KS, Ward NS, Stinear CM, Rosso C, Fisher RJ, et al. Biomarkers of stroke recovery: consensus-based core recommendations from the stroke recovery and rehabilitation roundtable. *Neurorehabil Neural Repair.* (2017) 31:864–76. doi: 10.1177/1545968317732680

7. Bernhardt J, Borschmann K, Boyd L, Carmichael ST, Corbett D, Cramer SC, et al. Moving rehabilitation research forward: developing consensus statements for rehabilitation and recovery research. *Neurorehabil Neural Repair.* (2017) 31:694–8. doi: 10.1177/1545968317724290

8. Hartwigsen G, Saur D. Neuroimaging of stroke recovery from aphasia - insights into plasticity of the human language network. *Neuroimage.* (2019) 190:14–31. doi: 10.1016/j.neuroimage.2017.11.056

9. Sinke MRT, van Tilborg GAF, Meerwaldt AE, van Heijningen CL, van der Toorn A, Straathof M, et al. Remote corticospinal tract degeneration after cortical stroke in rats may not preclude spontaneous sensorimotor recovery. *Neurorehabil Neural Repair.* (2021) 35:1010–19. doi: 10.1177/15459683211041318

10. Mattia D, Pichiorri F, Colamarino E, Masciullo M, Morone G, Toppi J, et al. The Promotoer, a brain-computer interface-assisted intervention to promote upper limb functional motor recovery after stroke: a study protocol for a randomized controlled trial to test early and long-term efficacy and to identify determinants of response. *BMC Neurol.* (2020) 20:254. doi: 10.1186/s12883-020-01826-w

11. Liu G, Tan S, Peng K, Dang C, Xing S, Xie C, et al. Network change in the ipsilesional cerebellum is correlated with motor recovery following unilateral pontine infarction. *Eur J Neurol.* (2019) 26:1266–73. doi: 10.1111/ene.13974

12. Wei W, Bai L, Wang J, Dai R, Tong RK, Zhang Y, et al. A longitudinal study of hand motor recovery after sub-acute stroke: a study combined FMRI with diffusion tensor imaging. *PLoS ONE.* (2013) 8:e64154. doi: 10.1371/journal.pone.0064154

13. Dang C, Liu G, Xing S, Xie C, Peng K, Li C, et al. Longitudinal cortical volume changes correlate with motor recovery in patients after acute local subcortical infarction. *Stroke.* (2013) 44:2795–801. doi: 10.1161/STROKEAHA.113.000971

14. Zarahn E, Alon L, Ryan SL, Lazar RM, Vry MS, Weiller C, et al. Prediction of motor recovery using initial impairment and fMRI 48 h poststroke. *Cereb Cortex.* (2011) 21:2712–21. doi: 10.1093/cercor/bhr047

15. Iorga M, Higgins J, Caplan D, Zinbarg R, Kiran S, Thompson CK, et al. Predicting language recovery in post-stroke aphasia using behavior and functional MRI. *Sci Rep.* (2021) 11:8419. doi: 10.1038/s41598-021-88022-z

16. Sebastian R, Long C, Purcell JJ, Faria AV, Lindquist M, Jarso S, et al. Imaging network level language recovery after left PCA stroke. *Restor Neurol Neurosci.* (2016) 34:473–89. doi: 10.3233/RNN-150621

17. Hartwigsen G. Adaptive plasticity in the healthy language network: implications for language recovery after stroke. *Neural Plast.* (2016) 2016:9674790. doi: 10.1155/2016/9674790

18. Jarso S, Li M, Faria A, Davis C, Leigh R, Sebastian R, et al. Distinct mechanisms and timing of language recovery after stroke. *Cogn Neuropsychol.* (2013) 30:454–75. doi: 10.1080/02643294.2013.875467

19. Lidzba K, Staudt M, Zieske F, Schwilling E, Ackermann H. Prestroke/poststroke fMRI in aphasia: perilesional hemodynamic activation and language recovery. *Neurology.* (2012) 78:289–91. doi: 10.1212/WNL.0b013e318243679a

20. Baldassarre A, Ramsey LE, Siegel JS, Shulman GL, Corbetta M. Brain connectivity and neurological disorders after stroke. *Curr Opin Neurol.* (2016) 29:706–13. doi: 10.1097/WCO.0000000000000396

21. Sexton EA-O, McLoughlin A, Williams DJ, Merriman NA, Donnelly N, Rohde DA-O, et al. Systematic review and meta-analysis of the prevalence of cognitive impairment no dementia in the first year post-stroke. *Eur Stroke J.* (2019) 4:160–71. doi: 10.1177/2396987318825484

22. Sun JH, Tan L, Yu JT. Post-stroke cognitive impairment: epidemiology, mechanisms and management. *Ann Transl Med.* (2014) 2:80. doi: 10.3978/j.issn.2305-5839.2014.08.05

23. Melkas S, Jokinen H, Hietanen M, Erkinjuntti T. Poststroke cognitive impairment and dementia: prevalence, diagnosis, and treatment. *Degener Neurol Neuromuscul Dis.* (2014) 4:21–7. doi: 10.2147/DNND.S37353

24. Ballard C, Rowan E, Stephens S, Kalaria R, Kenny RA. Prospective follow-up study between 3 and 15 months after stroke: improvements and decline in cognitive function among dementia-free stroke survivors >75 years of age. *Stroke.* (2003) 34:2440–4. doi: 10.1161/01.STR.0000089923.29724.CE

25. Liman TG, Heuschmann PU, Endres M, Flöel A, Schwab S, Kolominsky-Rabas PL. Changes in cognitive function over 3 years after first-ever stroke and predictors of cognitive impairment and long-term cognitive stability: the Erlangen Stroke Project. *Dement Geriatr Cogn Disord.* (2011) 31:291–9. doi: 10.1159/000327358

26. Desmond DW, Moroney JT, Sano M, Stern Y. Recovery of cognitive function after stroke. *Stroke.* (1996) 27:1798–803. doi: 10.1161/01.STR.27.10.1798

27. Obaid M, Flach C, Marshall I, C DAW, Douiri A. Long-term outcomes in stroke patients with cognitive impairment: a population-based study. *Geriatrics.* 5:32. (2020). doi: 10.3390/geriatrics5020032

28. Casolla B, Caparros F, Cordonnier C, Bombois S, Hénon H, Bordet R, et al. Biological and imaging predictors of cognitive impairment after stroke: a systematic review. *J Neurol.* (2019) 266:2593–604. doi: 10.1007/s00415-018-9089-z

