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Outcomes of stroke: Prediction and improvement

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Outcomes of stroke: Prediction and improvement

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Editorial: Outcomes of stroke: prediction and improvement

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Editorial on the Research Topic
Outcomes of stroke: prediction and improvement

Stroke remains the second-leading cause of death and the third-leading cause of disability worldwide (1). Of the two types of strokes, ischemic and hemorrhagic, the former accounts for more than 80% of strokes, while the latter is more disabling and fatal. Despite ongoing efforts to explore effective management and prevention strategies, the annual number and burden of stroke continue to grow worldwide, especially in low- and middle-income countries (2).

More and more attention has been paid to strategies that result in favorable outcomes for all types of strokes. Multiple therapies, including thrombolysis, mechanical thrombectomy, and surgical intervention, are used in clinical practice (3, 4). Despite the improvement in predictive ability, the choice of these therapies may be influenced by multiple factors, namely the time windows and may possibly bring out unpredictable adverse effects. Therefore, it will be more helpful if a predictor can rapidly identify the risks that affect the outcome and treatment effectiveness after stroke (5, 6). The management strategies chosen according to the effective prediction may provide more clinical benefits.

This Research Topic, entitled "Outcomes of Stroke: Prediction and Improvement," aims to investigate the predictors and effective management strategies that can predict and improve stroke outcomes. It consists of 37 articles, and a brief description of the research findings follows.

As ischemic stroke accounts for more than 80% of strokes, most of the articles deal with the predictors and therapeutic strategies of ischemic stroke. Some of the works explore useful predictors of the outcomes of ischemic strokes. Liu et al. demonstrated that the ratio of vascular endothelial growth factor to CBP/P300-interacting transactivator with Glu/Asp-rich C-terminal domain 2 (VEGF/CITED2) from peripheral blood mononuclear cells is an independent protective factor and has a potential predictive value in the collateral circulation of acute ischemic stroke (AIS) evaluated by diffusion-weighted imaging (DWI)-Alberta Stroke Program Early CT Score (ASPECTS). Liu et al. showed that the ratio of serum uric acid to serum creatinine (SUA/SCr) is negatively associated with the risk of early neurological deterioration (END) within 1 week in patients with branch atheromatous disease stroke. Another study also investigated biomarkers of END. Wang Z. et al. detected plasma neurofilament light chain (pNFL) via a novel ultrasensitive single-molecule array and found that pNFL is a promising biomarker of END in minor stroke with large vessel occlusion.

Meanwhile, Ag Lamat et al. illustrated how electroencephalogram (EEG) abnormalities are associated with stroke type and imaging characteristics. Additionally, NIHSS score and anterior circulation stroke can be considered predictors of focal EEG slowing.

Recurrent ischemic stroke (RIS) is associated with increased mortality and a poor outcome. Chung et al. investigated gender differences and risk factors for RIS. In their study, they found that hypertension and dyslipidemia were significant risk factors in both genders. Risk factors that differed between genders are smoking and alcohol consumption in men and diabetes in women. Another study by Wang H. et al. from Fudan University developed a radiomics-based DWI prediction model that performed well in terms of predicting 1-year RIS. Another prediction model for RIS was developed by Elhefnawy et al. from real-world data. In this model, incorporating time into predicting the risk of RIS can positively contribute to predicting the prognosis of RIS. The risk of RIS changes with time after the index ischemic stroke. In addition to concomitant diseases, secondary prevention time also plays a vital role in predicting the risk of RIS in the population. The COVID-19 pandemic has had a great impact on the treatment of AIS. Gu et al. demonstrated that the pandemic exacerbates certain time delays and plays a significant role in early adverse outcomes in patients with AIS. Atrial fibrillation (AF) is a common cardiac arrhythmia that is associated with an increased risk of ischemic stroke. Lu M. et al. performed a meta-analysis showing that the neutrophil-to-lymphocyte ratio (NLR), as an important inflammatory indicator, is associated with a higher risk of stroke in AF patients. The incidence of stroke in AF patients with NLR $\geq$3 was 1.4 times higher than in those with NLR $<$3. A comparative analysis performed by Bem Junior et al. showed that decompressive craniectomy is currently the most effective measure to control refractory ICH in cases of malignant ischemic stroke. In addition, Kosugi et al. conducted a study to create a cortical infarction using photothrombosis over the motor cortex of non-human primates to establish a reproducible deficit in the reaching and grasping tasks. This research suggests that different recovery speeds for each movement may be influenced by the extent to which cortical control is required to properly execute each movement.

Intravenous thrombolysis using recombinant tissue plasminogen activator (r-tPA) is effective for the treatment of AIS, although uncertainties remain about the balance of benefits and risks in some circumstances. In their study, Yuan et al. demonstrated that the non-standard dose of r-tPA (0.6 mg/kg $\leq$ dose $<$ 0.9 mg/kg) does not differ in safety and effectiveness compared with the standard dose (0.9 mg/kg). The findings suggest that, according to current evidence and guidelines, the standard dose should be regarded as the first choice, while the above non-standard dose could be an alternative option in the actual diagnosis and treatment process, considering the patient's clinical profile and financial condition. Meanwhile, Xu et al. evaluated the safety and efficacy of low-dose alteplase (0.55–0.65 mg/kg) compared with standard-dose (0.85–0.95 mg/kg) in Chinese patients with AIS using a real-world registry. The results indicate that low-dose alteplase has a significantly higher rate of death or disability at discharge without reducing the risk of symptomatic intracranial hemorrhage. The prediction of outcome after intravenous thrombolytic therapy was also included in our Research Topic. Jiang et al. showed that the difference in red blood cell distribution width from before to after rt-PA thrombolysis is an independent predictor of neurological outcome at 2 years after thrombolysis in patients with AIS. Fekete et al.'s work implies that older age, higher NIHSS, large vessel occlusion, and intra-arterial thrombolysis may correlate with intracranial hemorrhage after thrombolytic therapy, and such patients always have unfavorable outcomes in a prospective single-center study. Norata et al. demonstrated that the liver fibrosis-4 score is associated with poor 3-month outcome and symptomatic intracranial hemorrhage in AIS patients undergoing intravenous thrombolysis.

Endovascular thrombectomy has been shown to be effective in the treatment of acute ischemic stroke in patients with large vessel occlusion (7). Prediction of prognosis, especially in patients with successful revascularization, is critical. Shao et al. demonstrated that acidosis, including decreased HCO_3^- and pH levels detected by arterial blood gas (ABG) testing, is associated with clinical outcomes after endovascular therapy. Post-thrombectomy intracranial hemorrhage (PTIH) is a serious complication of AIS following mechanical thrombectomy. Rossi et al. analyzed 122 thrombi from 80 AIS patients and detected S100b expression by immunohistochemistry. The results show that S100b is co-localized with neutrophils, macrophages, and T-lymphocytes in clots and that higher S100b expression is associated with PTIH. In the study by Wagner et al., 714 AIS patients received intravenous thrombolysis followed by endovascular thrombectomy. Their results suggest that shorter intervals between intravenous thrombolysis and endovascular thrombectomy are associated with better 3-month functional outcomes. To reduce in-hospital workflow time, Zhou T. et al. developed a one-stop stroke management (OSSM) platform, and in their study, the OSSM transfer model significantly reduced the in-hospital delay in AIS patients compared to the traditional transfer model. Acute basilar artery occlusion (BAO) is one of the most fatal diseases, with a high risk of mortality and disability. Luo et al. aimed to determine the most effective rescue measure for acute BAO after the failure of mechanical thrombectomy. They found that balloon angioplasty, Wingspan stenting, and Apollo stenting rather than Solitaire stenting could be considered successful and safe rescue options. Lu Y. et al. investigated the optimal type of anesthesia management for acute vertebrobasilar artery occlusion (VBAO). Their findings reveal that similar effectiveness and safety were observed between general anesthesia (GA) and non-GA during endovascular treatment for VBAO, and GA may provide better successful reperfusion for a worse presenting GCS score ($\leq$8). Another study by Lee et al. also examined the effects of anesthesia management for endovascular thrombectomy on outcomes through a meta-analysis of randomized clinical trials and trial sequence analysis. The conclusion is that patients with acute anterior circulation ischemic stroke who receive GA are associated with a higher rate of successful recanalization and a better 3-month neurological outcome compared to those who receive conscious sedation. A systematic review and meta-analysis conducted by Duan et al. aims to evaluate the current evidence on the feasibility, efficacy, and safety of endovascular thrombectomy in patients with active cancer. They found that such patients are likely to have an unfavorable outcome and that

active cancer may increase the risk of mortality after endovascular thrombectomy. Another meta-analysis by Wu et al. revealed that the above-mentioned inflammatory marker NLR tested at admission and post-treatment can be used as a cost-effective and readily available biomarker to predict 3-month poor functional outcome, symptomatic intracerebral hemorrhage (sICH), and 3-month mortality in AIS patients undergoing reperfusion therapy. Hemorrhagic transformation is one of the most devastating complications of reperfusion therapy in AIS patients. A meta-analysis by Sun et al. identified several predictors of hemorrhagic transformation, including atrial fibrillation, a higher NIHSS score, older age, higher serum glucose levels, the number of thrombectomy procedures, and lower ASPECTS.

ICH accounts for ∼10%−20% of all stroke subtypes with high mortality, and the survivors always have a high degree of residual disability. Hematoma expansion (HE) is often observed in the early stages of ICH, which independently predicts poor outcomes, namely death and disability (8). Qin et al. investigated the association between leukocyte subpopulations and HE according to two definitions (Definition 1: volume increase ≥6 ml or 33%; Definition 2: volume increase ≥12.5 ml or 33%). The findings revealed that a higher monocyte count is associated with a higher risk of HE regardless of the two definitions, whereas an increased neutrophil count is associated with a decreased risk of HE according to Definition 1. Shi et al. retrospectively analyzed 105 patients with severe basal ganglia ICH and demonstrated that NLR was a better predictor of 30-day mortality than other risk factors. In addition, a systematic review and meta-analysis by Li J. et al. showed that higher low-density lipoprotein cholesterol (LDL-C) levels may reduce the risk of mortality in ICH patients. Elevated LDL-C levels are only inversely associated with 3-month mortality risks, not in-hospital mortality risks in these patients. The study by Coleman et al. enrolled 2,449 patients from the ERICH trial to reveal that the SAVED2 score, which is composed of several common predictors, is associated with unfavorable outcomes in patients with ICH. Finally, Li Z. et al. presented a review summarizing recent advances in therapeutic strategies and directions for ICH and discussing the barriers and issues that need to be overcome to improve the prognosis of ICH. Subarachnoid hemorrhage (SAH) is another devastating type of hemorrhagic stroke with high mortality and disability rates. Neuroinflammation is an important mechanism of injury after ICH (9). Zhang et al. included 3,173 aneurysmal SAH (aSAH) patients and compared the predictive effects of multiple inflammatory markers. The results indicated that one of these inflammatory markers, the neutrophil-to-albumin ratio (NAR) could improve the SAFIRE and SAHIT models for 3-month mortality. Furthermore, Ji et al. demonstrated that increased serum anion gap (AG) is an independent, significant, and robust predictor of all-cause in-hospital mortality. Venous thrombosis is another rare form of stroke. Zhou et al. contributed a review that provided a reference for a comprehensive understanding of venous thrombosis and a scientific understanding of various pathophysiological mechanisms and clinical features related to this condition.

Several articles in our Research Topic concern both ischemic and hemorrhagic stroke. Zhao et al. analyzed the data of 1,601 stroke patients and found that inflammatory biomarkers such as NLR, NAR, and the ratio of red cell distribution width to albumin (RA) were able to predict 30-day mortality in hemorrhagic stroke but not in ischemic stroke. Matuja et al. investigated the predictors of 30-day mortality in patients with stroke in northwestern Tanzania. Their findings suggested that NIHSS and mRS on admission, aspiration pneumonia, and electrocardiogram abnormalities were associated with 30-day mortality. Importantly, Zhu et al. used several highly interpretable machine learning models to predict stroke prognosis with the highest accuracy to date and to identify heterogeneous treatment effects of warfarin and human albumin in stroke patients.

Overall, the current Research Topic of 37 articles provides a comprehensive overview of the latest advances in this field. It includes neuroimaging and body fluid markers that predict stroke outcomes. Moreover, the articles also include the latest developments in medical and surgical therapies that improve stroke outcomes. These findings may provide new insights for clinical practice. Further efforts are needed to investigate more beneficial therapeutic strategies, perhaps with the help of effective predictors.

Author contributions

HC contributed to the drafting and writing of the editorial. HC, LL, BQ, and YT were responsible for proofreading, editing, and reviewing the manuscript. All authors approved the version submitted 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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Association between leukocyte subpopulations and hematoma expansion after spontaneous intracerebral hemorrhage: A retrospective cohort study

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Aims: To verify the association between leukocyte subpopulations and hematoma expansion (HE) determined by two definitions in Chinese individuals who experienced spontaneous intracerebral hemorrhage.

Methods: We enrolled 471 patients. The 1/2ABC formula was used to gauge hematoma volume. The outcome was whether HE appeared within 72 h. We used Definition 1 (volume increase ≥6 mL or 33%) and Definition 2 (volume increase ≥12.5 mL or 33%) to define HE, respectively. Binary logistic regression analysis was used to assess the association between leukocyte subpopulations and HE. For statistically significant leukocyte subpopulations, we also performed subgroup analyses to assess differences between subgroups.

Results: Among 471 patients, 131 (27.81%) and 116 (24.63%) patients experienced HE based on Definition 1 and Definition 2, respectively. After adjusting for confounding factors, elevated monocyte count was associated with a higher risk of HE-Definition 1 [adjusted odds ratio (aOR) 2.45, 95% confidence interval (CI) 1.02–5.88, $P = 0.0450$] and HE-Definition 2 (aOR 2.54, 95% CI 1.04–6.20, $P = 0.0399$). Additionally, we compared the results before and after adjusting for coagulation parameters. Monocyte count was significantly correlated with HE only after adjusting for coagulation parameters. Increased neutrophil count was associated with a lower risk of HE-Definition 1 (aOR 0.91, 95% CI 0.84–1.00, $P = 0.0463$). No correlations were observed between lymphocyte and leukocyte counts and HE ($P > 0.05$), and no subgroup interactions were observed (interaction $P > 0.05$).

Conclusion: A higher monocyte count is associated with a higher HE risk regardless of the two definitions, after excluding the influence of the coagulation parameters, which facilitates risk stratification. Moreover, an increased neutrophil count is associated with a decreased risk of HE in the context of HE-Definition 1, which reflects the importance of standardizing the definition of HE.

KEYWORDS

hematoma expansion, intracerebral hemorrhage, leukocyte, monocyte, neutrophil

Introduction

The number of deaths caused by stroke continues to increase globally, particularly in developing countries. Intracerebral hemorrhage (ICH) accounts for 10–15% of stroke cases and has the highest mortality rate (1). Accordingly, finding appropriate treatments for ICH is imperative. Early hematoma expansion (HE) after ICH is not only a potentially modifiable predictor of patient outcomes but also a promising therapeutic target (2–5). However, uncertainty regarding the risk factors for HE and the lack of specific predictors have led to the high disability and mortality related to ICH.

ICH is the rupture of diseased blood vessels and is characterized by the flow of blood into the brain parenchyma (5, 6). Once blood enters this region, the inflammatory response is activated (7). Within minutes of ICH onset, microglial cells are activated, marking the start of a potent inflammatory cascade (7, 8). Activated microglial cells initially exert their neuroprotective effects; however, hyperactive microglial cells release various cytokines and chemokines that promote the infiltration of peripheral leukocytes into the brain (7). Leukocytes infiltrating the hemorrhagic brain can produce reactive oxygen species, release pro-inflammatory mediators, and promote the breakdown of the blood–brain barrier (BBB), thereby aggravating ICH brain injury (7, 8). Previous studies reported that different leukocyte subpopulations have various effects on HE after ICH (9–11). However, to date, systematic clinical studies on the association of leukocytes and their subpopulations with HE among the Chinese population are lacking. Accordingly, this study aimed to retrospectively observe the association between leukocytes and their subpopulations with HE after spontaneous ICH.

Methods

Participant selection

We consecutively enrolled patients diagnosed with ICH using non-contrast computed tomography (NCCT) at Shenzhen Longhua District Central Hospital from May 2015 to November 2021. The inclusion criteria were as follows: (1) a diagnosis of spontaneous ICH detected by NCCT, (2) age at least 18 years old, and (3) leukocyte count obtained within 24 h of admission. In contrast, the exclusion criteria were as follows: (1) all secondary cerebral hemorrhage, including brain tumors, arteriovenous malformations, venous thrombosis, subarachnoid hemorrhage, thrombolysis-induced hemorrhage, and all trauma-related intracranial hemorrhage; (2) baseline NCCT and follow-up NCCT image artifacts significantly affecting volume measurement; and (3) hematoma surgery (including craniotomy and ventricular drainage) before follow-up NCCT.

We initially enrolled 558 patients with spontaneous ICH. Of these, 87 patients were excluded according to the following criteria: 22 patients whose follow-up NCCT images were missing, 33 who had undergone surgery before follow-up NCCT, 18 who exhibited leukocyte deficiency on admission, five who manifested vascular malformations, and nine whose images presented image artifacts. Ultimately, 471 patients were included (Figure 1).

This study was approved by the Ethical Review Committee of Shenzhen Longhua District Central Hospital (approval number: 2022-012-01). The need for informed consent was waived due to the study's retrospective design.

Clinical data acquisition

We collected basic patient information by retrospectively reviewing outpatient medical records, including age, sex, systolic blood pressure at admission, and time from onset to admission. We combined the outpatients' medical records with those of inpatients to determine the history of hypertension, diabetes, hyperlipidemia, mannitol use, and the Glasgow Coma Scale score.

Laboratory indicator collection

All laboratory indicators were assessed within 24 h of admission; when the patient had multiple test results, the first test result was preferentially selected for analysis. The laboratory indicators collected comprised thrombin time (TT); activated partial thromboplastin time (APTT); plasma fibrinogen determination (Fib); international standardized ratio; prothrombin time; red blood cell counts; hemoglobin levels; platelet counts; and leukocyte, lymphocyte, neutrophil, and monocyte counts. We also evaluated electrolyte levels that may impact ICH (potassium, chloride, and phosphorus levels) (12–14).

Imaging

The NCCT scan used axial technology and 5-mm thick slices at 120–140 kV (peak) and 10–500 mA. The first NCCT image obtained at admission was selected as the baseline image, whereas the last NCCT image obtained within 72 h was selected as the follow-up image.