29. Edlow BL, Claassen J, Schiff ND, Greer DM. Recovery from disorders of consciousness: mechanisms, prognosis and emerging therapies. *Nat Rev Neurol.* (2021) 17:135–56. doi: 10.1038/s41582-020-00428-x

30. Silva S, de Pasquale F, Vuillaume C, Riu B, Loubinoux I, Geeraerts T, et al. Disruption of posteromedial large-scale neural communication predicts recovery from coma. *Neurology.* (2015) 85:2036–44. doi: 10.1212/WNL.0000000000002196

31. Soch J, Richter A, Schütze H, Kizilirmak JM, Assmann A, Behnisch G, et al. A comprehensive score reflecting memory-related fMRI activations and deactivations as potential biomarker for neurocognitive aging. *Hum Brain Mapp.* (2021) 42:4478–96. doi: 10.1002/hbm.25559

32. Bhalla A, Wang Y, Rudd A, Wolfe CD. Differences in outcome and predictors between ischemic and intracerebral hemorrhage: the South London Stroke Register. *Stroke.* (2013) 44:2174–81. doi: 10.1161/STROKEAHA.113.001263

33. Wei JW, Heeley EL, Wang JG, Huang Y, Wong LK, Li Z, et al. Comparison of recovery patterns and prognostic indicators for ischemic and hemorrhagic stroke in China: the ChinaQUEST (QUality Evaluation of Stroke Care and Treatment) Registry study. *Stroke.* (2010) 41:1877–83. doi: 10.1161/STROKEAHA.110.586909

34. Sorensen AG, Ay H. Transient ischemic attack: definition, diagnosis, risk stratification. *Neuroimaging Clin N Am.* (2011) 21:303. doi: 10.1016/j.nic.2011.01.013

35. Abbott AL, Silvestrini M, Topakian R, Golledge J, Brunser AM, de Borst GJ, et al. Optimizing the definitions of stroke, transient ischemic attack, and infarction for research and application in clinical practice. *Front Neurol.* (2017) 8:537–7. doi: 10.3389/fneur.2017.00537

36. Gottesman RF, Hillis AE. Predictors and assessment of cognitive dysfunction resulting from ischaemic stroke. *Lancet Neurol.* (2010) 9:895–905. doi: 10.1016/S1474-4422(10)70164-2

37. Peng Y, Li Q, Qin L, He Y, Luo X, Lan Y, et al. Combination of serum neurofilament light chain levels and MRI markers to predict cognitive function in ischemic stroke. *Neurorehabil Neural Repair.* (2021) 35:247–55. doi: 10.1177/1545968321989354

38. Sagnier S, Catheline G, Munsch F, Bigourdan A, Poli M, Debruxelles S, et al. Severity of small vessel disease biomarkers reduces the magnitude of cognitive recovery after ischemic stroke. *Cerebrovasc Dis.* (2021) 50:456–63. doi: 10.1159/000513916

39. Sagnier S, Okubo G, Catheline G, Munsch F, Bigourdan A, Debruxelles S, et al. Chronic cortical cerebral microinfarcts slow down cognitive recovery after acute ischemic stroke. *Stroke.* (2019) 50:1430–36. doi: 10.1161/STROKEAHA.118.024672

40. Vicentini JE, Weiler M, Casseb RF, Almeida SR, Valler L, de Campos BM, et al. Subacute functional connectivity correlates with cognitive recovery six months after stroke. *Neuroimage Clin.* (2021) 29:102538. doi: 10.1016/j.nicl.2020.102538

41. Fruhwirth V, Enzinger C, Fandler-Höfler S, Kneihsl M, Eppinger S, Ropele S, et al. Baseline white matter hyperintensities affect the course of cognitive function after small vessel disease-related stroke: a prospective observational study. *Eur J Neurol.* (2021) 28:401–10. doi: 10.1111/ene.14593

42. Scharf AC, Gronewold J, Todica O, Moenninghoff C, Doeppner TR, de Haan B, et al. Evolution of neuropsychological deficits in first-ever isolated ischemic thalamic stroke and their association with stroke topography: a case-control study. *Stroke.* (2022) 53:1904–14. doi: 10.1161/STROKEAHA.121.037750

43. Sagnier S, Catheline G, Dilharreguy B, Munsch F, Bigourdan A, Poli M, et al. Admission brain cortical volume: an independent determinant of poststroke cognitive vulnerability. *Stroke.* (2017) 48:2113–20. doi: 10.1161/STROKEAHA.117.017646

44. Sung PS, Lee KP, Lin PY, Su HC, Yu RL, Tsai KJ, et al. Factors associated with cognitive outcomes after first-ever ischemic stroke: the impact of small vessel disease burden and neurodegeneration. *J Alzheimers Dis.* (2021) 83:569–79. doi: 10.3233/JAD-210587

45. Turunen KE, Kauranen TV, Laari SP, Mustanoja SM, Tatlisumak T, Poutiainen ET. Cognitive deficits after subcortical infarction are comparable with deficits after cortical infarction. *Eur J Neurol.* (2013) 20:286–92. doi: 10.1111/j.1468-1331.2012.03844.x

46. Zhang C, Wang Y, Li S, Pan Y, Wang M, Liao X, et al. Infarct location and cognitive change in patients after acute ischemic stroke: the ICONS study. *J Neurol Sci.* (2022) 438:120276. doi: 10.1016/j.jns.2022.120276

47. Aben HP, Biessels GJ, Weaver NA, Spikman JM, Visser-Meily JMA, de Kort PLM, et al. Extent to which network hubs are affected by ischemic stroke predicts cognitive recovery. *Stroke.* (2019) 50:2768–74. doi: 10.1161/STROKEAHA.119.025637

48. Aben HP, De Munter L, Reijmer YD, Spikman JM, Visser-Meily JMA, Biessels GJ, et al. Prediction of cognitive recovery after stroke: the value of diffusion-weighted imaging-based measures of brain connectivity. *Stroke.* (2021) 52:1983–92. doi: 10.1161/STROKEAHA.120.032033

49. Kuceyeski A, Navi BB, Kamel H, Raj A, Relkin N, Toglia J, et al. Structural connectome disruption at baseline predicts 6-months post-stroke outcome. *Hum Brain Mapp.* (2016) 37:2587–601. doi: 10.1002/hbm.23198

50. Lv H, Wang Z, Tong E, Williams LM, Zaharchuk G, Zeineh M, et al. Resting-state functional MRI: everything that nonexperts have always wanted to know. *AJNR Am J Neuroradiol.* (2018) 39:1390–9. doi: 10.3174/ajnr.A5527

51. Zhu Y, Bai L, Liang P, Kang S, Gao H, Yang H. Disrupted brain connectivity networks in acute ischemic stroke patients. *Brain Imaging Behav.* (2017) 11:444–53. doi: 10.1007/s11682-016-9525-6

52. Siegel JS, Ramsey LE, Snyder AZ, Metcalf NV, Chacko RV, Weinberger K, et al. Disruptions of network connectivity predict impairment in multiple behavioral domains after stroke. *Proc Natl Acad Sci USA.* (2016) 113:E4367–76. doi: 10.1073/pnas.1521083113

53. Tang C, Zhao Z, Chen C, Zheng X, Sun F, Zhang X, et al. Decreased functional connectivity of homotopic brain regions in chronic stroke patients: a resting state fMRI study. *PLoS ONE.* (2016) 11:e0152875. doi: 10.1371/journal.pone.0152875

54. Hohenfeld C, Werner CJ, Reetz K. Resting-state connectivity in neurodegenerative disorders: is there potential for an imaging biomarker? *Neuroimage Clin.* (2018) 18:849–70. doi: 10.1016/j.nicl.2018.03.013

55. Chan E, Altendorff S, Healy C, Werring DJ, Cipolotti L. The test accuracy of the Montreal Cognitive Assessment (MoCA) by stroke lateralisation. *J Neurol Sci.* (2017) 373:100–4. doi: 10.1016/j.jns.2016.12.028

56. Perna R, Temple J. Rehabilitation outcomes: ischemic versus hemorrhagic strokes. *Behav Neurol.* (2015) 2015:891651. doi: 10.1155/2015/891651

57. Reitz C, Bos MJ, Hofman A, Koudstaal PJ, Breteler MMB. Prestroke cognitive performance, incident stroke, and risk of dementia. *Stroke.* (2008) 39:36–41. doi: 10.1161/STROKEAHA.107.490334

58. Kandiah N, Chander RJ, Lin X, Ng A, Poh YY, Cheong CY, et al. Cognitive impairment after mild stroke: development and validation of the SIGNAL2 risk score. *J Alzheimers Dis.* (2016) 49:1169–77. doi: 10.3233/JAD-150736

59. Chander RJ, Lam BYK, Lin X, Ng AYT, Wong APL, Mok VCT, et al. Development and validation of a risk score (CHANGE) for cognitive impairment after ischemic stroke. *Sci Rep.* (2017) 7:12441. doi: 10.1038/s41598-017-12755-z

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Association of platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio with outcomes in stroke patients achieving successful recanalization by endovascular thrombectomy

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Objective: Serum inflammatory biomarkers play crucial roles in the development of acute ischemic stroke (AIS). In this study, we explored the association between inflammatory biomarkers including platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), and monocyte-to-lymphocyte ratio (MLR), and clinical outcomes in AIS patients who achieved successful recanalization.

Methods: Patients with AIS who underwent endovascular thrombectomy (EVT) and achieved a modified thrombolysis in the cerebral infarction scale of 2b or 3 were screened from a prospective cohort at our institution between January 2013 and June 2021. Data on blood parameters and other baseline characteristics were collected. The functional outcome was an unfavorable outcome defined by a modified Rankin Scale of 3–6 at the 3-month follow up. Other clinical outcomes included symptomatic intracranial hemorrhage (sICH) and 3-month mortality. Multivariable logistic regression analysis was performed to evaluate the effects of PLR, NLR, and MLR on clinical outcomes.

Results: A total of 796 patients were enrolled, of which 89 (11.2%) developed sICH, 465 (58.4%) had unfavorable outcomes at 3 months, and 168 (12.1%) died at the 3-month follow up. After adjusting for confounding variables, a higher NLR (OR, 1.076; 95% confidence interval [CI], 1.037–1.117; $p < 0.001$) and PLR (OR, 1.001; 95%CI, 1.000–1.003; $p = 0.045$) were significantly associated with unfavorable outcomes, the area under the receiver operating characteristic curve of NLR and PLR was 0.622 and 0.564, respectively. However, NLR, PLR,

and MLR were not independently associated with sICH and 3-month mortality (all adjusted $p > 0.05$).

Conclusion: Overall, our results indicate that higher PLR and NLR were independently associated with unfavorable functional outcomes in AIS patients with successful recanalization after EVT; however, the underlying mechanisms are yet to be elucidated.

KEYWORDS

acute ischemic stroke, platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, endovascular thrombectomy

Background

Previous randomized controlled trials have demonstrated that patients with acute ischemic stroke (AIS) secondary to large vessel occlusion could benefit from reperfusion therapy with endovascular thrombectomy (EVT) (1, 2). However, approximately half of patients who achieve successful recanalization of the occluded artery post-EVT have unfavorable outcomes at 90 days (3–5). The mechanisms underlying the mismatch of successful recanalization and good outcomes remain unclear (6).

The neuroinflammatory response has been increasingly recognized to be important in the pathophysiology of AIS (7). Activation of leukocytes, platelets, or other pro-inflammatory mediators plays a vital role in AIS neurological prognoses. The potential novel biomarkers of inflammation, platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), and monocyte-to-lymphocyte ratio (MLR), have recently been proposed as critical predictors of unfavorable outcomes in patients with AIS (8, 9). It has been found that NLR and PLR in AIS patients with a National Institutes of Health Stroke Scale (NIHSS) ≥6 were significantly higher than in patients with a NIHSS <6, indicating the severity of stroke was related to the value of NLR and PLR (10). In addition, higher NLR and MLR have been found to be positively correlated with stroke severity, adverse complications, and death (11, 12), while higher PLR predicted unfavorable functional outcomes with a higher modified Rankin Scale (mRS) and NIHSS scores (13). However, few studies support the predictive value of NLR, PLR, and MLR on clinical outcomes in AIS patients with successful recanalization (14). In this study we aimed to explore the association of PLR, NLR, and MLR with clinical outcomes in patients with AIS who underwent EVT and achieved successful recanalization.