Imaging assessments were performed independently by two attending radiologists, and investigators were blinded to all clinical and laboratory variables. When inconsistencies were observed, solutions were obtained through consultation and discussion with senior physicians. First, we determined the location of the ICH (deep, lobar, and infratentorial) and its

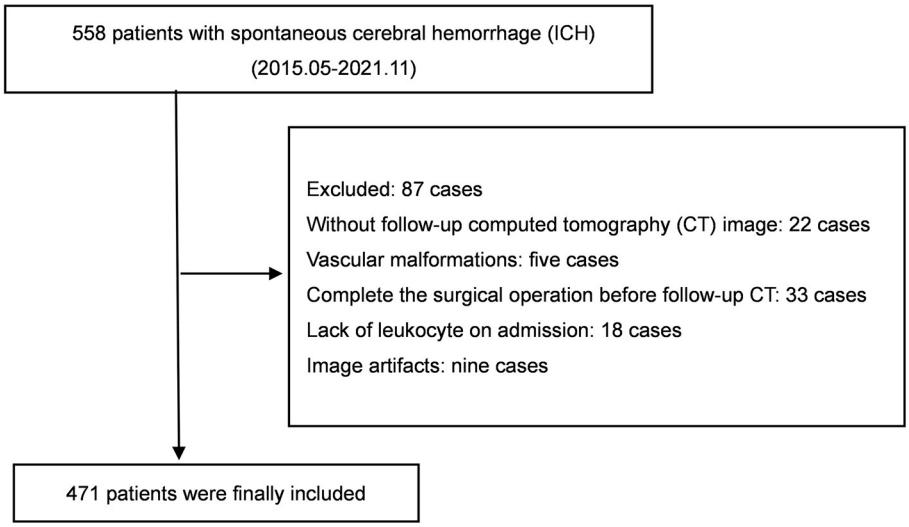

FIGURE 1
Flowchart of cohort selection.

penetration of the ventricular system on baseline NCCT images. Notably, we included ICH of the basal nucleus, internal capsule, external capsule, insula, and thalamus in deep ICH. Second, the two attending radiologists independently used the 1/2 ABC formula to gauge the hematoma volume on the baseline NCCT image and follow-up NCCT image. Specifically, we regarded the NCCT maximum bleeding diameter as A, the diameter perpendicular to A as B, and the approximation of the NCCT bleeding layer multiplied by the layer thickness as C (15). To avoid subjective differences, we measured follow-up hematoma volumes after completing baseline hematoma volume measurements for all patients and then averaged the two pre- and post-volume measurements observed by the two radiologists. Finally, we subtracted the average baseline hematoma volume from the average follow-up hematoma volume to obtain the difference. Accordingly, dividing the difference by the average baseline hematoma volume provided the percent change in hematoma volume.

Outcome measures

The study outcome was HE, which referred to the absolute and relative increase in hematoma volume at follow-up CT compared with the baseline hematoma volume. We used two methods recognized by researchers to define HE (16–21). For the convenience of expression, we termed these as HE-Definition 1 (absolute volume increase $\geq$6 mL or 33% relative volume increase) and HE-Definition 2 (absolute volume increase $\geq$12.5 mL or 33% relative volume increase).

Statistical analysis

Baseline characteristic variables are presented as mean $\pm$ standard deviation or median and interquartile range based on the consistency of the data with a normal distribution. Categorical variables are indicated as percentages. Depending on the type of data, one-way analysis of variance, the χ^2 test, or the Kruskal–Wallis *H*-test, was used to describe the normal distribution, categorical variables, or skewed distribution, respectively.

We used univariate analysis and a binary logistic regression model to assess the relationship between leukocytes and their subpopulations on HE. In the binary logistic regression model, the odds ratios (ORs) and 95% confidence intervals (CIs) of the unadjusted Model, Model I, and Model II were calculated. To control for confounders in Model I, we adjusted for sex, age, location, baseline hematoma volume, time from onset to admission, history of hypertension, hyperlipidemia, and the Glasgow Coma Scale score. To observe the effect of coagulation parameters on the results in Model II, we additionally adjusted for coagulation parameters (TT, Fib, and APTT) with $P < 0.1$ based on Model I.

We also performed subgroup analyses based on demographic characteristics (age <60 vs. $\geq$60 years; sex); clinical severity (Glasgow Coma Scale score <8 vs. $\geq$8 points; baseline hematoma volume <30 vs. $\geq$30 mL; systolic pressure <180 vs. $\geq$180 mmHg); and medical history (time from onset to admission <6 vs. $\geq$6 h). We evaluated the effect of subgroups on the relationship between monocyte or neutrophil counts and HE by adding an interaction term to the models. Data are presented as ORs and 95% CIs. Binary logistic regression was

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TABLE 1 Baseline characteristics of the included patients.

Variables	All cohort ($n = 471$)
Age, mean (SD), years	52.62 (12.28)
Baseline volume, mean (SD), mL	10.50 (5.10–22.87)
TT, mean (SD), s	18.09 (3.84)
Fib, mean (SD), g/L	2.92 (0.89)
APTT, mean (SD), s	24.08 (4.86)
INR, mean (SD)	0.96 (0.18)
PT, mean (SD), s	10.94 (2.13)
Total leukocyte count, mean (SD), $\times 10^9$/L	9.42 (3.45)
Neutrophil count, median (IQR), $\times 10^9$/L	5.58 (4.25–7.73)
Lymphocyte count, median (IQR), ×109/L	1.96 (1.34–2.89)
Monocyte count, median (IQR), ×109/L	0.45 (0.31–0.59)
Red blood cell, mean (SD) $\times 10^{12}$/L	4.82 (0.64)
Hemoglobin, mean (SD), g/L	142.38 (18.91)
Platelet, mean (SD), $\times 10^9$/L	225.91 (61.80)
Potassium, median (IQR), mmol/L	3.74 (3.46–4.11)
Chlorine, mean (SD), mmol/L	104.25 (4.05)
Phosphorus, mean (SD), mmol/L	1.03 (0.24)
Time from onset to admission, median (IQR), hours	1.00 (0.67–4.00)
Systolic pressure, mean (SD), mmHg	178.78 (32.30)
Glasgow coma scale score, mean (SD)	12.56 (3.24)
Sex, *n* (%)	
Male	327 (69.43)
Female	144 (30.57)
Baseline intraventricular hemorrhage, *n* (%)	
No	350 (75.11)
Yes	116 (24.89)
Location, *n* (%)	
Deep	355 (76.18)
Lobar	46 (9.87)
Infratentorial	65 (13.95)
Use mannitol before repeat CT, *n* (%)	
No	246 (53.59)
Yes	213 (46.41)
History of hypertension, *n* (%)	
No	76 (16.89)
Yes	374 (83.11)
History of diabetes, *n* (%)	
No	409 (91.29)
Yes	39 (8.71)
Hyperlipidemia, *n* (%)	
No	411 (91.74)
Yes	37 (8.26)
HE-Definition 1, *n* (%)	
No	340 (72.19)
Yes	131 (27.81)
HE-Definition 2, *n* (%)	
No	355 (75.37)
Yes	116 (24.63)

SD, standard deviation; TT, thrombin time; Fib, plasma fibrinogen assay; APTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time; IQR, interquartile range; CT, computed tomography; HE-Definition 1, hematoma expansion (relative volume ≥33% or absolute volume ≥6 mL); HE-Definition 2, hematoma expansion (relative volume ≥33% or absolute volume ≥12.5 mL).

used to examine associations between neutrophil or monocyte counts and HE in different subgroups, and *P*-values for the interaction were recorded.

A two-tailed *P*-value < 0.05 was considered as statistically significant in all analyses. The analyses were performed with R software, version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria); Empower, version 2.21 (X&Y Solutions, Boston, MA); and GraphPad Prism, version 9.0.0 (GraphPad Software, San Diego, CA).

Results

The average age of the study participants was 52.62 ± 12.28 years, and there were 327 men (69.43%) (Table 1). According to HE-Definition 1 and HE-Definition 2, 131 (27.81%) and 116 (24.63%) patients experienced HE, respectively.

Univariate analysis (Table 2) showed that baseline volume, TT, Fib, neutrophil count, lymphocyte count, history of hypertension, hyperlipidemia, and Glasgow Coma Scale score were associated with HE according to both definitions ($P < 0.05$). APTT, monocyte count, and red blood cells were only associated with HE-Definition 2 ($P < 0.05$).

Table 3 showed the results of binary logistic regression analysis. In the unadjusted Model, neutrophil count was associated with HE-Definition 1 (OR: 0.93, 95% CI: 0.87–1.00, $P = 0.0294$) and HE-Definition 2 (OR: 0.93, 95% CI: 0.87–1.00, $P = 0.0493$); similarly, lymphocyte count was associated with HE-Definition 1 (OR: 1.21, 95% CI: 1.06–1.39, $P = 0.0062$) and HE-Definition 2 (OR: 1.22, 95% CI: 1.06–1.41, $P = 0.0053$). Monocyte count was not associated with HE-Definition 1 (OR: 1.76, 95% CI: 0.91–3.42, $P = 0.0952$) but was associated with HE-Definition 2 (OR: 2.08, 95% CI: 1.05–4.14, $P = 0.0368$). Meanwhile, leukocyte count was not associated with HE-Definition 1 (OR: 0.99, 95% CI: 0.93–1.05, $P = 0.6972$) or HE-Definition 2 (OR: 1.00, 95% CI: 0.94–1.06, $P = 0.9295$).

In Model I, leukocyte count [adjusted OR (aOR): 0.92, 95% CI: 0.85–1.00, $P = 0.0411$] and neutrophil count (aOR: 0.87, 95% CI: 0.80–0.95, $P = 0.0015$) were associated with HE-Definition 1, whereas lymphocyte count (aOR: 1.14, 95%

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TABLE 2 Univariate analysis of hematoma expansion (HE) based on two definitions.

	HE-definition 1		HE-definition 2	
	OR, 95% CI	*P*-value	OR, 95% CI	*P*-value
Sex				
Male	ref		ref	
Female	0.86 (0.55–1.34)	0.4961	0.87 (0.55–1.39)	0.5673
Age (years)	0.99 (0.97–1.01)	0.2483	0.99 (0.97–1.00)	0.1289
Baseline intraventricular hemorrhage				
No	ref		ref	
Yes	1.10 (0.69–1.74)	0.6954	0.96 (0.59–1.57)	0.8763
Baseline volume (mL)	1.03 (1.02–1.04)	<0.0001*	1.02 (1.01–1.03)	0.0014*
Location				
Deep	Ref		ref	
Lobar	0.91 (0.45–1.83)	0.7974	0.87 (0.41–1.82)	0.7102
Infratentorial	1.15 (0.65–2.04)	0.6357	1.39 (0.78–2.48)	0.2656
TT (s)	1.27 (1.09–1.48)	0.0022*	1.23 (1.05–1.44)	0.0105*
Fib (g/L)	0.69 (0.53–0.90)	0.0068*	0.71 (0.54–0.94)	0.0165*
APTT (s)	1.04 (1.00–1.09)	0.0749	1.05 (1.00–1.10)	0.0298*
INR	1.39 (0.46–4.14)	0.5585	1.44 (0.47–4.39)	0.5215
PT (s)	1.04 (0.96–1.14)	0.3337	1.05 (0.96–1.14)	0.3198
Total leukocyte count ($\times 10^9$/L)	0.99 (0.93–1.05)	0.6972	1.00 (0.94–1.06)	0.9294
Neutrophil count ($\times 10^9$/L)	0.93 (0.87–0.99)	0.0294*	0.93 (0.87–1.00)	0.0493*
Lymphocyte count ($\times 10^9$/L)	1.21 (1.06–1.39)	0.0062*	1.22 (1.06–1.41)	0.0053*
Monocyte count ($\times 10^9$/L)	1.76 (0.91–3.42)	0.0952	2.08 (1.05–4.14)	0.0368*
Red blood cell ($\times 10^{12}$/L)	1.37 (0.99–1.88)	0.0566	1.43 (1.03–2.00)	0.0348*
Hemoglobin (g/L)	1.01 (1.00–1.02)	0.2120	1.01 (1.00–1.02)	0.1255
Platelet ($\times 10^9$/L)	1.00 (1.00, 1.00)	0.6290	1.00 (1.00–1.00)	0.5044
Potassium (mmol/L)	1.00 (0.99–1.00)	0.1581	1.00 (0.99–1.00)	0.1788
Chlorine (mmol/L)	0.97 (0.92–1.02)	0.2363	0.98 (0.93–1.04)	0.4882
Phosphorus (mmol/L)	0.41 (0.04–4.35)	0.4625	0.56 (0.05–6.05)	0.6291
Time from onset to admission (hours)	0.98 (0.97–1.00)	0.0551	0.99 (0.97–1.00)	0.1153
Mannitol use before follow-up CT				
No	ref		Ref	
Yes	0.71 (0.47–1.08)	0.1071	0.73 (0.47–1.11)	0.1420
Systolic blood pressure (mmHg)	1.00 (0.99–1.00)	0.2977	1.00 (0.99–1.00)	0.5759
History of hypertension (mmHg)				
No	ref		Ref	
Yes	2.93 (1.45–5.90)	0.0026*	3.75 (1.67–8.42)	0.0014*
History of diabetes				
No	ref		Ref	
Yes	0.88 (0.42–1.87)	0.7420	0.91 (0.42–1.99)	0.8226
Hyperlipidemia				
No	ref		Ref	
Yes	0.30 (0.10–0.86)	0.0249*	0.26 (0.08–0.85)	0.0266*
Glasgow Coma Scale score	0.87 (0.82–0.93)	<0.0001*	0.88 (0.83–0.94)	0.0002*

CI, confidence interval; OR, odds ratio; TT, thrombin time; Fib, plasma fibrinogen assay; APTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time; CT, computed tomography; HE-Definition 1, hematoma expansion (relative volume ≥33% or absolute volume ≥6 mL); HE-Definition 2, hematoma expansion (relative volume ≥33% or/and absolute volume ≥12.5 mL). Statistically significant values are in asterisks. Variables are presented as *n* (%) for nominal data and as mean ± standard deviation or median (interquartile range) for continuous data. The * symbol indicates the statistically significant values.

TABLE 3 Binary logistic regression of the relationship of leukocytes and their subpopulations with hematoma expansion (HE) based on two definitions.

Exposure	HE-definition 1			HE-definition 2		
	Non-adjusted OR, 95% CI, P	Model I OR, 95% CI, P	Model II OR, 95% CI, P	Non-adjusted OR, 95% CI, P	Model I OR, 95% CI, P	Model II OR, 95% CI, P
Total leukocyte count	0.99 (0.93–1.05) 0.6972	0.92 (0.85–1.00) 0.0411	0.97 (0.89–1.05) 0.4026	1.00 (0.94–1.06) 0.9295	0.93 (0.86–1.01) 0.0660	0.97 (0.89–1.06) 0.4841
Neutrophil count	0.93 (0.87–1.00) 0.0294	0.87 (0.80–0.95) 0.0015	0.91 (0.84–1.00) 0.0463	0.93 (0.87–1.00) 0.0493	0.88 (0.80–0.96) 0.0037	0.92 (0.84–1.01) 0.0774
Lymphocyte count	1.21 (1.06–1.39) 0.0062	1.14 (0.96–1.34) 0.1257	1.14 (0.96–1.34) 0.1414	1.22 (1.06–1.41) 0.0053	1.13 (0.95–1.33) 0.1598	1.12 (0.95–1.33) 0.1817
Monocyte count	1.76 (0.91–3.42) 0.0952	1.61 (0.75–3.44) 0.2178	2.45 (1.02–5.88) 0.0450	2.08 (1.05–4.14) 0.0368	1.73 (0.80–3.76) 0.1627	2.54 (1.04–6.20) 0.0399

OR, odds ratio; CI, confidence interval; HE-Definition 1, hematoma expansion (relative volume ≥33% or absolute volume ≥6 mL); HE-Definition 2, hematoma expansion (relative volume ≥33% or absolute volume ≥12.5 mL). Model I was adjusted for sex, age, location, baseline hematoma volume, time from onset to admission, history of hypertension, hyperlipidemia, and Glasgow Coma Scale score. Model II was adjusted for sex, age, location, baseline hematoma volume, time from onset to admission, history of hypertension, hyperlipidemia, Glasgow Coma Scale score, TT, Fib, and APTT.

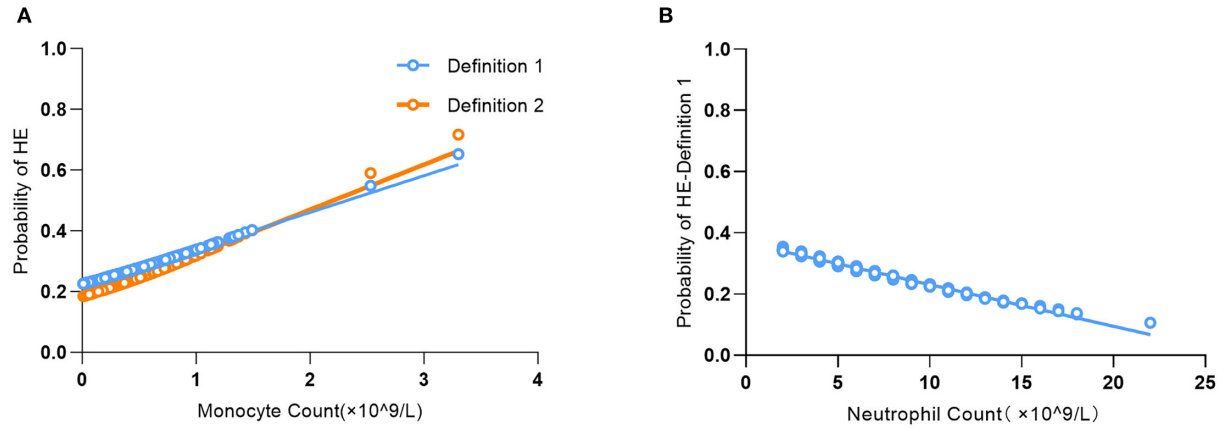

FIGURE 2
Association of monocyte count **(A)** and neutrophil count **(B)** with the probability of predicting hematoma expansion (HE).