Abbreviations: AIS, acute ischemic stroke; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; EVT, endovascular thrombectomy; mTICI, modified thrombolysis in cerebral infarction; mRS, modified Rankin Scale; CI, confidence interval; AUC, area under the receiver operating characteristic curve; SBP, systolic blood pressure; DBP, diastolic blood pressure; NIHSS, National Institute of Health Stroke Scale; ASPECTS, Alberta Stroke Program Early Computed Tomography Score; pc-ASPECTS, posterior circulation Alberta Stroke Program Early Computed Tomography Score; FBG, fasting blood glucose; LAA, large artery atherosclerosis; CE, cardio embolism; OTP, time interval from symptoms onset to puncture; OTR, time interval from symptoms onset to recanalization; IVT, intravenous thrombolysis; ICH, intracranial hemorrhage; sICH, symptomatic intracranial hemorrhage; SD, standard deviation; IQR, interquartile range; ROC, receiver operating characteristic.

Methods

Study design

Data for this study were obtained from a prospective cohort of consecutive patients with AIS who underwent EVT at our hospital between January 2013 and June 2021. Information on the prospective cohort, EVT procedure for AIS, and imaging evaluations have been described previously (15). This study was approved by the Ethics Committee of Xuanwu Hospital, and written informed consent was obtained from all patients or their legally authorized representatives.

Study population

The inclusion criteria for this study were as follows: (1) age ≥18 years, (2) treatment with EVT within 24 h and successful recanalization, defined as a modified Thrombolysis in Cerebral Infarction (mTICI) of 2b or 3. The exclusion criteria were as follows: (1) pre-stroke mRS > 2, (2) absence of blood parameters before EVT, and (3) lack of 3-month follow-up.

Data collection

Variables including demographics, vascular risk factors, baseline clinical assessment (admission systolic blood pressure [SBP], diastolic blood pressure [DBP], NIHSS, Alberta Stroke Program Early Computed Tomography Score [ASPECTS], or posterior circulation Alberta Stroke Program Early Computed Tomography Score [pc-ASPECTS]), laboratory tests (fasting blood glucose [FBG], NLR, PLR, MLR), lesion location, stroke etiology, treatment (general anesthesia, time interval from symptom onset to puncture [OTP], time interval from symptom onset to recanalization [OTR], intravenous thrombolysis [IVT]), intracranial hemorrhage (ICH), symptomatic intracranial hemorrhage (sICH), and clinical outcomes at 3 months were collected from the database and analyzed.

Assessment of NLR, PLR, and MLR

Blood samples were collected within 10 min of arrival at the hospital. Parameters including neutrophils, lymphocytes, monocytes, and platelets were analyzed using an automated blood cell counter (MEK-722K, NIHON, KOHEN, JAPAN). The NLR, PLR, and MLR were calculated by dividing the number of neutrophils, platelets, and monocytes by the number of lymphocytes.

Assessment of clinical outcomes

The functional outcome was an unfavorable outcome at 3 months defined as an mRS of 3–6 (16). Other clinical outcomes were sICH and mortality at the 3-month follow up. The sICH was diagnosed according to the European Cooperative Acute Stroke Study III (17) as ICH associated with any of the following conditions: (1) NIHSS score increased >4 points; (2) clinical deterioration determined by investigators, or adverse events including drowsiness and increase of hemiparesis (18, 19).

Statistical analyses

All enrolled patients were divided into favorable and unfavorable outcome groups according to their 3 months mRS score as previously described. Differences in baseline characteristics between the two groups were analyzed. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR). Analysis was performed using the *t*-test for independent samples or the Mann-Whitney *U*-test, respectively. Categorical variables were described as numbers (percentages) and analyzed using the chi-square test. Multivariable logistic regression analysis was performed to explore the effect of NLR, PLR, and MLR on 3-month functional outcomes, adjusting for age, sex, diabetes, hyperlipidemia, atrial fibrillation, admission DBP, NIHSS, ASPECTS, FBG, lesion location, general anesthesia, and sICH. Receiver operating characteristic (ROC) curves were used to test the discriminative ability of the NLR, MLR, and PLR for 3-month functional outcomes. In addition, the association between NLR, MLR, PLR, and sICH as well as mortality at 3 months was also analyzed.

Statistical analyses were performed using SPSS statistical software (version.26; IBM Corp., Armonk, NY, USA). Statistical significance was indicated by $p < 0.05$.

Results

A total of 960 patients with AIS who underwent EVT were screened, and 796 patients who fulfilled the inclusion criteria were included in the study (Figure 1). The mean age of the patients was 62.89 ± 12.22 years, and 566 (71.1%) were male. The median baseline NIHSS and ASPECTS/pc-ASPECTS scores were 16 and 9, respectively. Large-vessel occlusion in the anterior circulation was observed in 568 patients (71.4 %). A total of 270 patients (33.9%) underwent IVT before EVT. The median OTP and OTR were 380 and 458 min, respectively. sICH occurred in 89 (11.2%) patients. During the follow-up at 3 months, 465 (58.4%) patients had unfavorable functional outcomes and 168 (12.1%) patients died.

Univariate analyses of patients with favorable and unfavorable outcomes

A comparison of the detailed characteristics of the patients with favorable and unfavorable outcomes is shown in Table 1. In the univariable analysis, patients with unfavorable outcomes were much older (65.07 ± 11.93 vs. 59.83 ± 11.98, $p < 0.001$), had higher proportions of diabetes (34.0 vs. 21.1%, $p < 0.001$), hyperlipidemia (69.5 vs. 42.0%, $p < 0.001$), previous stroke (29.5 vs. 20.2%, $p = 0.003$), posterior circulation lesion (32.9 vs. 22.7%, $p = 0.002$), general anesthesia (42.2 vs. 29.9%, $p < 0.001$), ICH (44.1 vs. 22.7%, $p < 0.001$), and sICH (17.8 vs. 1.8%, $p < 0.001$). However, there was a lower proportion of current smokers (35.7 vs. 45.6%, $p = 0.005$).

The results showed that men were more likely to favorable outcomes (77.3 vs. 66.7%, $p < 0.001$). In addition, patients with unfavorable outcomes also had higher baseline SBP (150.67 ± 24.15 vs. 143.55 ± 22.58 mmHg, $p < 0.001$), higher DBP (85.27 ± 14.79 vs. 83.03 ± 14.43 mmHg, $p = 0.033$), higher NIHSS score (median, 18 vs. 13, $p < 0.001$), lower ASPECTS/pc-ASPECTS score (median, 8 vs. 9, $p = 0.006$). For laboratory tests, patients in the unfavorable outcome group had higher FBG

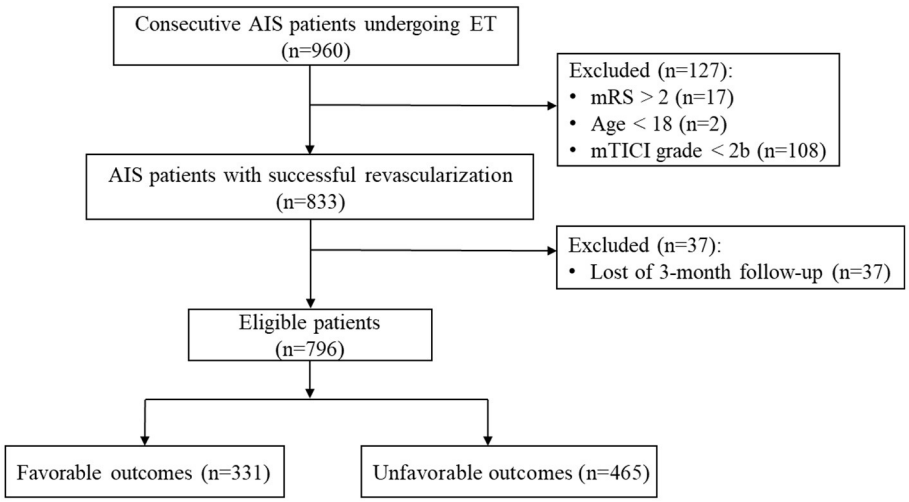

FIGURE 1
Study flow chart. AIS, acute ischemic stroke; EVT, endovascular thrombectomy; mRS, modified Rankin Scale; mTICI, modified Thrombolysis in Cerebral Infarction.

(median, 7.96 vs. 6.89 mmol/L, $p < 0.001$), NLR (median, 6.57 vs. 4.85, $p < 0.001$), PLR (median, 168.89 vs. 153.90, $p = 0.002$), and MLR (median, 0.32 vs. 0.28, $p < 0.001$).

Effect of NLR, PLR, and MLR on 3-month functional outcomes

After adjusting for potential confounders (age, sex, diabetes, hyperlipidemia, atrial fibrillation, admission DBP, NIHSS, ASPECTS, FBG, lesion location, general anesthesia, and sICH), NLR (OR, 1.076; 95% CI, 1.037–1.117; $p < 0.001$), and PLR (OR, 1.001; 95% CI, 1.000–1.003; $p = 0.045$) were found as independent predictors of unfavorable outcomes. Nevertheless, MLR was not significantly associated with unfavorable outcomes (OR, 1.052; 95% CI, 0.954–2.365; $p = 0.079$) (Table 2).

The areas under the receiver operating characteristic curves (AUC) of NLR, PLR, and MLR were 0.622 (95% CI, 0.583–0.661; $p < 0.001$), 0.564 (95% CI, 0.524–0.604; $p = 0.002$), and 0.576 (95% CI, 0.536–0.616; $p < 0.001$), respectively (Figure 2).

Effect of NLR, PLR, and MLR on sICH and 3-month mortality

We also analyzed the relationship between NLR, PLR, MLR, and sICH as well as 3-month mortality. After adjusting for potential confounders, NLR, PLR, and MLR was neither significantly associated with sICH [NLR (OR: 1.010, 95% CI: 0.980–1.042, $p = 0.500$), PLR (OR: 1.000, 95% CI: 0.998–1.001, $p = 0.601$), MLR (OR: 1.059, 95% CI: 0.630–1.778, $p = 0.830$)] nor mortality at 3 months [NLR (OR: 1.023, 95% CI: 0.997–1.049, $p = 0.082$), PLR (OR: 1.001, 95% CI: 1.000–1.002, $p = 0.268$), MLR (OR: 1.213, 95% CI: 0.847–1.737, $p = 0.292$)].

Discussion

In this study, we found that approximately half (58.4%) of the patients with successful recanalization still had unfavorable outcomes at follow-up after 3 months. Moreover, higher NLR and PLR before EVT were significantly associated with unfavorable functional outcomes in patients with AIS who achieved successful recanalization after EVT.

Currently, EVT is recognized as the most effective reperfusion therapy for the treatment of AIS secondary to the occlusion of large vessels (20). Despite EVT yielding a successful recanalization rate of >80% compared with traditional therapies, around half of the patients who achieved successful recanalization still suffer from unfavorable functional outcomes (3), as was observed in this study. Possible causes include subsequent secondary brain injury from cerebral edema (CED), hemorrhagic transformation, and infarct growth due to impaired microvascular reperfusion mediating early neurological deterioration and 3-month unfavorable functional outcomes (21, 22). The inflammatory response plays an essential role in the pathophysiology and predicting the prognosis of ischemia or hemorrhagic stroke (23, 24). In acute ischemic stroke, the inflammatory response may worsen the CED, ICH, and delay cerebral ischemia thereby leading to poor prognosis (8, 9, 21).

In this study, we found that NLR and PLR before EVT were significantly associated with unfavorable functional

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TABLE 1 Characteristics of patients with favorable and unfavorable outcomes.