CI: 0.96–1.34, $P = 0.1257$) and monocyte count (aOR: 1.61, 95% CI: 0.75–3.44, $P = 0.2178$) were not significantly associated with HE-Definition 1. However, leukocyte count (aOR: 0.93, 95% CI: 0.86–1.01, $P = 0.0660$), lymphocyte count (aOR: 1.13, 95% CI: 0.95–1.33, $P = 0.1598$), and monocyte count (aOR: 1.73, 95% CI: 0.80–3.76, $P = 0.1627$) were not associated with HE-Definition 2, whereas neutrophil count was significantly associated with HE-Definition 2 (aOR: 0.88, 95% CI: 0.80–0.96, $P = 0.0037$).

In Model II, for every 1-unit increase in monocyte count, the risk of HE-Definition 1 increased by 1.45 times (aOR: 2.45, 95% CI: 1.02–5.88, $P = 0.0450$), and the risk of HE-Definition 2 increased by 1.54 times (aOR: 2.54, 95% CI: 1.04–6.20, $P = 0.0399$). Each unit increase in the neutrophil count was associated with a 9% reduction in the risk of HE-Definition 1 (aOR: 0.91, 95% CI: 0.84–1.00, $P = 0.0463$); however, it was not associated with HE-Definition 2 (aOR: 0.92, 95% CI: 0.84–1.01, $P = 0.0774$). The relationship between monocyte and neutrophil counts and HE is shown in Figure 2. Total leukocyte count and lymphocyte count were not significantly associated with HE-Definition 1 and HE-Definition 2 (all $P > 0.05$).

Subgroup analysis showed no interaction between age, sex, baseline hematoma volume, Glasgow Coma Scale score, systolic blood pressure, or time from onset to admission, regardless of HE-Definition 1 or HE-Definition 2 in the relationship of monocyte (Figure 3) and neutrophil counts (Figure 4) with HE (interaction $P > 0.05$).

Discussion

Our findings suggest that an elevated monocyte count is related to a higher risk of HE regardless of the two definitions of HE, and the relationship between monocyte count and the risk of HE is independent of coagulation parameters. Moreover, an increased neutrophil count is associated with a decreased risk

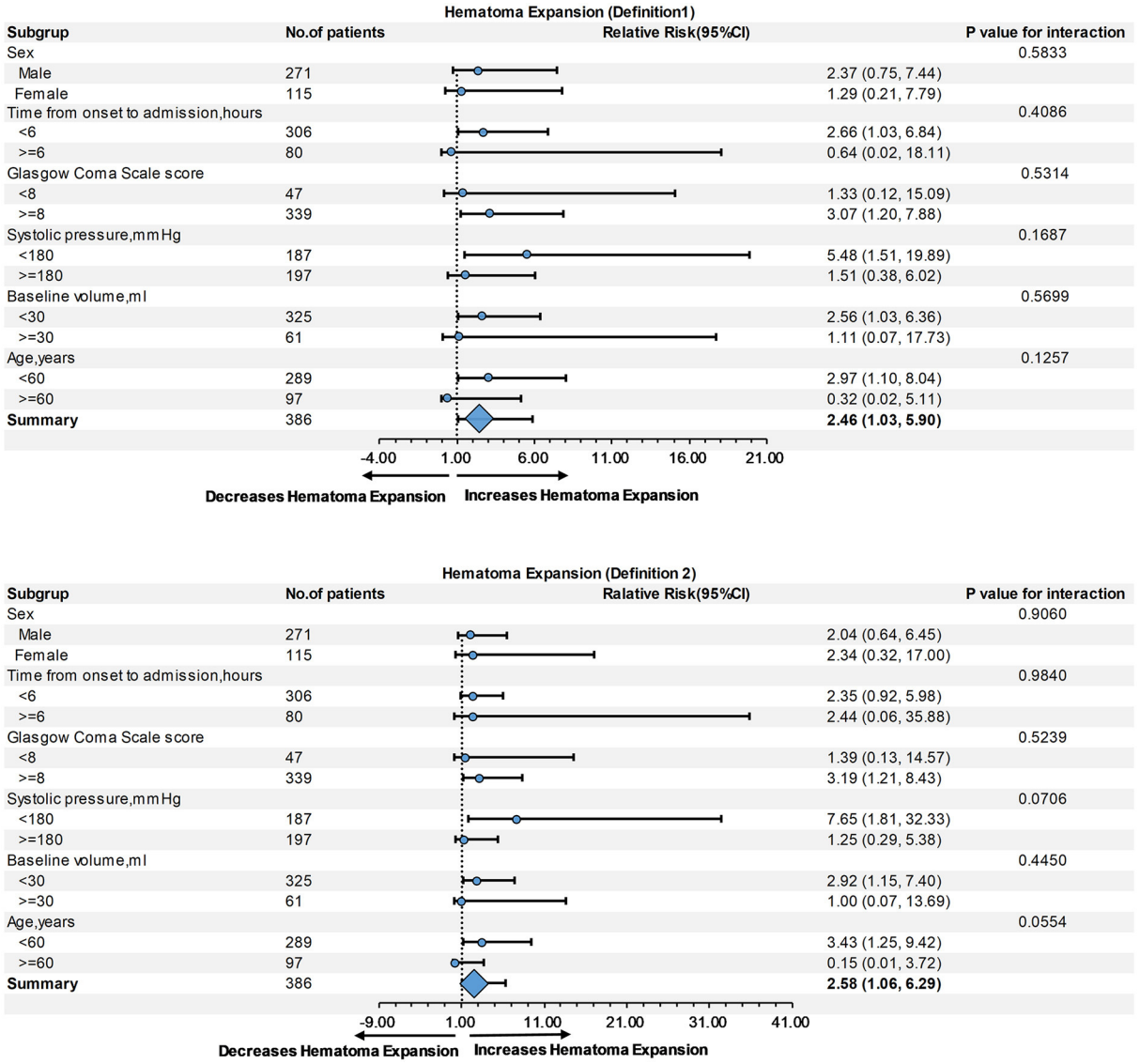

FIGURE 3
Subgroup analysis of the relationship of monocyte count and hematoma expansion (HE) according to Definitions 1 and 2.

of HE in the context of HE-Definition 1, and this relationship is independent of coagulation parameters.

Morotti et al. previously reported a relationship between leukocyte count and HE in a population from Boston (10); the present results verify some of these results and expand them to include a Chinese population. The most significant similarities between the two studies are the positive correlation of monocyte count with HE and the lack of a relationship between lymphocyte count and HE. However, there are also two important differences. First, after adjusting for coagulation parameters, we found that the total leukocyte count was no longer associated with HE, and the relationship between monocyte count and HE was first presented; however, the relationship between neutrophil count and HE was not affected by coagulation parameters. These results suggest an interaction between leukocyte count and coagulation parameters, and different leukocyte subsets responded differently to coagulation parameters. Second, we only observed a relationship between neutrophil count and HE-Definition 1, which reflects the influence of the method used to define HE on the results.

Evidently, both definitions of HE resulted in consistent conclusions regarding the relationship between monocyte count and HE, thereby improving the reliability of our conclusions. Our results are consistent with a previous study, which suggested that the correlation between elevated monocyte levels and HE is mainly regulated by coagulation (10). Therefore, we compared

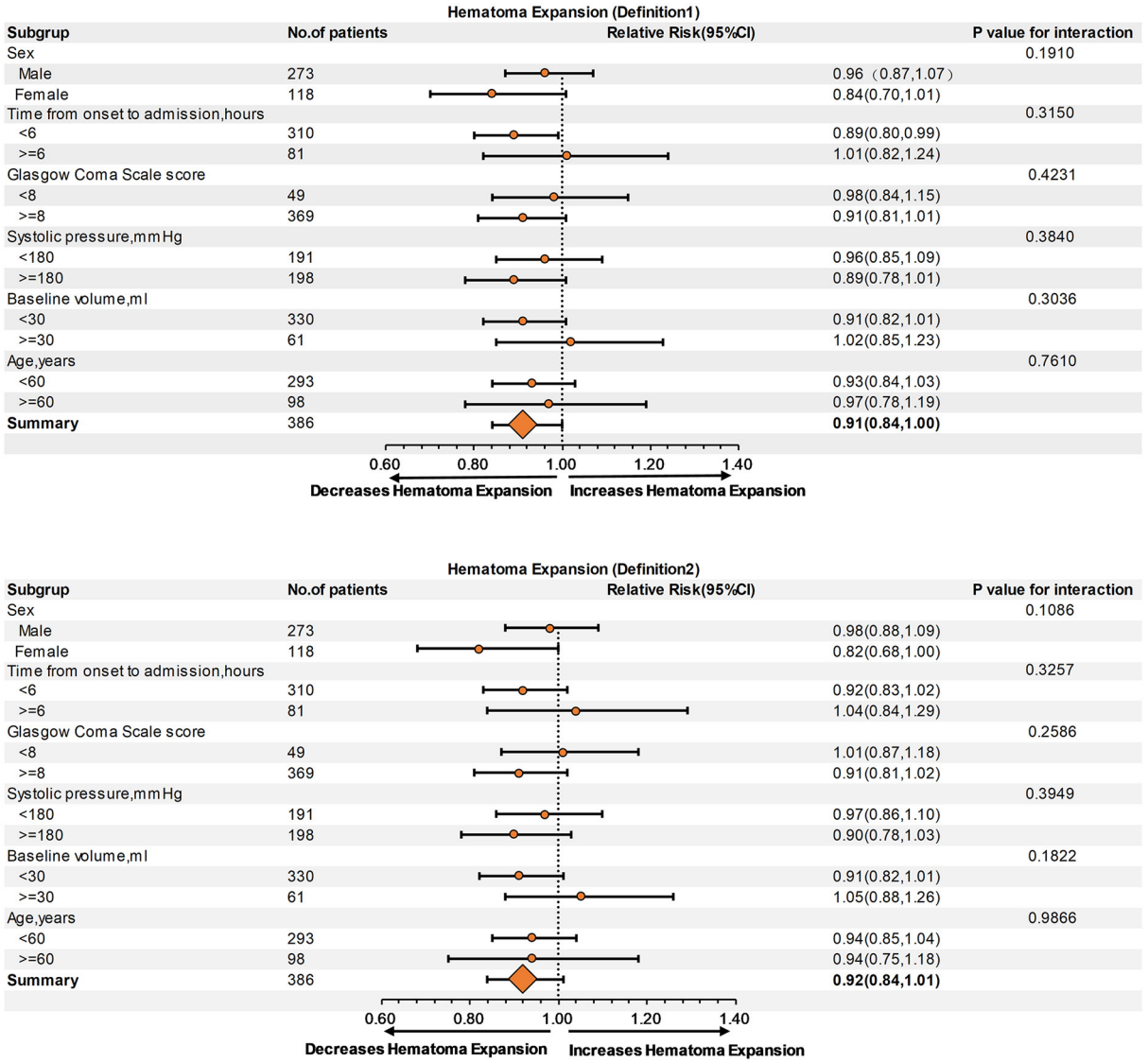

FIGURE 4
Subgroup analysis of the relationship of neutrophil count and hematoma expansion (HE) according to Definitions 1 and 2.

the results before and after adjusting for the coagulation parameters (TT, Fib, and APTT). The evidence shows that the relationship between monocytes and the coagulation process is complex, with simultaneous procoagulant and anticoagulant properties. Under normal physiological regulations, the two are relatively balanced, and inflammatory stimulation can change the balance (22–24). Conversely, the expression of monocyte-derived tissue factors can promote thrombin and stable fibrinogen production (22, 24). In contrast, thrombomodulin on the surface of monocytes inhibits procoagulant activity, interferes with thrombus formation, and destabilizes fibrin (23); previous studies have used this mechanism to explain the positive correlation between monocyte count and HE (10, 25). However, our study showed that coagulation parameters had a great impact on the relationship between monocyte count and HE, but the coagulation parameters were not the main reason for high monocyte count being associated with increased HE risk, because only after adjusting these for these coagulation parameters that the monocyte count significantly correlated with HE. We speculate that this may be due to the predominance of coagulation characteristics of monocytes over anticoagulation characteristics within 72 h after ICH; however, this needs to be proven by future research. Besides, Mei et al. (26) reported that the proportion of monocytes in peripheral blood increased during the acute phase of ICH (6 h after onset). Another study also suggested that changes in monocytes may be closely related

to the process of ICH (27). However, there was no interaction between the subgroups (time from onset to admission <6 h vs. ≥6 h) in our study. In summary, our study provides clinical evidence that higher monocyte count is associated with an increased risk of HE.

Our study shows that elevated neutrophil count reduces the risk of HE only for Definition 1, which is somewhat consistent with the findings of the Boston study (10). The mechanism may be related to the release of lactoferrin from neutrophils, prompting an increase in iron clearance (11) and the marked procoagulant properties of activated neutrophils (10). The association between neutrophil count and HE-Definition 2 was almost statistically significant ($P = 0.07$). This highlights the impact of using different methods of defining HE on the results. Since there is no consensus definition for HE yet, it is difficult to determine which definition of HE is closer to the actual clinical phenomena. We observed that neutrophil count might have less influence on HE than monocyte count because of the impact of the HE definition. Hence, when compared with other studies wherein Definition 1 was used for HE, neutrophil count was as valuable as other risk factors for HE (28).

Morotti et al. (10, 29) reported that leukocyte count might reduce HE by enhancing coagulation because of the interactions between leukocytes and platelets, endothelial cells, and coagulation factors. In our study, after adjusting for TT, Fib, and APTT, the leukocyte count was not associated with HE. This confirms that leukocytes limit HE by interacting with the coagulation process rather than directly affecting HE processes.

Preclinical studies have shown that certain lymphocyte subsets can disrupt the BBB by reducing the expression of claudin, destroying endothelial cells and their tight junctions, and mediating endothelial cell apoptosis (26, 30); hence, the disruption of the BBB contributes to HE (31). These results suggest that lymphocytes participate in the disruption of the BBB, which inevitably leads to HE. However, to the best of our knowledge, this hypothesis has not been confirmed in clinical studies. Our study showed that lymphocyte count was not significantly associated with HE either.

The following five points describe the strengths of our study: first, our study demonstrates that the earlier findings reported by Morotti et al. are also applicable in China. Second, we adopted two definitions for HE to improve the study's accuracy. Third, our findings apply to a wider population because we did not limit the time from onset to patient admission. Most previous studies only included patients with HE within 24 h from onset (21, 32), and a few studies expanded this to 48 h (10). Fourth, other Chinese studies are limited to the role of a single leukocyte type in ICH, which makes it difficult to exclude population differences when interpreting the results of different studies. However, our study is valuable for future research because of the simultaneous analysis of the role of the three leukocyte subsets in HE in the same population. Finally, leukocyte count is a readily available laboratory indicator during admission that can be obtained cost-effectively and is easily instituted, making our methodology and results easily translatable to clinical settings.

In summary, our study contributes to the risk stratification of HE in an acute clinical setting. For patients with a higher monocyte count and lower neutrophil count, physicians should shorten the time for NCCT revision and provide more attentive care. Furthermore, considering the diametrically opposed representations of monocytes and neutrophils in HE, physicians should be cautious of employing traditional holistic anti-inflammatory approaches for treating patients with ICH and consider individualizing the strengths of different subpopulations.

Regardless, our study had the following limitations. First, our study was limited by the retrospective nature of the cohort; therefore, some patients were excluded because of a lack of routine blood tests and follow-up NCCT. Second, the results cannot be extrapolated to patient groups excluded from the study. Third, we did not exclude other systemic diseases that might affect leukocytes and their subpopulations, which might slightly weaken our findings; however, we still obtained results that were somewhat similar to previous studies. Fourth, the 1/2ABC formula has been proven to overestimate the hematoma volume (33). Hence, even if the 1/2ABC formula was proved to be accurate in the change of hematoma volume (33), it might still affect the results. Fifth, our study lacked multi center data. Finally, our research population was limited to a Chinese population with certain geographical and ethnic restrictions, which may affect the generalizability of the results.

In conclusion, after excluding the influence of coagulation parameters, a higher monocyte count is associated with higher HE risks regardless of the two HE definitions, which facilitates risk stratification. Nevertheless, the underlying pathophysiological mechanisms requires further investigation. Moreover, an increased neutrophil count is associated with a decreased risk of HE in the context of HE-Definition 1, which reflects the importance of standardizing the definition of HE. More clinical studies are needed to explore a standard for HE.

Data availability statement

Study data are not publicly available due to patient confidentiality. Study data are available from the corresponding author upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Review Committee of Shenzhen Longhua District Central Hospital (approval number: 2022-012-01).

Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JQ and HW contributed to the research planning, data collection, and drafting of the manuscript. YL participated in data collection, contributed to the analysis of data, and assessed the risk of bias in the study. LD and JX provided professional guidance for this study and performed a final check of the manuscript. All authors contributed to the study described in this manuscript 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

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General anesthesia but not conscious sedation improves functional outcome in patients receiving endovascular thrombectomy for acute ischemic stroke: A meta-analysis of randomized clinical trials and trial sequence analysis

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Background: This study aimed at comparing the difference in prognostic outcomes between patients receiving general anesthesia (GA) and conscious sedation (CS) for endovascular thrombectomy after acute ischemic stroke.

Methods: Databases from Medline, Embase, Google scholar, and Cochrane library were searched for randomized controlled studies (RCTs) comparing patients undergoing GA and CS for endovascular thrombectomy following anterior circulation ischemic stroke. The primary outcome was frequency of 90-day good functional outcome [defined as modified Rankin Scale score of ≤2], while secondary outcomes included successful recanalization rate (SRR) [i.e., modified thrombolysis in cerebral infarction = 2b or 3], mortality risk, symptomatic intracranial hemorrhage (ICH), procedure-related complications, hypotension, pneumonia, neurological outcome at post-procedure 24–48 h, and puncture-to-recanalization time.

Results: Six RCTs including 883 patients published between 2016 and 2022 were included. Merged results revealed a higher SRR [risk ratio (RR) = 1.11, 95% CI: 1.03–1.2, $p = 0.007$; $I^2 = 29\%$] and favorable neurological outcomes at 3-months (RR = 1.2, 95% CI: 1.01–1.41, $p = 0.04$; $I^2 = 8\%$) in the GA group compared to CS group, without difference in the risk of mortality (RR = 0.88), symptomatic ICH (RR = 0.91), procedure-related complications (RR = 1.05), and pneumonia (RR = 1.9) as well as post-procedure neurological outcome (MD = −0.21) and successful recanalization time (MD = 3.33 min). However, GA was associated with a higher risk of hypotension compared with that of CS.

Conclusion: Patients with acute anterior circulation ischemic stroke receiving GA were associated with a higher successful recanalization rate as well as a better 3-month neurological outcome compared to the use of CS. Further investigations are warranted to verify our findings.