Factors	Total number ($n = 796$)	Favorable outcomes ($n = 331$)	Unfavorable outcomes ($n = 465$)	*P*-value
Demographics				
Age, y, mean ± SD	62.89 ± 12.22	59.83 ± 11.98	65.07 ± 11.93	<0.001*
Male, *n* (%)	566 (71.1%)	256 (77.3%)	310 (66.7%)	<0.001*
Vascular risk factors				
Hypertension, *n* (%)	558 (70.1%)	220 (66.5%)	338 (72.7%)	0.059
Diabetes, *n* (%)	228 (28.6%)	70 (21.1%)	158 (34.0%)	<0.001*
Hyperlipidemia, *n* (%)	462 (58.0%)	139 (42.0%)	323 (69.5%)	<0.001*
Current smoking, *n* (%)	317 (39.8%)	151 (45.6%)	166 (35.7%)	0.005*
Atrial fibrillation, *n* (%)	259 (32.5%)	95 (28.7%)	164 (35.3%)	0.051
Previous stroke, *n* (%)	204 (25.6%)	67 (20.2%)	137 (29.5%)	0.003*
Baseline clinical assessment				
Admission SBP (mmHg), mean ± SD	146 ± 33	143.55 ± 22.58	150.67 ± 24.15	<0.001*
Admission DBP (mmHg), mean ± SD	83 ± 15	83.03 ± 14.43	85.27 ± 14.79	0.033*
Admission NIHSS, median (IQR)	16 (12–21)	13 (10–17)	18 (14–26)	<0.001*
Admission ASPECTS/pc-ASPECTS, median (IQR)	9 (7–10)	9 (8–10)	8 (7–10)	0.006*
Laboratory test				
FBG (mmol/L), median (IQR)	7.37 (6.15–9.49)	6.89 (5.71–8.25)	7.96 (6.56–10.48)	<0.001*
NLR, median (IQR)	5.87 (3.39–9.82)	4.85 (2.79–7.76)	6.57 (4.00–11.21)	<0.001*
PLR, median (IQR)	161.79 (108.46–241.67)	153.90 (105.04–218.23)	168.89 (114.38–254.48)	0.002*
MLR, median (IQR)	0.30 (0.22–0.42)	0.28 (0.21–0.37)	0.32 (0.22–0.47)	<0.001*
Lesion location				0.002*
Anterior circulation, *n* (%)	568 (71.4%)	256 (77.3%)	312 (67.1%)	
Posterior circulation, *n* (%)	228 (28.6%)	75 (22.7%)	153 (32.9%)	
Stroke etiology				
LAA, *n* (%)	479 (60.2%)	210 (63.4%)	269 (57.8%)	0.088
CE, *n* (%)	281 (35.3%)	103 (31.1%)	178 (38.3%)	
Others, *n* (%)	36 (4.5%)	18 (5.4%)	18 (3.9%)	
Treatment				
General anesthesia, *n* (%)	295 (37.1%)	99 (29.9%)	196 (42.2%)	<0.001*
OTP (min), median (IQR)	380 (284–528)	389 (286–540)	375 (282–520)	0.775
OTR (min), median (IQR)	458 (358–600)	450 (360–597)	465 (353–602)	0.507
IVT, *n* (%)	270 (33.9%)	112 (33.8%)	158 (34.0%)	0.967
Clinical outcomes				
ICH, *n* (%)	280 (35.2%)	75 (22.7%)	205 (44.1%)	<0.001*
sICH, *n* (%)	89 (11.2%)	6 (1.8%)	83 (17.8%)	<0.001*

*$P < 0.05$. SBP, systolic blood pressure; DBP, diastolic blood pressure; NIHSS, National Institute of Health Stroke Scale; ASPECTS, Alberta Stroke Program Early Computed Tomography Score; pc-ASPECTS, posterior circulation Alberta Stroke Program Early Computed Tomography Score; FBG, fast blood glucose; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; LAA, large artery atherosclerosis; CE, cardio embolism; OTP, time interval from symptoms onset to puncture; OTR, time interval from symptoms onset to recanalization; IVT, intravenous thrombolysis; ICH, intracranial hemorrhage; sICH, symptomatic intracranial hemorrhage.

TABLE 2 Multivariable analysis of NLR, PLR, MLR in predicting clinical outcomes.

Variable	β	SE	Adjusted OR	Adjusted 95% CI		*P*-value
Unfavorable outcomes at 3 months						
NLR[@]	0.074	0.019	1.076	1.037	1.117	<0.001*
PLR[@]	0.001	0.001	1.001	1.000	1.003	0.045*
MLR[@]	0.407	0.232	1.502	0.954	2.365	0.079
sICH						
NLR[&]	0.010	0.015	1.010	0.980	1.042	0.500
PLR[&]	0.001	0.001	1.000	0.998	1.001	0.601
MLR[&]	0.057	0.265	1.059	0.630	1.778	0.830
Mortality at 3 months						
NLR[@]	0.022	0.013	1.023	0.997	1.049	0.082
PLR[@]	0.001	0.001	1.001	1.000	1.002	0.268
MLR[@]	0.193	0.183	1.213	0.847	1.737	0.292

$*P < 0.05$. [@]Adjusting for age, sex, diabetes, hyperlipidemia, atrial fibrillation, admission DBP, NIHSS, ASPECTS/pc-ASPECTS, FBG, lesion location, general anesthesia, and sICH. [&]Adjusting for age, admission SBP, FBG, lesion site, TOAST, and IVT.

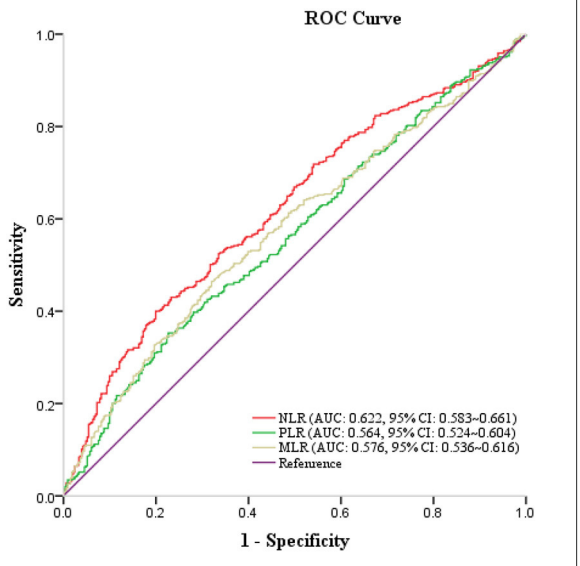

FIGURE 2
Receiver operating characteristic curve of NLR, PLR, MLR in predicting unfavorable outcomes at 3 months.

outcomes in patients with AIS after successful recanalization with EVT. Firstly, to determine whether these inflammatory indexes increased the prognosis of poor function by increasing sICH, we analyzed the relationship between PLR, NLR, MLR, and sICH. However, no significant association between inflammatory indexes and sICH was found. Secondly, recent imaging studies have shown that the no-reflow phenomenon, which indicates incomplete microvascular reperfusion of the tissue despite successful macrovascular revascularization, provides insights into the underlying mechanisms of this unfavorable prognosis of successfully recanalized stroke (25). However, advanced perfusion imaging for the evaluation of microvascular tissue reperfusion is too time-consuming for timely treatment, thus difficult to implement in the clinic. In patients with acute myocardial infarction treated with percutaneous coronary intervention, composite inflammatory biomarkers have been shown to be strong predictors of both the no-reflow phenomenon and unfavorable functional outcomes (26). We hypothesized that NLR, PLR, and MLR may mediate neurological outcomes through microvascular no-reflow mechanism in patients with AIS treated with EVT. Theoretically, ischemic brain tissues can release various cytokines and chemokines to guide the proliferation and migration of peripheral leukocytes (27). Elevated levels of peripheral leukocytes transmigrating and infiltrating to the ischemic tissues may cause thrombosis, aggravate endothelial edema, and lead to microvascular occlusion, thereby participating in the microvascular no-reflow phenomenon (25). Therefore, composite inflammation indexes such as NLR, PLR, and MLR, are easy-to-acquire biomarkers, which mayserve as potential predictors of the no-reflow phenomenon, and could be associated with unfavorable functional outcomes in successfully recanalized patients with AIS (28, 29). In the present study, NLR and PLR were found to be independently correlated with functional outcome; however, MLR was not significantly associated with functional outcome. Further investigations are needed to explore the relationship between inflammatory

indices, the no-reflow phenomenon, and functional outcomes in human ischemic stroke.