Systematic review registration: www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022342483, identifier: CRD42022342483.

KEYWORDS

stroke, endovascular thrombectomy, general anesthesia, sedation, prognosis

Introduction

Endovascular thrombectomy (EVT) has revolutionized the treatment of acute ischemic stroke (AIS) with large-vessel occlusion in the anterior circulation since 2015 when several clinical trials demonstrated its efficacy for reperfusion (1, 2). Several prognostic factors have been identified for the achievement of better neurologic outcome of EVT, including a shorter reperfusion time and a stable hemodynamic condition during the procedure (3–5). Consistently, one observational study reported a 10% reduction in the probability of a good outcome for every 15-min delay in EVT reperfusion (6), highlighting the importance of shortening the door-to-reperfusion time. For patients receiving EVT, the most common anesthetic modalities include general anesthesia (GA) and conscious sedation (CS), both of which have their pros and cons (7–9). The choice of the optimal anesthetic approach to EVT is still under debate. Observational studies comparing GA with other strategies (i.e., local anesthesia or CS) have reported poorer outcomes in patients receiving GA for EVT (10–12). In contrast, pooled evidence from a recent meta-analysis (7) focusing on five randomized controlled trials (RCTs) (13–18) demonstrated favorable successful recanalization rate (SRR) and functional outcomes associated with GA compared to CS. Nevertheless, the limited sample size in that meta-analysis (i.e., 498 patients) (7) may impair the robustness of their findings. Recently, one multicenter RCT involving 351 patients from France showed comparable functional outcomes between patients receiving GA and those undergoing CS for EVT (19). Taking into account the limitations of the previous meta-analysis (7) and the availability of updated data, we conducted this systematic review and meta-analysis to provide more evidence for clinical decision.

Abbreviations: EVT, endovascular thrombectomy; AIS, acute ischaemic stroke; CS, conscious sedation; GA, general anesthesia; mRS, modified Rankin Scale; TICI, thrombolysis in cerebral infarction; NIHSS, National Institutes of Health Stroke Scale; RR, risk ratio; MD, mean difference; RCT, randomized controlled study; SRR, successful recanalization rate; ICH, intracranial hemorrhage.

Methods

This review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines and was registered at the PROSPERO international database (CRD42022342483).

Search strategy and studies selection

We searched the databases of Embase, Medline, and the Cochrane controlled trials register for peer-reviewed RCTs comparing the prognostic outcomes between GA with CS in patients requiring EVT using the keywords "general anesthesia," "conscious sedation," "stroke" or "thrombectomy" and their synonyms as well as controlled vocabulary from inception to June 28, 2022. There were no restrictions on age, language, gender, publication date, sample size, and geographic location during literature search. We also reviewed relevant meta-analyses to retrieve related articles. The syntax and search strategies for one of these databases (i.e., Medline) is illustrated in Supplementary Table 1.

After removal of duplicated citations, two independent authors examined the titles and abstracts of the remaining records to determine the eligibility for inclusion before a full-text review. Disagreements between the two authors were settled by consensus or discussion with a third author.

Study selection criteria

Studies were considered to be eligible for inclusion if the following criteria were fulfilled: (a) Population: adult patients (i.e., ≥18 years) receiving EVT for acute anterior circulation ischemic stroke regardless of timing of symptom onset (i.e., <6 h or ≥6 h), (b) Intervention: use of GA as an anesthetic approach (GA group) regardless of the thrombectomy technique, (c) Comparison: CS with or without the use of local anesthetics (CS group). CS was defined as the use of sedative or/and analgesic

agents via intravenous route to provide sedative, amnesic, analgesic, or anxiolytic effects, (d) Outcomes: prognostic outcomes including successful recanalization rate and neurological outcomes.

Primary outcome, secondary outcomes, and definitions

Primary outcome

- Frequency of good functional outcome (i.e., functional independence), which was defined as one with a modified Rankin Scale (mRS) score of 0–2, at 3-month follow-up.

Secondary outcomes

- SRR following EVT. Successful recanalization referred to an achievement of an extended or modified thrombolysis in cerebral infarction (TICI) scale of 2b or 3.
- Risk of mortality within 3-months.
- Risk of symptomatic intracranial hemorrhage (ICH) during hospitalization.
- Risk of procedure-related complications.
- Risk of hypotension, the definition of which was according to that defined in individual studies.
- Risk of pneumonia.
- Neurological outcome at post-procedure 24–48 h assessed with the NIHSS.
- Time from puncture to successful recanalization, which referred to the period from groin puncture to arterial reperfusion.

Analysis and assessment of risk of bias

Cochrane Review Manager (RevMan 5.3; Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014) was used for data synthesis. Risk ratios (RRs) or mean difference (MD) with 95% confidence intervals (CIs) were calculated based on a random effects model assuming heterogeneity across studies (20, 21). Heterogeneity was assessed with I^2 statistics [i.e., low (I^2 < 50%), moderate (I^2 = 50–75%), and high (I^2 > 75%)]. For studies with a high heterogeneity (I^2 > 50%), a leave-one-out sensitivity analysis was conducted to evaluate stability of results (22). A probability value of <0.05 was considered statistically significant for all (including subgroup) analyses.

Two authors independently assessed the risk of bias for each study using the revised Cochrane risk-of-bias tool for randomized trials (RoB 2.0) (23) based on five domains, namely, possible bias from the randomization process, deviations from intended interventions, outcome measurement, missing outcome, and selection of the reported results.

To minimize false-positive results attributed to multiple testing and sparse data, trial sequential analysis (TSA) with TSA viewer version 0.9.5.10 Beta (www.ctu.dk/tsa) was conducted to test the robustness of the cumulative evidence as previously reported (24). Following the calculation of the required information size (RIS) and the trial sequential monitoring boundaries, the correlation between the cumulative Z curve and the TSA boundary was examined. To calculate the RIS for dichotomous outcomes, two-sided tests were adopted with a type I error, power, and a relative risk reduction being set at 5, 80, and 20%, respectively.

Results

Study selection and characteristics of included studies

Figure 1 shows the various reasons for study exclusion after full-text screening. Finally, six RCTs involving 883 patients undergoing EVT published from 2016 to 2022 were included in this meta-analysis (14–19). Study characteristics are described in Table 1. The median or mean age of the participants ranged from 60 to 73 years with a proportion of male gender being 44–66%. The six studies that provided details regarding the baseline NIHSS score (range: 13–20) all reported no difference between the GA and CS groups (14–19). The sample size of individual RCT varied between 40 and 345. In the CS group, the anesthetic conversion rates were between 4.5 and 20% with a pooled incidence of 10.3% (Supplementary Figure 1). The reasons for conversion are demonstrated in Supplementary Table 2, revealing that patient agitation was the most common reason for conversion. In the GA group, anesthetic agents for maintenance of anesthesia included sevoflurane (one study) (15) and propofol (four studies) (16–19) with the use of remifentanil, while one study did not provide relevant details (14). In the CS group, propofol with or without short-acting opioids was used in the three studies (16–18). The two other trials only adopted short-acting opioids (i.e., remifentanil) to provide CS (15, 19), and one study did not report this information (14). Of the six studies included in the present meta-analysis, two provided information about brain infarct volume 3 days following acute stroke. One of the studies demonstrated a notable reduction in final infarct volume in the GA group compared to that in the CS group (22.3 vs. 38.0 mL, respectively, p = 0.04) despite a lack of significant difference in the initial infarct volume (10.5 vs. 13.3 mL) and infarct growth (8.2 vs. 19.4 mL) (16), while the other only showed comparable final infarct volume between the two groups (i.e., 20 vs. 20 mL) (15). The risks of bias of individual studies are summarized in Figure 2. The

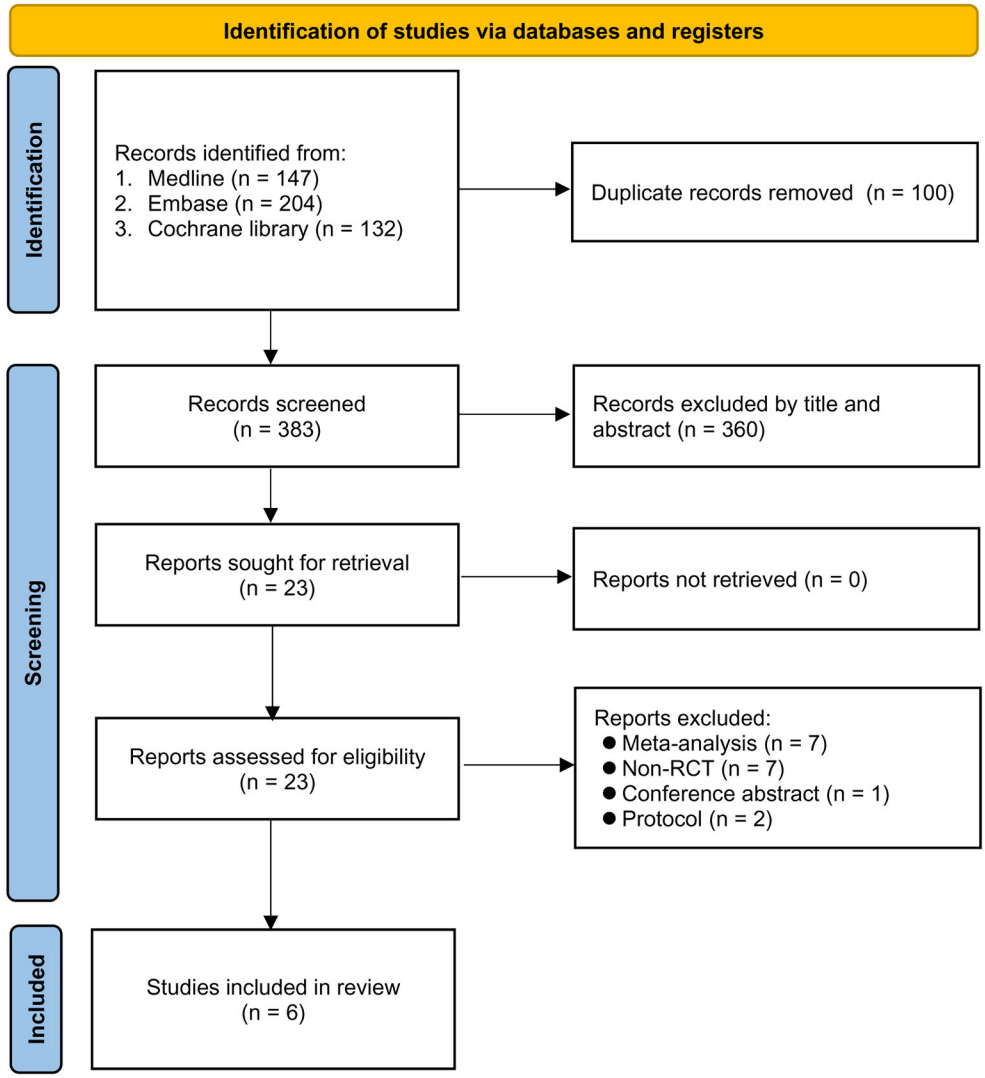

FIGURE 1
PRISMA flow diagram of study selection for the current meta-analysis.

anesthetic conversion rate was 5–10% and ≥10% in two (16, 18) and three (14, 15, 17) studies, respectively. Accordingly, the risk of these studies were considered to be uncertain or high.

Outcomes

Primary outcomes

Pooled analysis showed a high frequency of good functional outcomes (RR = 1.2, 95% CI: 1.01 to 1.41, $p = 0.04$; $I^2 = 8\%$) (Figure 3A) in the GA group compared to the CS group (14–19). Sensitivity analyses were not performed due to a low heterogeneity. Crossing of the cumulative Z-curve over RIS on TSA suggested sufficient evidence to reach a sound conclusion for this primary outcome (Figure 3B).

Secondary outcomes: Procedure-related outcomes

Regarding procedural outcomes, the merged results revealed a higher SRR (RR = 1.11, 95% CI: 1.03 to 1.2, $p = 0.007$; $I^2 = 29\%$) in the GA group than that in the CS group (Figure 4A). However, patients receiving GA also had a higher hypotension risk compared to those undergoing CS for EVT (RR = 1.59, 95% CI: 1.2 to 2.1, $p = 0.001$, $I^2 = 78\%$) (Figure 4B). Sensitivity analysis for this outcome demonstrated a consistent finding when certain studies were removed one at a time.

TABLE 1 Characteristics of studies ($n = 6$).

Author-year (trial)	Age (years)	Male (%)	NIHSS Score at baseline	IV t-PA (%)	Endovascular technique			GA group	CS group	N	Conversion Rate (%)¶	Country
					A (%)	B (%)	C (%)					
Löwhagen Hendén et al. (15) (Anstroke)	73 vs. 72	58 vs. 51	20 vs. 17	73 vs. 80	NR	NR	NR	S, R	R	90	15.6	Sweden
Maurice et al. (19) (GASS)	71 vs. 73	47 vs. 44	16 vs. 16	66 vs. 65	NR	NR	NR	P,R	R	345	4.5	France
Ren et al. (18)	69 vs. 69	54 vs. 57	14 vs. 14	77 vs. 81	NR	NR	NR	P, R, D	P,D,F	130	9.5	China
Schönenberger et al. (14) (SIESTA)	72 vs. 71	66 vs. 55	17 vs. 17	63 vs. 65	82 vs. 86	8 vs. 5	22 vs. 16	NR	NR	150	14.3	Germany
Simonsen et al. (16) (GOLIATH)	71 vs. 72	55 vs. 48	18 vs. 17	77 vs. 73	22 vs. 19	39 vs. 38	17 vs. 16	P,R	P,F	128	6.3	Demark
Sun et al. (17) (CANVAS)	67 vs. 60	65 vs. 65	14 vs. 13	45 vs. 55	10 vs. 15	40 vs. 45	50 vs. 40	P,R	P,Su	40	20	China

NR, not reported; GA, general anesthesia; CS, conscious sedation; IV, intravenous; NIHSS; A: stent retriever; B: direct aspiration; C: stent retriever combined with direct aspiration; ¶Conversion to general anesthesia; NIHSS, National Institutes of Health Stroke Scale; P, propofol; F, fentanyl; R, remifentanil; D, dexmedetomidine; S, sevoflurane; Su, sufentanil.

FIGURE 2
Summary of different categories of risk of bias of the included studies. Green: low risk of bias; yellow: moderate risk of bias; red: high risk of bias.

Nevertheless, the cumulative duration of hypotension episode was comparable between the two groups (MD = 0.86 min, 95% CI: −1.78 to 3.5, $p = 0.52$, $I^2 = 0$) (Figure not shown). No significant difference was noted in the duration from puncture to reperfusion (MD = 3.33, 95% CI: −4.87 to 11.53, $p = 0.43$, $I^2 = 50\%$) (Figure 4C) and the risk of procedure-related complications (RR = 1.05, 95% CI: 0.64 to 1.74, $p = 0.85$, $I^2 = 0\%$) between the two groups (Figure 4D). Sensitivity analyses were not performed for other outcomes because of a low heterogeneity.

Crossing of the cumulative Z-curve over the trial sequential monitoring boundary in two outcomes (i.e., SRR, and risk of hypotension) (Supplementary Figures 2, 3) on TSA indicated sufficient evidence for these three outcomes to reach a firm conclusion. In contrast, TSA for puncture to reperfusion time and procedure-related complications demonstrated a failure of interaction between the cumulative Z-curve and the futility boundary (Supplementary Figures 4, 5), implicating insufficient evidence for a robust conclusion.

Secondary outcomes: Other prognostic outcomes

Forest plots demonstrated no significant difference in NIHSS score at 24–48 h (MD = −0.21, 95% CI: −1.12 to

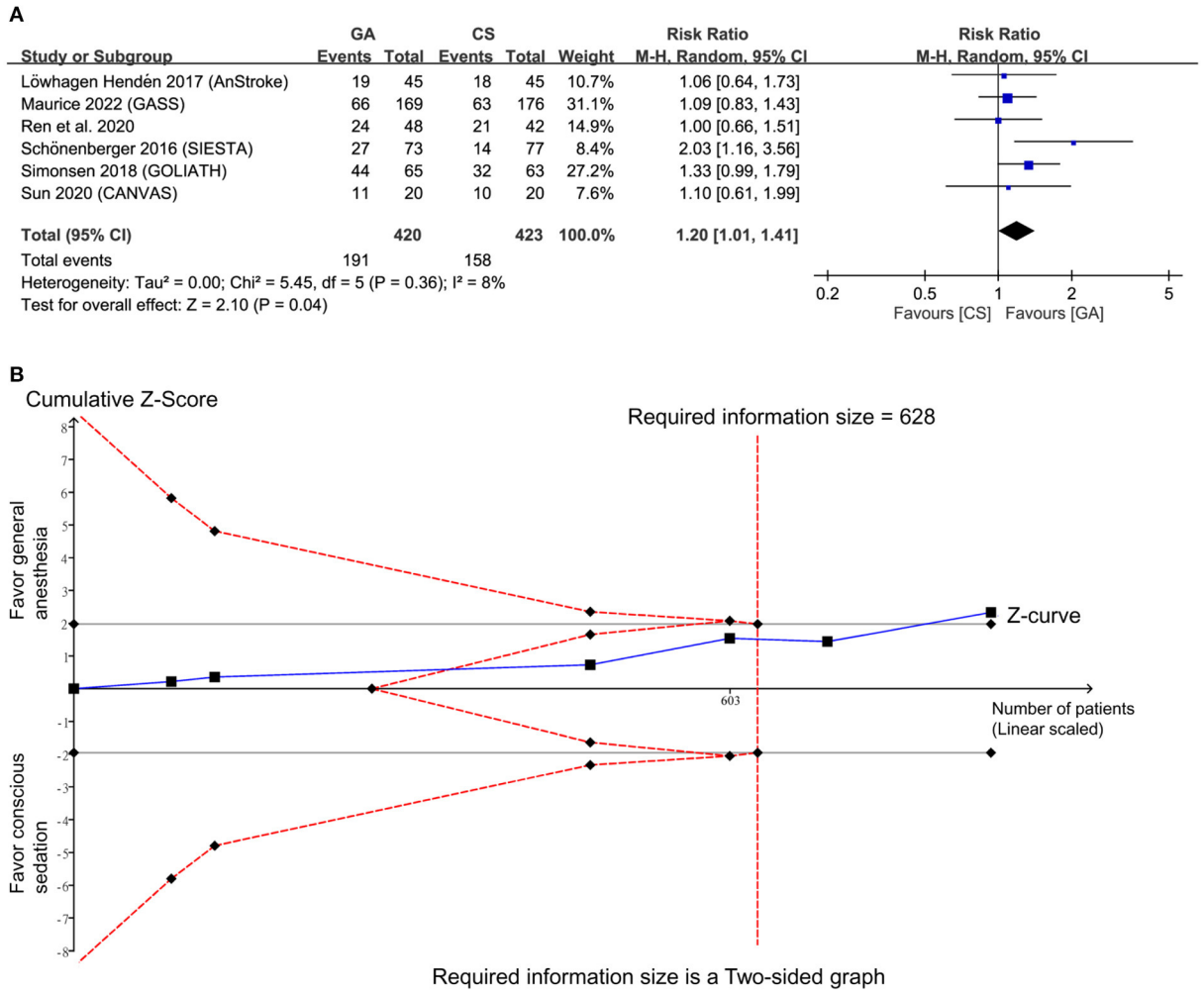

FIGURE 3
(A) Forest plot comparing the risk of good functional outcome between general anesthesia (GA) and conscious sedation (CS) groups. M-H, Mantel-Haenszel; CI, confidence interval; **(B)** Trial sequential analysis of risk of good functional outcome. The risk of type I error was set at 5% with a power of 80%.

0.69, $p = 0.65$, $I^2 = 0$) (Figure 5A) as well as the risks of pneumonia (RR = 1.9, 95% CI: 0.96 to 3.77, $p = 0.07$, $I^2 = 37\%$) (Figure 5B), symptomatic ICH (RR = 0.91, 95% CI: 0.64 to 1.28, $p = 0.58$, $I^2 = 0$) (Figure 5C), and mortality at 3-month follow-up (RR = 0.88, 95% CI 0.64 to 1.22, $p = 0.44$; $I^2 = 12\%$) (Figure 5D) between the two groups. Sensitivity analyses were not performed due to a low heterogeneity for all the outcomes. TSA for difference in NIHSS score was ignored because of inadequate information for TSA boundary construction (Supplementary Figure 6). For the risks of pneumonia, symptomatic ICH, and mortality rate, failure of the cumulative Z-curve to cross the trial sequential monitoring boundary or the futility boundary suggested inconclusive evidence for these outcomes (Supplementary Figures 7–9).

Discussion

Focusing on patients with AIS undergoing EVT, our results revealed a higher successful recanalization rate in GA compared with CS groups (85.7% vs. 75.7%, respectively) with similar duration of puncture to reperfusion and risk of procedure-related complications. There was also no difference in the immediate neurological outcome (i.e., NIHSS score at 24–48 h) between the GA and CS groups. Nevertheless, the 3-month neurological prognosis (i.e., functional independence) was better in the former than the latter (45.5% vs. 37.4%, respectively) without significant differences in the risks of 3-month mortality, symptomatic ICH, and pneumonia between the two groups. Despite a higher incidence of hypotension episodes with the use

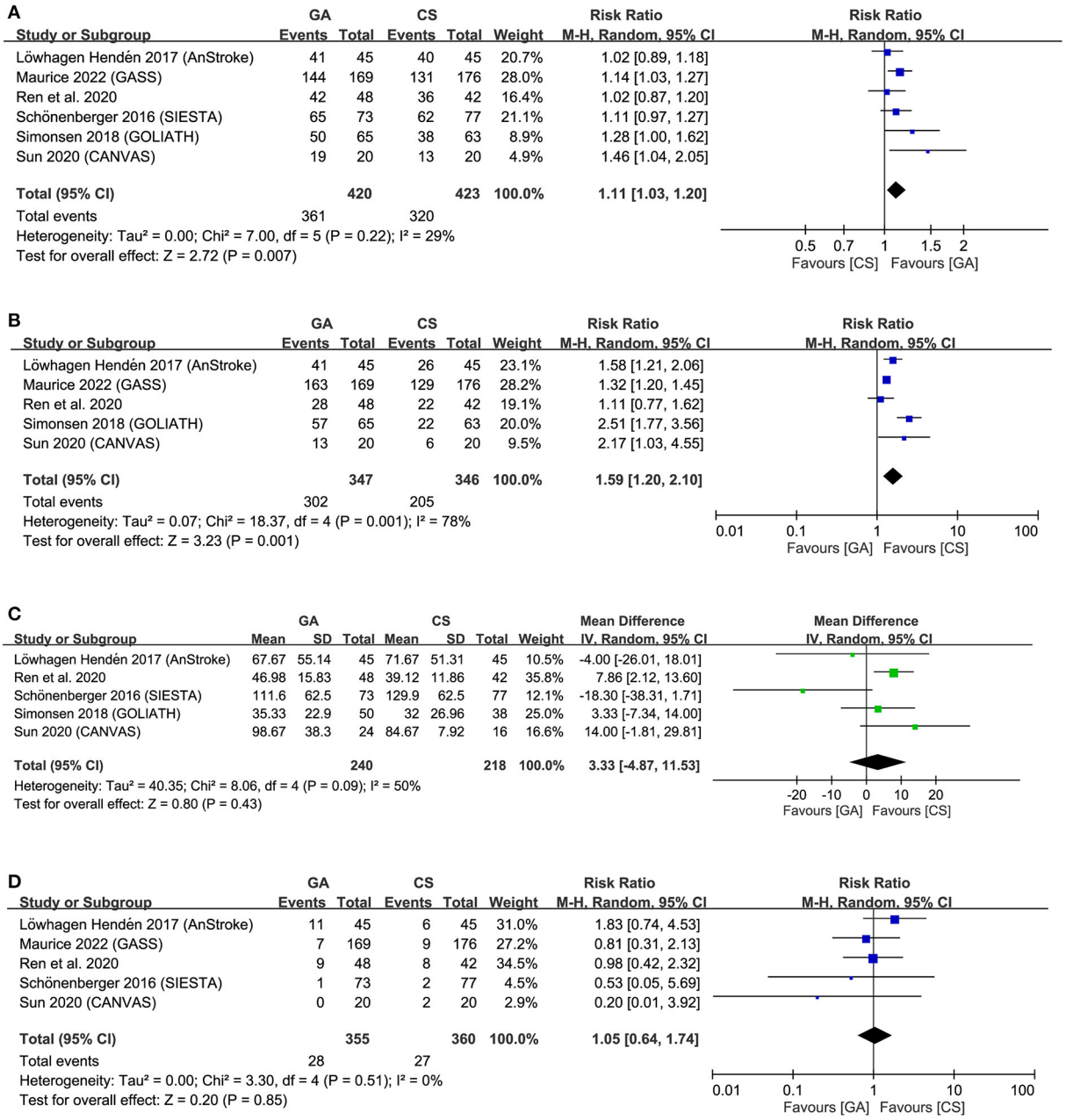

FIGURE 4
Forest plot comparing **(A)** successful recanalization rate, **(B)** hypotension risk, **(C)** duration from puncture to reperfusion, and **(D)** risk of procedure-related complications between general anesthesia (GA) and conscious sedation (CS) groups. IV, inverse variance; CI, confidence interval; M-H, Mantel-Haenszel.

of GA, there was no difference in the accumulative period of hypotension between the two groups. The pooled conversion rate from CS to GA was 10.3%.

Our updated meta-analysis including six RCTs demonstrated that the use of GA was associated with a higher recanalization rate and more favorable functional outcome compared to the use of CS during EVT. The lack of a significant difference in baseline NIHSS score between the two groups together with our findings of better outcomes in the GA group compared to the CS group further supported the superiority of GA to CS in this clinical setting. Indeed, the worse treatment outcome among patients with acute stroke who underwent GA than in those receiving CS in early studies has been found to be attributable to selection bias as those

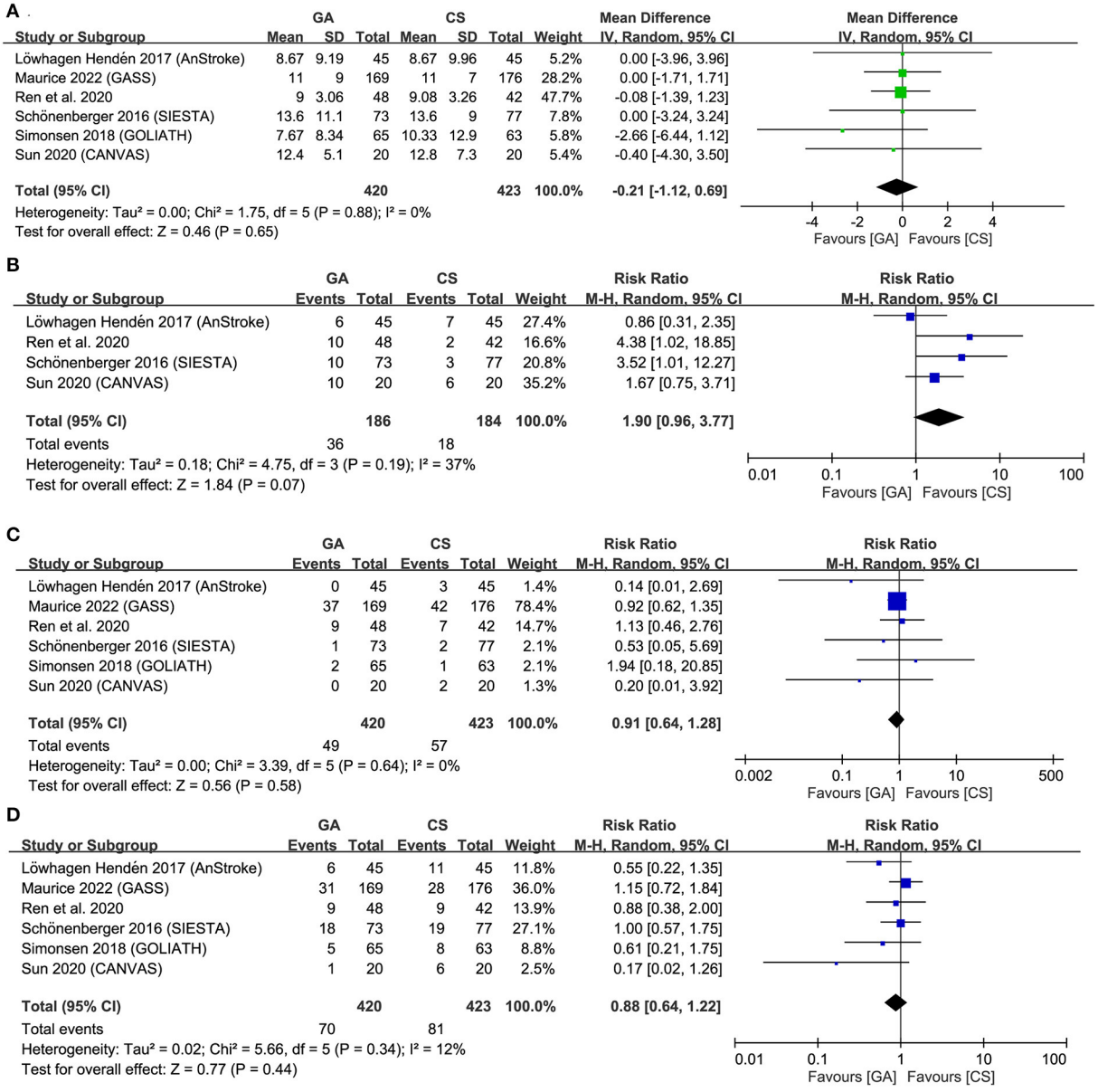

FIGURE 5
Forest plot comparing **(A)** National Institutes of Health Stroke Scale (NIHSS) score at 24–48 h, **(B)** risk of pneumonia, **(C)** symptomatic intracranial hemorrhage (ICH), and **(D)** mortality at 3-month follow-up between general anesthesia (GA) and conscious sedation (CS) groups. IV, inverse variance; CI, confidence interval.

with a more severe condition tended to receive GA for EVA (25). Although our main findings were consistent with those of a previous meta-analysis (7), the advantages of the present investigation included the enrollment of more participants (i.e., 883 patients) and the use of TSA to examine the robustness of our evidence. Our findings on functional outcome were inconsistent with those of a recent meta-analysis that included seven RCTs (anterior cranial circulation, $n = 6$; posterior cranial circulation, $n = 1$) for comparing the impact between GA and non-GA in patients with AIS receiving EVT. While the current study showed a significant association between GA with 3-month functional outcome compared with CS, the recent meta-analysis demonstrated no difference between the GA and non-GA groups (i.e., conscious sedation, local anesthesia, monitored anesthesia care) (26). The discrepancy in results may be explained by the differences in the number of RCTs included for functional outcome analysis; while the current study extracted relevant information from six RCTs (14–19), the

recent meta-analysis only focused on four of our six included studies (14, 15, 17, 19). Further examination of the adequacy of patient sample size of the previous meta-analysis (26) with TSA indicated no crossing between the z-curve and the RIS (data not shown), suggesting insufficient evidence to reach a sound conclusion for this outcome (26). In contrast, TSA of the current study demonstrated a satisfactory sample size for reaching a robust conclusion (Figure 3).

Several factors may contribute to the favorable neurological outcome (i.e., functional independence at 3-months) in the GA group in the current study. Although a number of studies have reported an association between 3-month neurological outcome and recanalization rate (2, 7, 8, 27), the higher recanalization rate in the GA group may only be one of the possible explanations for our promising outcome. In fact, some authors suggested that the possible neuroprotective effects of anesthetic agents being used in GA may be more important contributors to a better outcome compared to a high recanalization rate (28). Consistently, a previous meta-analysis demonstrated that the use of GA was associated with a better neurological outcome compared to those with CS for patients with recanalization failure, supporting the potentially neuroprotective effect of GA (28). Such a neuroprotective action of GA against ischemic brain infarct was further underscored by a reduced final infarct volume and infarct growth in one of our included trials despite the lack of statistical significance of the latter (16). In concert with this proposal, previous clinical and animal studies have reported a neuroprotective effect of anesthetic agents (29). For instance, propofol has been found to be protective against ischemia-reperfusion injury through suppressing oxidative stress-related astrocyte injuries, reinforcing astroglial-mediated neuronal defense (29), reducing cerebral metabolism, enhancing antioxidant ability, and redirecting cerebral blood flow to focal ischemic penumbra area (30). Nevertheless, one of the novel findings of the current meta-analysis was a higher recanalization rate in the GA group compared to that in the CS group without a significant difference in immediate post-procedural NIHSS between the two groups. Although the subsequent more significant improvement in neurological outcome at 3-months in the GA group than that in the CS group may still support a long-term beneficial influence of anesthetics as propofol was used for anesthetic maintenance in four out of our six included studies (16–19), the higher EVT recanalization rate in the former may contribute to the favorable outcome.

Despite potential benefit from propofol, concurrent use of opioid with propofol may lead to respiratory depression and subsequent hypercapnia in the CS group in the current meta-analysis. A hypocapnic state has been shown to widen the plateau region of the autoregulatory curve (31), thereby improving cerebrovascular autoregulatory capacity to maintain a constant CBF in the face of fluctuations in cerebral perfusion pressure (32). Improving the autoregulatory capacity of cerebrovascular is of particular importance in disease situations such as acute stroke in which the patient may experience extremes of cerebral perfusion pressure from rising intracranial pressures or uncontrolled hypertension (32). In this way, our finding of a poorer 3-month neurological outcome in the CS group compared to patients undergoing GA for EVT may support this argument, taking into consideration the possibility of respiratory depression-induced hypercapnia in patients receiving CS.

Several retrospective studies reported that hypotension during the procedure is a poor prognostic factor for EVT (3, 4, 33). In the present meta-analysis, although the risk of hypotension was higher in the GA compared to the CS groups, there was no difference in the cumulative duration of hypotension attack. Our apparently contradictory finding of more significantly improved functional outcome in the GA group compared to patients subjected to CS may suggest a relatively minor role of hypotension provided that there was no prolonged hypotensive episode as well as related complications. Accordingly, our results implied that the beneficial effect of GA may outweigh its associated risk of hypotension given that the patients are monitored under strict protocols. Therefore, in patients scheduled for EVT under GA, a well-designed management strategy for hemodynamic instability should be incorporated into the peri-procedural care protocol to optimize neurological outcome.

Despite a substantially lower anesthetic conversion rate compared with patients receiving local anesthesia without sedation (i.e., 17.5%) (34), the conversion rate in the current meta-analysis remained high at ∼10.3%, which was comparable to that reported in a previous meta-analysis of retrospective studies (i.e., 8.8%) (34). Conversion from a non-GA approach to GA is known to prolong the procedural time and have a theoretical detrimental effect on neurological outcomes as described in a retrospective clinical report on the effect of conversion from CS to GA (9). Taking into account the high conversion rate from a non-GA approach, GA may be the first choice for patients who are scheduled for EVT to minimize the risk of procedural delay. Nevertheless, despite our finding of a tendency of an increased pneumonia risk in patients receiving GA compared to CS based on a random-effects model, it failed to reach statistical significance. Our result was inconsistent with that of a previous meta-analysis that used a fix-effects model and demonstrated a significant increase in risk of pneumonia among patients receiving GA for EVT (7). Because TSA in the current meta-analysis suggested inconclusive evidence, this finding remains a concern for patients receiving GA for EVT.

There are some limitations in our study. First, the sample size of only six RCTs was not large enough to reach a sound conclusion. Besides, most were single-center studies with well-trained neuro-anesthetic teams which may not be available in a real world scenario. Second, because the depth of sedation varies with the goals of CS, the lack of a standardized sedation goal for EVT may affect the conversion rate from CS to GA

which, in turn, could influence the risk of poor clinical outcome (9). Third, the use of different anesthetic agents may bias our results. For instance, there were four RCTs using propofol and one choosing sevoflurane for anesthetic maintenance, while the other did not give details regarding anesthetic agents (14–19). Volatile agents such as sevoflurane have a vasodilatory effect which may worsen clinical outcome by diverting intracranial blood flow away from the ischemic penumbra area, especially in the presence of systemic hypotension (30). Nevertheless, the low heterogeneity across our studies suggested robustness of our findings. Fourth, a previous meta-analysis reported that although the choice of thrombectomy technique (i.e., direct aspiration approach vs. stent-retriever) had no influence on the rate of successful recanalization, a better functional outcome at 3-months was noted in patients receiving direct aspiration (35). In the current meta-analysis, the proportion of patients receiving direct aspiration ranged from 8 to 40% in three trials, while the other three studies did not provide relevant details. Therefore, potential confounding effects from the use of different thrombectomy techniques across our included studies cannot be ruled out. Finally, because we did not include one ongoing trial (Sedation vs. General Anesthesia for Endovascular Therapy in Acute Ischemic Stroke; SEGA, NCT 03263117) in the present meta-analysis because of the unavailability of data for analysis, the impact of the outcomes of that study on the pooled results remains to be elucidated.