In addition, inflammatory biomarkers have been found to predict functional outcomes in patients with intracerebral (23) and subarachnoid hemorrhage (24). Therefore, in either ischemic or hemorrhagic stroke, inflammatory biomarkers may share common mechanisms in mediating secondary brain injury following acute vascular events. Moreover, anti-inflammatory therapy targeting their common pathways may help improve the neurological prognosis of patients with acute stroke (27).

This study had some limitations. Firstly, the cohort included subjects from only one region of China, which would have introduced selection bias. Therefore, further exploration using larger multicenter prospective studies is warranted to substantiate our findings. Second, covariates related to AIS could not be completely collected due to data limitations. Thirdly, the area under the ROC curve values of NLR, PLR, and MLR for outcome prediction were relatively low and need further exploration. Finally, only preoperative inflammatory indicators were evaluated without post-operative indicators; therefore, post-operative inflammatory indicators will need to be evaluated in future studies.

Conclusion

This study showed that NLR and PLR before EVT were significantly associated with 3-month functional outcomes in patients with AIS who achieved successful recanalization after EVT. Further studies are needed to confirm these results and explore the underlying mechanisms.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Xuanwu Hospital. Written informed consent to participate in this study was provided by the patient/participants or patient/participants' legal guardian/next of kin.

Author contributions

JM and WG conceived of the study idea, collected and analyzed the data, and drafted the manuscript. JX, LW, and WZ participated in the data collection and analysis. XJ, SL, CR, CW, CL, JC, JD, QM, and HS participated in the coordination of the study. WZ and XJ helped to interpret the data and modify the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Zi W, Qiu Z, Li F, Sang H, Wu D, Luo W, et al. Effect of endovascular treatment alone vs intravenous alteplase plus endovascular treatment on functional independence in patients with acute ischemic stroke: the DEVT randomized clinical trial. *JAMA*. (2021) 325:234–43. doi: 10.1161/str.52.suppl_1.44

2. Leng T, Xiong ZG. Treatment for ischemic stroke: from thrombolysis to thrombectomy and remaining challenges. *Brain Circ.* (2019) 5:8–11. doi: 10.4103/bc.bc_36_18

3. Zhou T, Yi T, Li T, Zhu L, Li Y, Li Z, et al. Predictors of futile recanalization in patients undergoing endovascular treatment in the DIRECT-MT trial. *J Neurointerv Surg.* (2021). doi: 10.1136/neurintsurg-2021-017765

4. Lattanzi S, Norata D, Divani AA, Di Napoli M, Broggi S, Rocchi C, et al. Systemic inflammatory response index and futile recanalization in patients with ischemic stroke undergoing endovascular treatment. *Brain Sci.* (2021) 11:1164. doi: 10.3390/brainsci11091164

5. Deng G, Xiao J, Yu H, Chen M, Shang K, Qin C, et al. Predictors of futile recanalization after endovascular treatment in acute ischemic stroke: a meta-analysis. *J Neurointerv Surg.* (2021) 14:881–5. doi: 10.1136/neurintsurg-2021-017963

6. Kitano T, Todo K, Yoshimura S, Uchida K, Yamagami H, Sakai N, et al. Futile complete recanalization: patients characteristics and its time course. *Sci Rep.* (2020) 10:4973. doi: 10.1038/s41598-020-61748-y

7. Belayev L, Obenaus A, Mukherjee PK, Knott EJ, Khoutorova L, Reid MM, et al. Blocking pro-inflammatory platelet-activating factor receptors and activating cell survival pathways: a novel therapeutic strategy in experimental ischemic stroke. *Brain Circ.* (2020) 6:260–8. doi: 10.4103/bc.bc_36_20

8. Lux D, Alakbarzade V, Bridge L, Clark CN, Clarke B, Zhang L, et al. The association of neutrophil-lymphocyte ratio and lymphocyte-monocyte ratio with 3-month clinical outcome after mechanical thrombectomy following stroke. *J Neuroinflammation.* (2020) 17:60. doi: 10.1186/s12974-020-01739-y

9. Ozgen E, Guzel M, Akpinar CK, Yucel M, Demir MT, Baydin A. The relationship between neutrophil/lymphocyte, monocyte/ /lymphocyte, platelet/lymphocyte ratios and clinical outcomes after ninety days in patients who were diagnosed as having acute ischemic stroke in the emergency room and underwent a mechanical thro. *Bratisl Lek Listy.* (2020) 121:634–9. doi: 10.4149/BLL_2020_102

10. Sung PH, Chen KH, Lin HS, Chu CH, Chiang JY, Yip HK. The correlation between severity of neurological impairment and left ventricular function in patients after acute ischemic stroke. *J Clin Med.* (2019) 8:190. doi: 10.3390/jcm8020190

11. Kömürcü HF, Gözke E, Ak PD, Aslan IK, Salt I, Bi ÇIÖ. Changes in neutrophil, lymphocyte, platelet ratios and their relationship with NIHSS after rtPA and/or thrombectomy in ischemic stroke. *J Stroke Cerebrovascular Dis.* (2020) 29:105004. doi: 10.1016/j.jstrokecerebrovasdis.2020.105004

12. Cheng H-R, Song J-Y, Zhang Y-N, Chen Y-B, Lin G-Q, Huang G-Q, et al. High monocyte-to-lymphocyte ratio is associated with stroke-associated pneumonia. *Front Neurol.* (2020) 11:575809. doi: 10.3389/fneur.2020.575809