Conclusion

Among patients with acute ischemic stroke from large vessel occlusion in the anterior circulation, endovascular thrombectomy under general anesthesia was associated with a higher successful recanalization rate as well as a better 3-month neurological outcome compared to the use of conscious sedation. Because of the small effect size and the tendency toward an increased pneumonia risk related to general anesthesia, whether the benefit of general anesthesia outweighs its risk remains to be elucidated.

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

C-WL and K-CH: conceptualization, literature search, and data extraction. Y-TH and S-ZW: methodology. Y-PC and Y-TH: trial selection. C-HH and M-HC: data analysis. K-CH, C-WL, and C-KS: writing—original draft preparation. K-CH and C-KS: writing—review and editing. All authors have read and agreed to the published version of the manuscript. All authors contributed to the article and approved the submitted version.

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.2022.1017098/full#supplementary-material

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VEGF to CITED2 ratio predicts the collateral circulation of acute ischemic stroke

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Objective: The research objective was to evaluate the predicting role of the vascular endothelial growth factor to CBP/P300-interacting transactivator with Glu/Asp-rich C-terminal domain 2 Ratio (VEGF/CITED2) from peripheral blood mononuclear cells (PBMCs) in the collateral circulation of acute ischemic stroke (AIS).

Methods: In an observational study of patients with AIS, the western blot was applied to test the protein expression of VEGF and CITED2. Then, we calculated the VEGF/CITED2 and collected other clinical data. Binary logistic regression analysis between collateral circulation and clinical data was performed. Finally, receiver operating characteristic (ROC) curve analysis was used to explore the predictive value of VEGF/CITED2.

Results: A total of 67 patients with AIS were included in the study. Binary logistic regression analysis indicated the VEGF/CITED2 (OR 165.79, 95%CI 7.25–3,791.54, $P = 0.001$) was an independent protective factor. The ROC analyses showed an area under the ROC curve of the VEGF/CITED2 was 0.861 (95%CI 0.761–0.961). The optimal cutoff value of 1.013 for VEGF/CITED2 had a sensitivity of 89.1% and a specificity of 85.7%.

Conclusion: In patients with AIS, the VEGF/CITED2 was related to the establishment of collateral circulation. The VEGF/CITED2 is a potentially valuable biomarker for predicting collateral circulation.

Clinical trial registration: ClinicalTrials.gov, identifier: NCT05345366.

KEYWORDS

collateral circulation, VEGF/CITED2, AIS, PBMCs, biomarker

Introduction

Acute ischemic stroke (AIS) is a common neurological event of disability and death (1). Its clinical prognosis varies greatly, such as complete recovery, neurological deficit, and death. This diversity is mainly accounted for by collateral circulation (2). When a cerebral vessel develops stenosis or occlusion, the blood can reach the surrounding peri-infarct zone (referred to as the penumbra) through collateral circulation so that the local ischemic brain tissue can be saved (3). Thus, collateral circulation has a significant impact on the recovery of neurological function and clinical prognosis (4).

Imaging examination is the major method to evaluate collateral circulation, such as transcranial Doppler, traditional single-phase CT angiography (CTA), MR angiography, and digital subtraction angiography (DSA), which depends on specialty devices and professional technicians (5). At present, there is still a lack of an effective and sensitive biomarker to predict collateral circulation. Therefore, the search for the biomarker is beneficial for predicting collateral circulation and judging clinical prognosis.

At present, the establishment of collateral circulation is a hot topic. Ischemic brain tissues recruit peripheral blood mononuclear cells (PBMCs) during the establishment of collateral circulation, among which PBMCs play a significant role by secreting vascular endothelial growth factor (VEGF) (6–8). Multiple studies have observed the high expression of VEGF can promote vasculature remodeling, promote the formation of angiogenesis, and reduce brain infarct volume in the middle cerebral artery occlusion rat model (9, 10). Of those, the most widely studied pathway is the hypoxia-inducible factor-1α (HIF-1α)—VEGF pathway.

Bhattacharya et al. found that CBP/P300-interacting transactivator with Glu/Asp-rich C-terminal domain 2 (CITED2) acts as a molecular switch in the HIF-1α-VEGF pathway (11). CITED2 is a nuclear protein widely expressed in mammalian cells and plays a significant role in the development and growth of cells (12). CITED2 is mainly expressed on peripheral blood mononuclear cells (PBMCs) (13). Under hypoxic conditions, CITED2 involves in the HIF-1α-mediated VEGF angiogenesis pathway (11). In addition, CITED2 may activate the peroxisome proliferator-activated receptor (PPAR) pathway which inhibits VEGF expression (14). However, the mechanism of the action of CITED2 is yet to be elucidated in AIS. Thus, we suggest that CITED2 regulates the VEGF-mediated angiogenesis pathway. The VEGF/CITED2 was better at predicting collateral circulation due to the combined effect of VEGF and CITED2. However, there is no direct evidence that the VEGF/CITED2 in PBMCs can predict the collateral circulation of AIS.

Alberta Stroke Program Early CT Score (ASPECTS), a semiquantitative approach in non-contrast CT, was originally developed to evaluate infarct size, clinical prognosis, and the probability of hemorrhagic transformation in patients with AIS (15). Because of the close relationship between clinical prognosis and collateral circulation, ASPECTS can also be used as an indirect score for evaluating collateral circulation (16). With the imaging methods developing, ASPECTS is implemented into diffusion-weighted imaging (DWI) to evaluate the collateral circulation. Furthermore, DWI is indicated with ischemic tissue of early AIS. Thus, as compared to ASPECTS, DWI-ASPECTS more clearly reflects the collateral circulation of AIS. Yuan et al. evaluated the predictive value of DWI-ASPECTS and conducted a receiver operating characteristic (ROC) curve analysis in 178 patients with AIS. They found the area under the ROC curve (AUC) of 0.932, the sensitivity of 81%, and a specificity of 94.1% (17). In contrast to DSA, CTA, CTP, and perfusion-weighted imaging (PWI), DWI-ASPECTS offers the advantages of being non-invasive and does not injection of contrast agents. At the same time, it has a few disadvantages, such as time-consuming and patient cooperation. According to practical conditions at our hospital, most patients with AIS were examined by DWI. Only a few patients were examined by DSA, CTA, CTP, and PWI. Therefore, we explored the relationship between VEGF/CITED2 and collateral circulation by DWI-ASPECTS. In this study, it provides an objective basis for VEGF/CITED2, as an effective biomarker, to predict the collateral circulation of AIS.

Methods

Study design

The institutional ethics committee of the hospital approved this study according to the principles of the Declaration of Helsinki. This was a single-center, prospective, observational study. The patients would perform diffusion-weighted imaging (DWI). Based on the DWI-ASPECTS, we classified patients into two groups: the good collateral group and the poor collateral group. All the patients were collected venous blood samples in the early morning the next day and sent to the laboratory within 1 h. The blood samples had been stored in a 4°C refrigerator before sending to the laboratory. Moreover, the patients were evaluated by the National Institute of Health Stroke Scale (NIHSS) score and the Modified Barthel Index (MBI) score on day 0 and day 7 of hospitalization. Follow-up was carried out after 3 months and recorded clinical outcomes by the Modified Rankin Scale (mRS) score (Figure 1).

Patient selection

Patients were diagnosed with AIS in the neurology department of our hospital from November 2020 to November 2021. The data of 90 AIS patients were obtained in this study. However, 23 patients were excluded due to various reasons. Of these, 10 patients were excluded due to the onset time>3 days, three patients were excluded because they combined with a tumor, five patients were excluded because they refused to have blood drawn, three patients were excluded because they were not completed the MRI+DWI, and two patients were excluded because the infarct had not been found in DWI. Thus, a total of 67 patients were included in this study (Figure 2).

The inclusion criteria are as follows: ① the onset time <3 days; ② age≥18 years old; and ③ in accordance with the

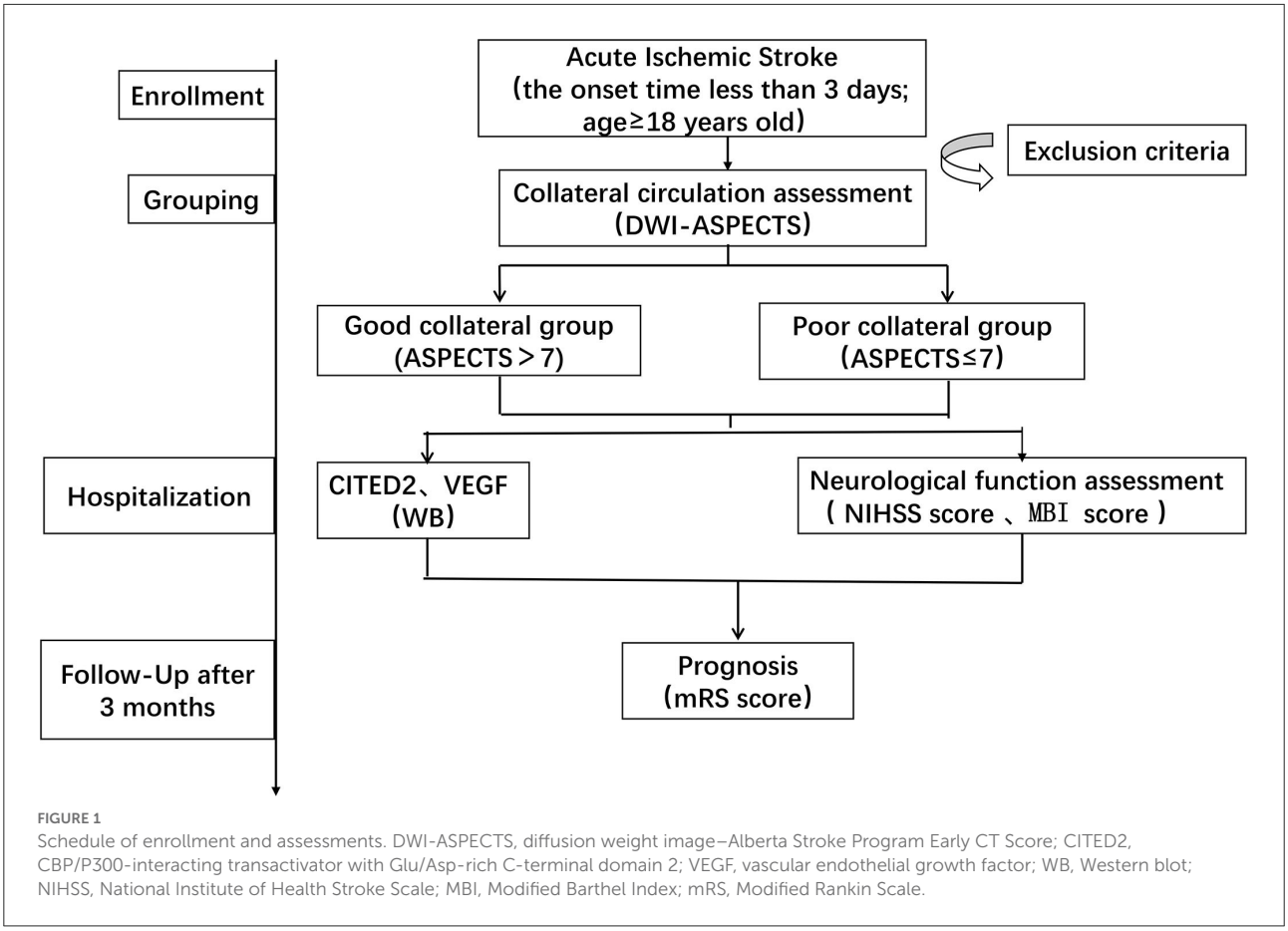

FIGURE 1
Schedule of enrollment and assessments. DWI-ASPECTS, diffusion weight image–Alberta Stroke Program Early CT Score; CITED2, CBP/P300-interacting transactivator with Glu/Asp-rich C-terminal domain 2; VEGF, vascular endothelial growth factor; WB, Western blot; NIHSS, National Institute of Health Stroke Scale; MBI, Modified Barthel Index; mRS, Modified Rankin Scale.

diagnostic criteria in the Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke in China 2018 (18). The exclusion criteria are as follows: ① the patients had received treatment of thrombolysis or thrombectomy; ② the patients with AIS combined with other diseases that may affect the CITED2 and VEGF expressions, such as acute myocardial infarction, peripheral artery occlusion disease, congenital heart defect, and tumor; ③ the patients exhibited serious heart, liver, or kidney diseases; ④ disturbance of consciousness or mental illness; and ⑤ pregnant and lactating patients.

Grouping definition

The total score of DWI-ASPECTS is 10 points. The midbrain or pons with high signal was decreased by two points, and other each area was one point. According to infarct lesions involving different circulation, the DWI-ASPECTS corresponding to the circulation was adopted (19, 20). Scores >7 points were defined as the good collateral group, and scores ≤ 7 points were defined as the poor collateral group.

Peripheral blood mononuclear cell preparation

The 4 ml venous blood samples were collected from all patients within 24 h after hospitalization. The blood samples were diluted with 4 ml of phosphate-buffered saline (Boster, Wuhan) free of calcium and magnesium. Then, 8 ml of venous blood was slowly added to the surface of 8 ml of Lymphocyte Separation Solution (TBD, Tianjin) and centrifuged at 1000 x g for 30 min. The second layer, called the white film lays, was collected and transferred to a centrifuge tube. The 5 ml phosphate-buffered saline free of calcium and magnesium was added to the centrifuge tube and centrifuged at 300 x g for 10 min, and the supernatant was discarded. This step of centrifugation was repeated three times to obtain purified PBMCs.

Western blot

PBMCs (1 x 10^7 cells) were lysed by 200 μl RIPA lysis buffer (CWBIO, Beijing) supplement with protease inhibitor cocktail (CWBIO, Beijing) and phosphatase

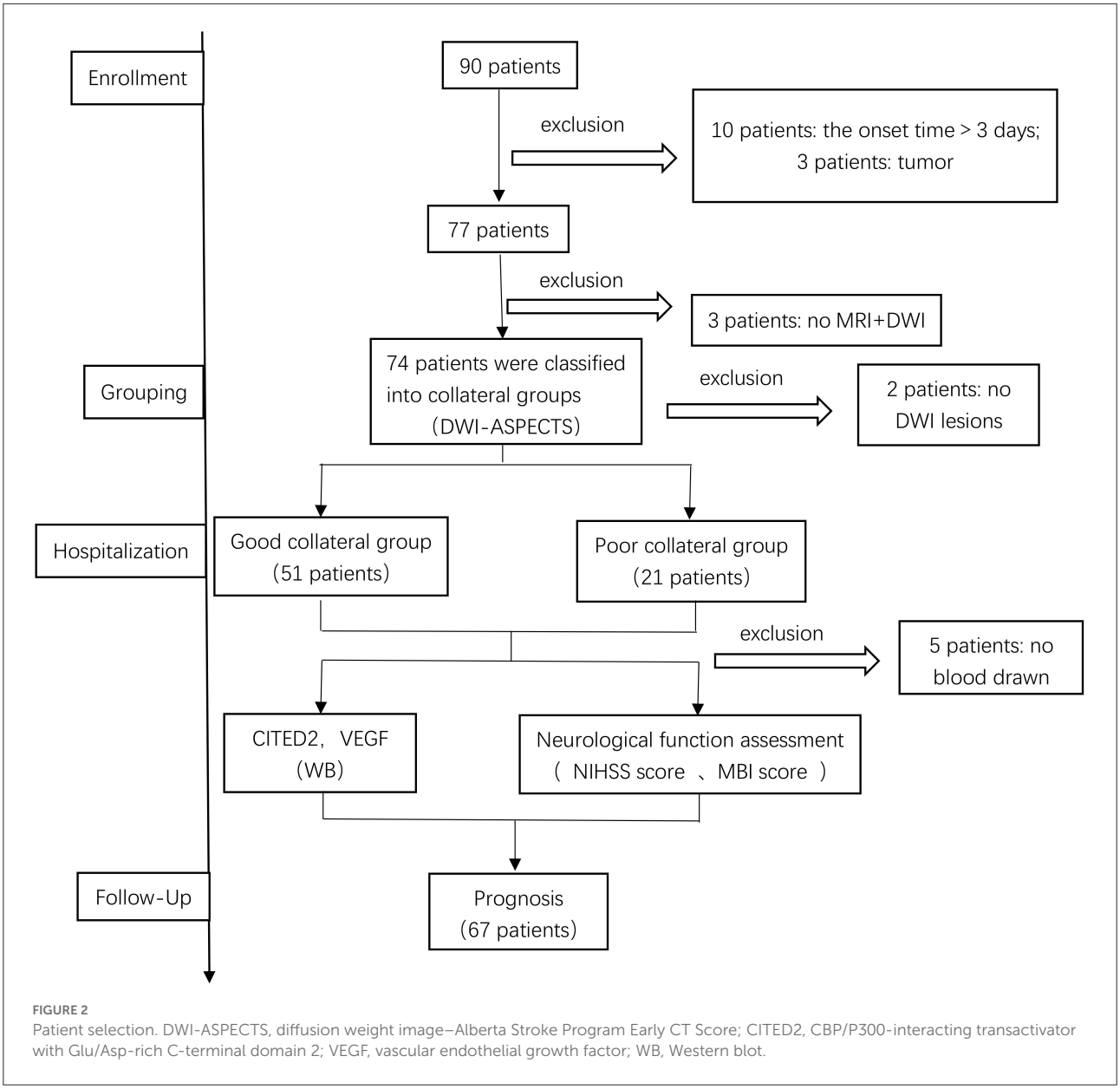

FIGURE 2
Patient selection. DWI-ASPECTS, diffusion weight image–Alberta Stroke Program Early CT Score; CITED2, CBP/P300-interacting transactivator with Glu/Asp-rich C-terminal domain 2; VEGF, vascular endothelial growth factor; WB, Western blot.

inhibitor cocktail (CWBIO, Beijing). After lysis at 4°C for 15 min, the proteins were centrifuged at 8000 x g for 15 min. The protein concentration in the supernatant of cell extracts was determined using a bicinchoninic acid protein assay kit (Beyotime, Shanghai). Then, proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Beyotime, Guangdong) and transferred to polyvinylidene difluoride membranes (Millipore, American). The membranes were blocked with 5% no-fat powdered milk (Sangon, Shanghai) in tris-buffered saline (Solarbio, Beijing) with 1% tween (Solarbio, Beijing) for 2 h at room temperature. The membrane was probed with diluted primary antibodies CITED2 (Abclonal, 1:1,000), VEGF (Bioss, 1:1,000), and β-actin (Bioworld, 1:10,000) overnight at 4°C. On the next day, the membrane was re-probed with a secondary antibody, goat anti-rabbit antibody IgG (CWBIO, 1:20,000), labeled by enhanced chemiluminescence hypersensitive luminescent solution (Millipore, American) for 2 h at room temperature, and quantified by densitometry.

TABLE 1 General data of the included patients.

General data	Good collateral group ($N = 46$)	Poor collateral group ($N = 21$)	t/x^2	P-value
Age [mean (SD), y]	64.04 (10.06)	60.38 (13.94)	−1.220	0.227
Gender [*n* (%)]			0.082	0.774
Male	29 (63%)	14 (66.7%)		
Female	17 (37%)	7 (33.3%)		
Hypertension [*n* (%)]			0.165	0.684
Yes	35 (76.1%)	15 (71.4%)		
No	11 (23.9%)	6 (28.6%)		
Diabetes [*n* (%)]			0.295	0.587
Yes	23 (50%)	12 (57.1%)		
No	23 (50%)	9 (42.9%)		
Hyperlipidemia [*n* (%)]			0.000	1.000
Yes	7 (15.2%)	3 (14.3%)		
No	39 (84.8%)	18 (85.7%)		
History of coronary heart disease [*n* (%)]			-	0.301
Yes	4 (8.7%)	0 (0%)		
No	42 (91.3%)	21 (100%)		
History of stroke [*n* (%)]			0.069	0.792
Yes	4 (8.7%)	3 (14.3%)		
No	42 (91.3%)	18 (85.7%)		
Smoking history [*n* (%)]			0.165	0.684
Yes	11 (23.9%)	6 (28.6%)		
No	35 (76.1%)	15 (71.4%)		
Drinking history [*n* (%)]			-	1.000
Yes	1 (1.5%)	0		
No	45 (97.8%)	21 (100%)		

Neurological deficit score

NIHSS was used to quantitatively score the neurological deficit of AIS patients on days 0 and 7 of hospitalization, ranging from 0 to 42 points. The higher the score, the more severe the symptoms.

Daily life ability score

MBI was used to quantitatively score the daily life ability of AIS patients on day 0 and day 7 of hospitalization, ranging from 0 to 100 points. The higher the score, the better the daily life ability of the patients.

Prognostic neurological function score

After 3 months, the patient follow-up was carried out by telephone and recorded clinical outcomes of AIS patients by mRS score. A score of 0–2 was defined as a good prognosis of neurological function, and a score of 3–6 was defined as a poor prognosis of neurological function.

Statistical analysis

Data were analyzed by using SPSS 26.0 software. Quantitative data meeting normal distribution were expressed as the mean ± standard deviation and applied an independent t-test in the difference analysis of the two groups. If quantitative data did not conform to the normal distribution, data were expressed as the median (interquartile range) and applied the Mann–Whitney U-test in the difference analysis of the two groups. The counting data were expressed as the cases (percentage) and analyzed by the chi-square test. The correlation between VEGF/CITED2 and collateral circulation was evaluated using binary logistic regression analysis. ROC curve analysis was used to determine the cutoff point, sensitivity, and specificity of VEGF/CITED2. Hypothesis tests were all two-tailed tests, and $P < 0.05$ indicated statistical significance.

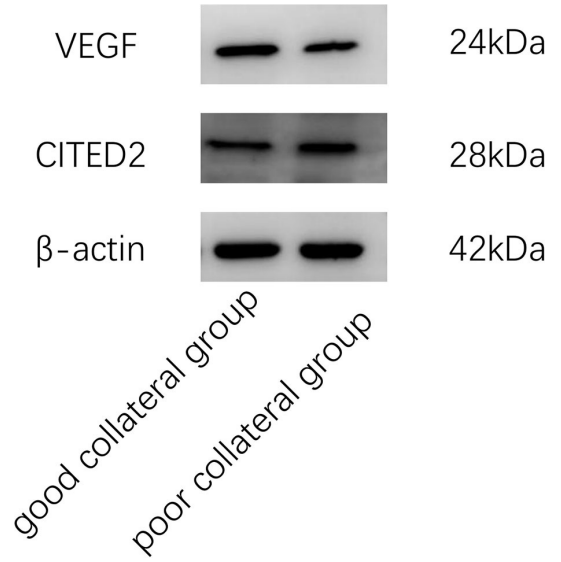

FIGURE 3
Protein expression of CITED2 and VEGF.

Results

Baseline characteristics

The good collateral group included 46 patients, and the poor collateral group included 21 patients. There was no statistically significant difference between the two groups in general data such as age, gender, hypertension, diabetes, hyperlipidemia, history of coronary heart disease, history of stroke, smoking history, and drinking history ($P > 0.05$) (Table 1). In clinical data, WBC count, neutrophil count, Fib, Hcy, and VEGF/CITED2 were found to be significantly different (Figure 3) ($P < 0.05$). In addition, there was no statistically significant difference between the two groups in other clinical data such as lymphocyte count, neutrophil-to-lymphocyte ratio (NLR), uric acid (UA), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glycated hemoglobin A1c (HbA1c), CITED2, and VEGF($P > 0.05$) (Table 2).

Clinical prognosis

Compared with day 0, the NIHSS score was decreased and the MBI score was increased in the two groups on day 7. The good collateral group could significantly decrease in NIHSS score and increase in MBI score more than the poor collateral group. The good collateral group has a high percentage (93.5%) of good prognosis and a low percentage (6.5%) of poor prognosis. The poor collateral group has a low percentage (47.6%) of good prognosis and a high percentage (52.4%) of poor prognosis. The differences in clinical prognosis between the two groups were statistically significant ($P < 0.05$) (Table 3).

The correlation between VEGF/CITED2 and collateral circulation

Univariate logistic regression analysis was conducted by taking collateral circulation as the dependent variable and age, gender, hypertension, diabetes, hyperlipidemia, history of coronary heart disease, history of stroke, smoking history, drinking history, WBC count, neutrophil count, lymphocyte count, NLR, UA, TC, TG, HDL, LDL, Hcy, Fib, HbA1c, CITED2, VEGF, and VEGF/CITED2 as independent variables. The results demonstrated the WBC count (OR = 0.77, 95%CI 0.63–0.95, $P = 0.016$), neutrophil count (OR = 0.78, 95%CI 0.63–0.95, $P = 0.016$), NLR (OR = 0.83, 95%CI 0.68–1.02, $P = 0.075$), Hcy (OR = 0.87, 95%CI 0.75–1.00, $P = 0.054$), and Fib (OR = 0.59, 95%CI 0.34–1.02, $P = 0.059$) were the risk factors, and VEGF/CITED2 (OR = 190.13, 95%CI 11.04–3,273.83, $P = 0.000$) was the protective factor (Table 4).

To adjust confounding factors, variables with $P < 0.1$ (WBC count, neutrophil count, NLR, Hcy, Fib, and VEGF/CITED2) in the univariate logistic regression analysis were tested in further multivariable logistic regression analysis. The results showed that VEGF/CITED2 was an independent protective factor for collateral circulation (Table 5).

The predictive value of VEGF/CITED2

Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive value of VEGF/CITED2 in the collateral circulation of AIS. ROC analyses showed an AUC of 0.861 (95%CI 0.761–0.961). The optimal cutoff value of 1.013 for VEGF/CITED2 had a sensitivity of 89.1% and a specificity of 85.7% (Figure 4 and Table 6).

Discussion

By contrasting the good collateral group to the poor collateral group in AIS, we did not only find the good collateral group had a good clinical prognosis, but also that VEGF/CITED2 had a prognostic value for predicting the establishment of collateral circulation in AIS. Moreover, WBC count, neutrophil count, NLR, Hcy, and Fib were the risk factors for collateral circulation and significant differences between the groups.

Collateral circulation was a decisive factor for clinical prognosis in AIS. Our results revealed patients in the good collateral group developed mild neurological deficits, high daily living ability, and good clinical prognosis. These findings had

TABLE 2 Clinical data of the included patients.

Clinical data	Good collateral group	Poor collateral group	t/x^2	*P*-value
WBC count($\times 10^9$ cells/L)	8.02 ± 1.93	9.98 ± 3.85	2.217	0.036**
Neutrophil count($\times 10^9$ cells /L)	5.16 ± 1.95	7.13 ± 3.83	2.234	0.035**
Lymphocyte count($\times 10^9$ cells /L)	2.03 ± 0.65	2.02 ± 0.94	−0.010	0.992
NLR	2.99 ± 2.21	4.55 ± 3.99	1.674	0.106
UA(μmol/L)	324.48 ± 80.27	339.00 ± 105.40	0.621	0.537
TC(mmol/L)	4.94 ± 1.55	4.85 ± 1.01	−0.241	0.811
TG(mmol/L)	1.88 ± 1.46	1.76 ± 1.10	−0.327	0.745
HDL(mmol/L)	1.03 ± 0.25	1.05 ± 0.22	0.382	0.704
LDL(mmol/L)	3.18 ± 1.41	3.10 ± 0.80	−0.246	0.807
Hcy(μmol/L)	12.28 ± 3.69	14.22 ± 3.50	2.028	0.047**
Fib(g/L)	3.18 ± 0.82	3.75 ± 1.36	2.120	0.038**
HbA1c(%)	7.22 ± 1.92	7.11 ± 1.79	−0.213	0.832
CITED2	1.16 ± 1.77	1.17 ± 0.64	0.034	0.973
VEGF	1.23 ± 0.68	1.02 ± 0.64	−1.182	0.241
VEGF /CITED2	1.41 ± 0.97	0.89 ± 0.26	−2.453	0.017**
TOAST classification			3.102	0.078*
Large atheromatous	20 (43.5%)	14 (66.7%)		
Non- large atheromatous				
Cardiogenic embolism	0 (0%)	0 (0%)		
Small artery occlusion	24 (52.2%)	4 (19.0%)		
Other defined etiology	2 (4.3%)	3 (14.3%)		
Unknown etiology	0 (0%)	0 (0%)		

WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; UA, uric acid; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hcy, homocysteine; Fib, fibrinogen; HbA1c, glycated hemoglobin A1c; CITED2, CBP/P300-interacting transactivator with Glu/Asp-rich C-terminal domain 2; VEGF, vascular endothelial growth factor; TOAST, Trial of ORG 10172 in Acute Stroke Treatment.
*$P < 0.1$; **$P < 0.05$.

TABLE 3 Clinical outcome of the included patients.

Evaluation indicators	Good collateral group	Poor collateral group	t	*P*-value
NIHSS score at day 0	5.76 ± 3.09	10.62 ± 7.34	2.918	0.008**
NIHSS score at day 7	2.72 ± 2.21	9.48 ± 9.86	3.107	0.005**
MBI score at day 0	74.83 ± 22.83	50.76 ± 21.80	−4.058	0.000**
MBI score at day 7	87.99 ± 16.39	60.11 ± 25.31	−4.623	0.000**
mRS score at 3 months	0.49 ± 1.13	2.52 ± 2.16	4.067	0.000**
Prognosis			15.675	0.000**
Good	43 (93.5%)	10 (47.6%)		
Poor	3 (6.5%)	11 (52.4%)		

NIHSS, National Institute of Health Stroke Scale; MBI, Modified Barthel Index; mRS, Modified Rankin Scale.
**$P < 0.05$.

been similar to the result of previous studies (21, 22). This may be explained by the mechanism that good collateral circulation was able to augment brain tissue perfusion and promote the recovery of neurological function, thereby improving the quality of life of patients. It also suggested the DWI-ASPECTS we adopted to evaluate collateral circulation was basically in line with actual collateral status.

VEGF/CITED2 was a protective factor of collateral circulation of AIS. Contrasting with the poor collateral group in clinical data, we found that the VEGF/CITED2 was higher in the good collateral group. Then, we adjusted confounding factors by binary logistic regression analysis and found VEGF/CITED2 was a protective factor of collateral circulation of AIS. However, its correlation related to the collateral circulation of AIS has

TABLE 4 Univariate logistic regression analysis of collateral circulation-related factors in AIS.

	P-value	OR	95% CI	
			LCI	UCI
Age(y)	0.226	1.03	0.98	1.08
Gender	0.774	0.85	0.29	2.53
Hypertension(yes/no)	0.685	0.79	0.26	2.52
Diabetes(yes/no)	0.588	1.33	0.47	3.77
Hyperlipidemia(yes/no)	0.921	0.93	0.22	4.01
History of coronary heart disease(yes/no)	0.999	0.00	0.00	-
History of stroke(yes/no)	0.492	1.75	0.36	8.63
Smoking history(yes/no)	0.685	1.27	0.40	4.08
Drinking history(yes/no)	1.000	0.00	0.00	-
WBC count($\times 10^9$ cells/L)	0.016**	0.77	0.63	0.95
Neutrophil count($\times 10^9$ cells/L)	0.016**	0.78	0.63	0.95
Lymphocyte count($\times 10^9$cells/L)	0.992	1.00	0.50	2.01
NLR	0.075*	0.83	0.68	1.02
UA(μmol/L)	0.531	1.00	0.99	1.00
TC(mmol/L)	0.807	1.05	0.72	1.54
TG(mmol/L)	0.741	1.07	0.72	1.60
HDL(mmol/L)	0.699	0.66	0.08	5.56
LDL(mmol/L)	0.803	1.06	0.69	1.63
Hcy(μmol/L)	0.054*	0.87	0.75	1.00
Fib(g/L)	0.059*	0.59	0.34	1.02
HbA1c(%)	0.829	1.03	0.78	1.37
CITED2	0.972	0.99	0.71	1.40
VEGF	0.249	1.82	0.66	4.98
VEGF /CITED2	0.000**	190.13	11.04	3,273.83

WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; UA, uric acid; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hcy, homocysteine; Fib, fibrinogen; HbA1c, glycated hemoglobin A1c; CITED2, CBP/P300-interacting transactivator with Glu/Asp-rich C-terminal domain 2; VEGF, vascular endothelial growth factor.
*$P < 0.1$; **$P < 0.05$.

not been investigated. Previous researches have confirmed that the VEGF and CITED2 are related to collateral circulation. The most widely accepted explanation is that CITED2 plays a negative role in the HIF-1α-VEGF angiogenesis pathway in hypoxia (11, 23, 24). Under hypoxia, CITED2 binds to CBP/P300 competitively with HIF-1α and forms CITED2-CBP/P300 complex. The complex inhibits the expression of VEGF gene encoding protein (25). In addition, it has been proposed that CITED2, as an anti-inflammatory cytokine, may play a role in angiogenesis inhibition through the PPAR pathway (26). The activated PPAR can inhibit the expression of VEGF through the HIF-1α pathway (27).

In general, CITED2 plays a negative role in the VEGF-mediated angiogenesis pathway that has been verified (28–30). VEGF is a potent angiogenic factor. VEGF/CITED2, a comprehensive ratio of VEGF and CITED2, can predict the collateral circulation of AIS more objectively. Indeed, VEGF/CITED2 has not been reported in the literature. Our study implied that VEGF/CITED2 in the good collateral circulation was higher than the poor collateral circulation, which was a statistically significant difference. We speculated that VEGF/CITED2 may be a balance point in the collateral circulation. When good collateral circulation is established, the inhibition of CITED2 is weaker and VEGF mainly plays a pro-angiogenic effect. Conversely, when poor collateral circulation is established, VEGF plays a weaker pro-angiogenic effect and CITED2 mainly exerts an effect in negative regulation. On the contrary, VEGF and CITED2 are expressed in mononuclear macrophages (13, 31). After AIS, the brain parenchyma generates the cytokines and chemokines leading to inflammatory cells in peripheral blood being attracted to infiltrate the ischemic area (32, 33). Thus, we detected VEGF/CITED2 of PBMCs by Western blot. Our results showed significant differences in the VEGF/CITED2 of PBMCs between these two groups.

TABLE 5 Multivariate logistic regression analysis of collateral circulation-related factors in AIS.

	P-value	OR	95% CI	
			LCI	UCI
WBC count ($\times 10^9$ cells/L)	0.855	1.11	0.37	3.34
Neutrophil count ($\times 10^9$ cells/L)	0.806	0.84	0.21	3.34
NLR	0.720	0.91	0.53	1.56
Hcy(μmol/L)	0.219	0.89	0.74	1.07
Fib(g/L)	0.561	0.79	0.36	1.74
VEGF /CITED2	0.001**	165.79	7.25	3,791.54

WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; Hcy, homocysteine; Fib, fibrinogen; CITED2, CBP/P300-interacting transactivator with Glu/Asp-rich C-terminal domain 2; VEGF, vascular endothelial growth factor.
*$P < 0.1$; **$P < 0.05$.

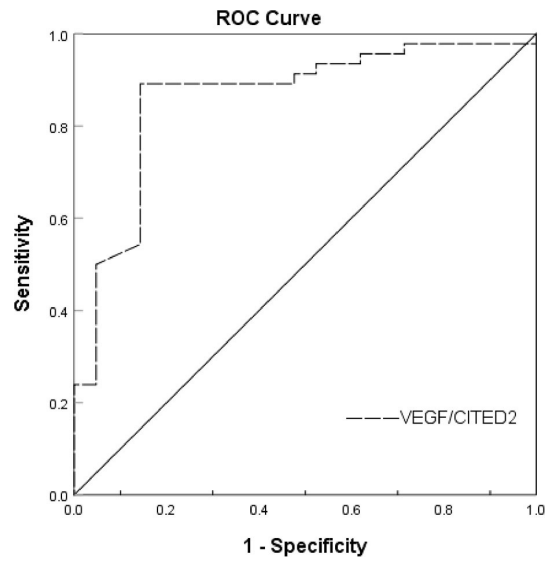

FIGURE 4
ROC curve analysis of the VEGF/CITED2 in the collateral circulation of AIS.