13. Xu J-H, He X-W, Li Q, Liu J-R, Zhuang M-T, Huang F-F, et al. Higher platelet-to-lymphocyte ratio is associated with worse outcomes after intravenous thrombolysis in acute ischaemic stroke. *Front Neurol.* (2019) 10:1192. doi: 10.3389/fneur.2019.01192

14. Li X, Wu F, Jiang C, Feng X, Wang R, Song Z, et al. Novel peripheral blood cell ratios: Effective 3-month post-mechanical thrombectomy prognostic biomarkers for acute ischemic stroke patients. *J Clin Neurosci.* (2021) 89:56–64. doi: 10.1016/j.jocn.2021.04.013

15. Zhao W, Che R, Shang S, Wu C, Li C, Wu L, et al. Low-dose tirofiban improves functional outcome in acute ischemic stroke patients treated with endovascular thrombectomy. *Stroke.* (2017) 48:3289–94. doi: 10.1161/STROKEAHA.117.019193

16. Ha SH, Kim BJ Ryu JC, Bae JH, Kim JS. Basilar artery tortuosity may be associated with early neurological deterioration in patients with pontine infarction. *Cerebrovas Dis.* (2022) 51:594–9. doi: 10.1159/000522142

17. Neuberger U, Möhlenbruch MA, Herweh C, Ulfert C, Bendszus M, Pfaff J. Classification of bleeding events: comparison of ECASS III (European Cooperative Acute Stroke Study) and the new Heidelberg bleeding classification. *Stroke.* (2017) 48:1983–5. doi: 10.1161/STROKEAHA.117.016735

18. Zhang X, Xie Y, Wang H, Yang D, Jiang T, Yuan K, et al. Symptomatic intracranial hemorrhage after mechanical thrombectomy in Chinese ischemic stroke patients: the ASIAN score. *Stroke.* (2020) 51:2690–6. doi: 10.1161/STROKEAHA.120.030173

19. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). *Second Eur Aust Acute Stroke Study Invest Lancet.* (1998) 352:1245–51. doi: 10.1016/S0140-6736(98)08020-9

20. Warner JJ, Harrington RA, Sacco RL, Elkind M. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke. *Stroke.* (2019) 50:3331–2. doi: 10.1161/STROKEAHA.119.027708

21. Ferro D, Matias M, Neto J, Dias R, Moreira G, Petersen N, et al. Neutrophil-to-lymphocyte ratio predicts cerebral edema and clinical worsening early after reperfusion therapy in stroke. *Stroke.* (2021) 52:859–67. doi: 10.1161/STROKEAHA.120.032130

22. Gong P, Liu Y, Gong Y, Chen G, Zhang X, Wang S, et al. The association of neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, and lymphocyte to monocyte ratio with post-thrombolysis early neurological outcomes in patients with acute ischemic stroke. *J Neuroinflammation.* (2021) 18:51. doi: 10.1186/s12974-021-02090-6

23. Lattanzi S, Cagnetti C, Rinaldi C, Angelocola S, Provinciali L, Silvestrini M. Neutrophil-to-lymphocyte ratio improves outcome prediction of acute intracerebral hemorrhage. *J Neurol Sci.* (2018) 387:98–102. doi: 10.1016/j.jns.2018.01.038

24. Chen L, Pandey S, Shen R, Xu Y, Zhang Q. Increased systemic immune-inflammation index is associated with delayed cerebral ischemia in aneurysmal subarachnoid hemorrhage patients. *Front Neurol.* (2021) 12:745175. doi: 10.3389/fneur.2021.745175

25. Schiphorst AT, Charron S, Hassen WB, Provost C, Naggara O, Benzakoun J, et al. Tissue no-reflow despite full recanalization following thrombectomy for anterior circulation stroke with proximal occlusion: a clinical study. *J Cereb Blood Flow Metab.* (2021) 41:253–66. doi: 10.1177/0271678X20954929

26. Esenboa K, Kurtul A, Yamantürk Y, Tan TS, Tutar DE. Systemic immune-inflammation index predicts no-reflow phenomenon after primary percutaneous coronary intervention. *Acta Cardiologica.* (2021) 77:1–8. doi: 10.1080/00015385.2021.1884786

27. Maida CD, Norrito RL, Daidone M, Tuttolomondo A, Pinto A. Neuroinflammatory mechanisms in ischemic stroke: focus on cardioembolic stroke, background, and therapeutic approaches. *Int J Mol Sci.* (2020) 21:6454. doi: 10.3390/ijms21186454

28. Chen C, Gu L, Chen L, Hu W, Feng X, Qiu F, et al. Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio as potential predictors of prognosis in acute ischemic stroke. *Front Neurol.* (2020) 11:525621. doi: 10.3389/fneur.2020.525621

29. Lee S-H, Jang MU, Kim Y, Park SY, Kim C, Kim YJ, et al. The neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios predict reperfusion and prognosis after endovascular treatment of acute ischemic stroke. *J Pers Med.* (2021) 11:696. doi: 10.3390/jpm11080696

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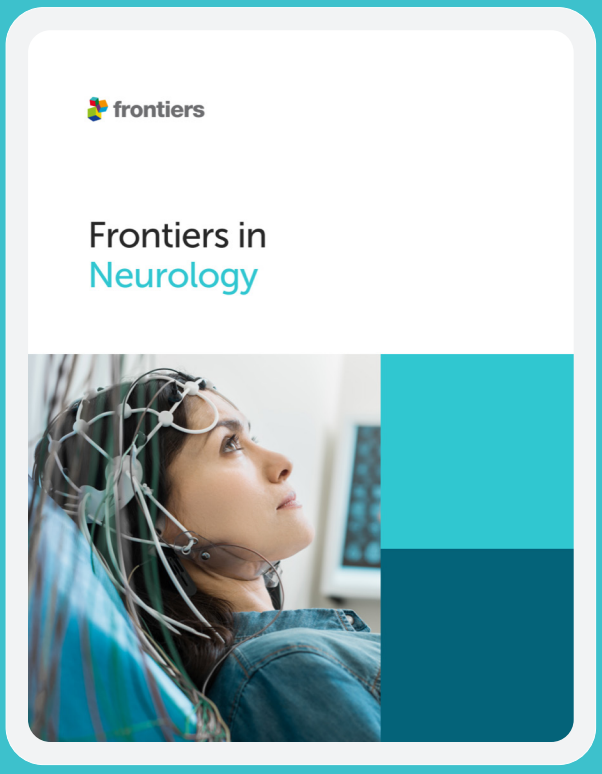

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