Further analysis of the ROC curve results showed that the VEGF/CITED2 of PBMCs was an independent predictor of AIS. The optimal cutoff value of 1.013 means the AIS patients are more likely to have good collateral circulation if VEGF/CITED2>1.013. At this point, the VEGF/CITED2 had a sensitivity of 89.1% and a specificity of 85.7%. Due to the lack of studies on the correlation between VEGF/CITED2 and cerebral collateral circulation, it was difficult to make a direct comparison in the optimal cutoff value of VEGF/CITED2. At the same time, we looked through the literature on biomarkers of cerebral collateral circulation. The sphinganine-1- phosphate (S1P) is a bioactive lipid that acts on receptors to mediate various cellular processes, including cell growth, differentiation, angiogenesis, and immunoregulation (34, 35). Recently, Fang Yu et al. classified AIS patients into two groups (the good collateral group and the poor collateral group) by the Tan score of CTA. They tested S1P in plasma and found that the AUC of S1P was 0.738 (95%CI 0.60–0.85) (36). They considered that the S1P was an independent predictor of cerebral collateral circulation. Because of different study populations and collateral circulation grouping, the comparison could not be performed for S1P and VEGF/CITED2. Thus, the biomarker of cerebral collateral circulation has been poorly studied, which still needs to be further explored in future.

Besides, it was found that the differences between the good collateral group and the poor collateral group were significant in AIS such as WBC count, neutrophil count, NLR, Fib, and Hcy. In univariate logistic regression analysis, they also were the risk factors for collateral circulation. There are the following mechanisms that may be involved: ① After AIS, the WBC is attracted to infiltrate the ischemic area. Among these, neutrophils are the first immune cells to the ischemic tissue and further aggravate ischemic damage (37). A few reports have shown that neutrophils inhibit angiogenesis by releasing elastase and α-defensins (also known as human neutrophil peptides) (27, 38). The leukocyte has been considered to be a marker of inflammatory response after stroke and NLR is an objective indicator of neutrophil and lymphocyte. Thus, the high levels of the inflammatory response are deleterious to collateral circulation. ② Hcy can cause damage to vascular endothelial cells, leading to vascular endothelial cell dysfunction and inhibition of collateral circulation (39, 40). ③ The vascular endothelial cells are required for the localized degradation of the fibrin matrix so that they can proliferate, migrate, and form capillaries. A high level of Fib can inhibit this process, which in turn inhibits collateral circulation (41–43). However, when in the multivariate logistic regression analysis, WBC count, neutrophil count, Fib, and Hcy were found no statistically significant (P>0.05). We speculate that there are two possibilities. First, the small sample size may lead to false-negative results. Second, there may exist an association among these factors such as WBC count, neutrophil count, NLR, and VEGF/CITED2. The VEGF and CITED2, secreted by inflammatory cells, play an

TABLE 6 ROC analysis of the poor collateral circulation in AIS.

	AUC	*P*-value	95% CI		Optimal cutoff value	Specificity	Sensitivity
			LCI	UCI			
VEGF/CITED2	0.861	0.000**	0.761	0.961	1.013	85.7%	89.1%

CITED2, CBP/P300-interacting transactivator with Glu/Asp-rich C-terminal domain 2; VEGF, vascular endothelial growth factor.
**$P < 0.05$.

anti-inflammatory effect (13, 44). When the VEGF/CITED2 was controlled by multivariate logistic regression analysis, the statistical correlation of WBC count, neutrophil count, and NLR disappears.

In this study, we were pleasantly surprised that VEGF/CITED2 is related to the collateral circulation of AIS. As a predictor for collateral circulation, VEGF/CITED2 is also an independent protective factor. However, there are some limitations to this study. For one thing, we could not further adjust confounders due to the small sample size. For another, the DWI-ASPECTS might have certain effects on patients' grouping. Although this score was performed for an initial evaluation of clinical prognosis, further evaluation of the predictive value of VEGF/CITED2 is still needed by adopting DSA or other precise methods.

Conclusion

Collateral circulation was a decisive factor for clinical prognosis in AIS. Moreover, VEGF/CITED2 in PBMCs had been associated with the collateral circulation of AIS. It was an independent protective factor and has a potential predictive value in the collateral circulation of AIS.

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 Institutional Ethics Committee of the Zhujiang Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ML: investigation, methodology, writing—original draft, and writing—reviewing and editing. YL and ZX: investigation, methodology, and writing—original draft. XY: data analysis and investigation. JC: investigation and supervision. ST: data analysis and supervision. PZ: conceptualization, resources, and writing—reviewing and editing. YG: conceptualization, resources, writing—reviewing and editing, supervision, and project administration. 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.

Publisher's note

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Plasma anion gap and risk of in-hospital mortality in patients with spontaneous subarachnoid hemorrhage

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Background: The association between the serum anion gap (AG) and prognosis of patients with spontaneous subarachnoid hemorrhage (SAH) remains unknown. Thus, this study aimed to explore the association between AG levels and mortality in patients with SAH in the intensive care unit (ICU).

Methods: This was a retrospective analysis of data stored in the Medical Information Mart for Intensive Care–IV and eICU Collaborative Research databases. Critically ill patients diagnosed with spontaneous SAH were included. The primary outcome measure was in-hospital all-cause mortality. A multivariate Cox proportional hazards regression model and a restricted cubic spline were used to evaluate the relationship between AG concentration and outcomes. Kaplan–Meier curves were used to compare cumulative survival among patients with AG levels.

Results: A total of 1,114 patients were enrolled. AG concentration was significantly associated with in-hospital all-cause mortality [hazard ratio ([HR], 1.076 (95% confidence interval (CI), 1.021–1.292; $p = 0.006$)]. The risk of mortality was higher in the Category 2 group (AG ≥10 mmol/L and <13 mmol/L; HR, 1.961; 95% CI, 1.157–3.324; $p = 0.0$) and the Category 3 group (AG ≥13 mmol/L; HR, 2.151; 95% CI, 1.198–3.864; $p = 0.010$) than in the Category 1 group (AG < 10 mmol/L). Cumulative survival rates were significantly lower in patients with higher AG levels (log-rank $p < 0.001$).

Conclusions: In-hospital and ICU mortalities increase with increasing AG concentration in patients with SAH. An increased serum AG level is an independent, significant, and robust predictor of all-cause mortality. Thus, serum AG levels may be used in the risk stratification of SAH.

KEYWORDS

subarachnoid hemorrhage, anion gap, intensive care unit, in-hospital mortality, ICU mortality

Introduction

Spontaneous subarachnoid hemorrhage (SAH) accounts for 5–10% of all strokes (1). Patients with SAH tend to be younger than patients with other stroke subtypes, thus leading to an enormous burden of premature mortality (2). Half of surviving SAH patients experience long-term neuropsychological complications and lower quality of

life (3). Early identification and appropriate treatment regimens can improve the overall survival of patients with SAH. Thus, a robust and easily accessible clinical indicator for determining prognosis is needed for patients with SAH.

The plasma anion gap (AG) is a mathematical derivation parameter calculated using the formula $Na^{+}+(Cl^{-}+HCO^{3-})$. AG has been widely applied in diagnosing various forms of metabolic acidosis for more than 50 years (4). Previous research has found relationships between AG and mortality in patients with many different diseases, such as acute renal failure (5), cerebral infarction (6), acute myocardial infarction (7), acute ischemic stroke (8), coronary artery disease (9), and aortic aneurysms (10). Furthermore, in the general population, which is essentially free of these diseases, higher levels of AG might be of prognostic significance because an increase in AG has been associated with insulin resistance (11), hypertension (12), and low cardiorespiratory fitness (13). However, it is still unknown whether such changes in AG during the course of SAH are associated with a risk difference in mortality. Therefore, this study aimed to investigate the relationship between AG and SAH using publicly accessible clinical databases.

Methods

Study design and population

This retrospective study analyzed data from the Medical Information Mart for Intensive Care (MIMIC)-IV database (version:1.0) (14) and eICU Collaborative Research Database (15). The MIMIC-IV database, as an update to the MIMIC-III database, contains de-identified health-related data of over 40,000 unique patients who were admitted to the critical care units of Beth Israel Deaconess Medical Center between 2008 and 2019. The eICU database is a multicenter database (208 hospitals) comprising de-identified health data associated with over 200,000 ICU admissions between 2014 and 2015 across the United States. Adult patients with spontaneous SAH, as defined according to the International Classification of Diseases, Ninth and Tenth Revision (ICD-9 and ICD-10) codes and Acute Physiology and Chronic Health Evaluation admission codes, were included in the study. Patients meeting the following criteria were excluded: (1) age <18 or >90 years, (2) with insufficient AG data, and (3) with diagnoses related to traumatic SAH. One of the authors who passed the Collaborative Institutional Training Initiative exam and accessed database for data extraction (X.T. certification number:43334826).

Data extraction and outcome measures

The following patient characteristics were collected: (1) comorbidities including myocardial infarct, congestive heart failure, peripheral vascular disease, chronic pulmonary disease, rheumatic disease, peptic ulcer disease, liver disease, diabetes, renal disease, and malignant cancer; (2) Sequential Organ Failure Assessment (SOFA) score; (3) first day vital signs, including temperature, systolic blood pressure, diastolic blood pressure, and respiratory rate heart rate; (4) and first day laboratory test results, including AG, bicarbonate, creatinine, chloride, glucose, hematocrit, hemoglobin, lactate, platelets, potassium, sodium, blood urea nitrogen (BUN), white blood cell (WBC), and calcium. The overall Charlson Comorbidity Index (CCI) (16) was calculated using 18 categories of medical conditions identified in the medical records. For patients with multiple intensive care unit (ICU) admissions, we collected information only on the first ICU admission.

The primary outcome measure was in-hospital mortality, and the secondary outcome measure was ICU mortality. The patients were classified into three groups based on the three AG categories (Category 1, AG < 10 mmol/L; Category 2, AG ≥ 10 mmol/L and <13 mmol/L; and Category 3, AG ≥ 13 mmol/L). Previous studies have shown that an increase in AG levels is associated with poor clinical outcomes in several diseases. To

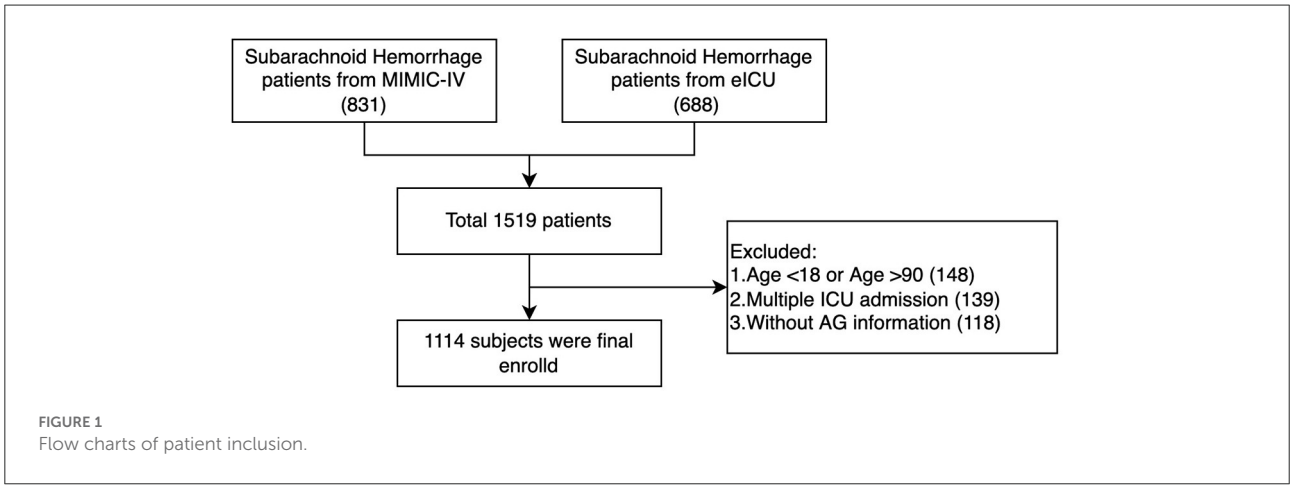

FIGURE 1
Flow charts of patient inclusion.

TABLE 1 Patient characteristics by anion gap concentration subgroup.

Characteristics	Anion gap subgroups (mmol/L)			*P* value
	Category 1 (<10, *n* = 283)	Category 2 (≥10, <14; *n* = 476)	Category 3 (≥14; *n* = 236)	
Age, years [mean (SD)]	57.32 (15.60)	59.52 (14.13)	59.10 (14.42)	0.125
Male (%)	123 (43.5)	192 (40.3)	108 (45.8)	0.359
Days in Hospital [mean (SD)]	14.34 (19.74)	13.16 (10.52)	11.95 (10.94)	0.149
Days in ICU [mean (SD)]	8.32 (7.37)	9.17 (8.89)	7.90 (7.73)	0.115
Hospital mortality (%)	40 (14.1)	80 (16.8)	58 (24.6)	0.006
ICU mortality (%)	29 (10.2)	58 (12.2)	47 (19.9)	0.003
Vital signs [mean (SD)]				
Heart rate	61.69 (12.78)	63.27 (12.49)	64.03 (15.34)	0.121
Systolic blood pressure	97.41 (15.46)	97.68 (14.72)	99.97 (19.54)	0.144
Diastolic blood pressure	49.45 (9.86)	48.11 (10.13)	49.80 (11.54)	0.075
Respiratory rate	11.39 (4.02)	11.90 (3.58)	12.49 (3.75)	0.005
Temperature	36.25 (0.78)	36.40 (0.60)	36.39 (0.71)	0.013
SPO2	91.82 (10.70)	93.05 (7.44)	90.58 (11.91)	0.007
Comorbidities [*n* (%)]				
Myocardial infarction	4 (1.4)	20 (4.2)	11 (4.7)	0.072
Congestive heart failure	7 (2.5)	22 (4.6)	15 (6.4)	0.097
Peripheral vascular disease	6 (2.1)	17 (3.6)	15 (6.4)	0.04
Chronic pulmonary disease	15 (5.3)	57 (12.0)	25 (10.6)	0.01
Rheumatic disease	2 (0.7)	6 (1.3)	5 (2.1)	0.367
Peptic ulcer disease	1 (0.4)	3 (0.6)	2 (0.8)	0.765
Mild liver disease	6 (2.1)	12 (2.5)	4 (1.7)	0.774
Severe liver disease	1 (0.4)	4 (0.8)	1 (0.4)	0.648
Diabetes	7 (2.5)	36 (7.6)	19 (8.1)	0.008
Renal disease	15 (5.3)	13 (2.7)	19 (8.1)	0.006
Paraplegia	4 (1.4)	30 (6.3)	15 (6.4)	0.005
Malignant cancer	8 (2.8)	9 (1.9)	5 (2.1)	0.694
Charlson Comorbidity Index [mean (SD)]	3.62 (1.81)	4.04 (2.01)	4.20 (2.13)	0.002
SOFA score [mean (SD)]	3.16 (2.80)	3.49 (2.69)	3.68 (2.74)	0.083
Laboratory results [mean (SD)]				
Anion gap	7.06 (1.75)	11.73 (1.09)	16.72 (2.18)	<0.001
Bicarbonate	23.23 (3.32)	22.68 (3.31)	20.57 (3.18)	<0.001
Creatinine	0.83 (0.48)	0.78 (0.39)	1.03 (1.67)	0.002
Chloride	104.29 (4.81)	103.49 (4.72)	102.12 (4.70)	<0.001
Glucose	120.78 (33.32)	127.32 (35.57)	139.60 (50.88)	<0.001
Hematocrit	35.80 (5.85)	35.81 (4.95)	36.79 (5.60)	0.052
Hemoglobin	11.99 (2.11)	12.02 (1.80)	12.35 (1.98)	0.062
Platelets	210.84 (70.99)	211.63 (71.56)	232.58 (89.81)	0.001
Potassium	3.66 (0.49)	3.63 (0.43)	3.72 (0.51)	0.042
Sodium	137.41 (3.96)	138.34 (4.23)	138.12 (3.63)	0.008
BUN	13.88 (9.22)	13.24 (7.12)	17.50 (18.63)	<0.001
WBC count	10.95 (13.11)	10.72 (3.92)	12.08 (4.58)	0.09
Calcium	8.23 (0.86)	8.31 (0.73)	8.51 (0.74)	<0.001

SAH, subarachnoid hemorrhage; SD, standard deviation; BUN, blood urea nitrogen; WBC, white blood cell.

thoroughly analyze whether this trend also occurred in patients with SAH, we only collected the minimum results for patients with a multi-lab test in the first 24 h of ICU admission.

Statistical analysis

Continuous variables were presented as the mean and standard deviation, while categorical variables were presented as proportions in each category, substratified by AG concentrations. Chi-square or Fisher's exact tests were used to compare categorical variables, and the *t*-test or one-way analysis of variance was used to compare continuous variables. Multivariate Cox proportional hazards regression models adjusted for potential confounders were used to assess hazard ratios (HRs) of mortality for AG concentration in SAH patients. Three cox models were performed as follows: model 1, without adjusting for any confounders; model 2, adjusting for demographic information, vital signs and score system which have p values < 0.2 in the univariate analysis; model 3, adjusting for all confounders with p values < 0.2 in the univariate analysis. Restricted cubic spline (RCS) models fitted with three knots at the 10^{th}, 50^{th}, and 90^{th} percentiles of AG were used for multivariate Cox proportional hazards regression model 3 to show the association between AG and in-hospital mortality in patients with SAH. The Kaplan–Meier method was employed to calculate the absolute risk of in-hospital and ICU mortalities for each subgroup of different AG concentrations. The data were reported as HRs with 95% confidence intervals (CIs). All statistical analyses were performed using R software (version 4.0.1). All tests were two sided, and statistical significance between two or more groups was set at $p < 0.05$.

Results

Patient characteristics

A total of 1,519 patients were confirmed to have spontaneous SAH, including 831 patients from the MIMIC-IV database and 688 patients from the eICU database. After exclusion, 1,114 patients were finally included in the analysis (Figure 1). The mean age of the total cohort was 58.74 ± 14.51 years, and 42.5% (474/1,114) were men. The in-hospital mortality and ICU mortality rates were 17.4% (194/1,114) and 13.2% (147/1,114), respectively. The basic patient characteristics by category are summarized in Table 1. The mean AG concentrations of the total cohort and Categories 1, 2, and 3 were 11.84 ± 3.70 mmol/L, 7.06 ± 1.75 mmol/L, 11.73 ± 1.09 mmol/L, and 16.72 ± 2.18 mmol/L, respectively. In general, patients with higher AG levels also had higher in-hospital (14.1 vs. 16.8 vs. 25.6%, $p = 0.006$) and ICU mortality (10.2 vs. 12.2 vs. 19.9%, $p = 0.003$). Patients with higher AG levels also had larger vital signs indices and a higher proportion of chronic pulmonary disease, diabetes, paraplegia and renal disease.

Association between AG and outcomes

The mean hospital stay duration for the survivor and the non-survivor groups was 14.76 ± 14.40 days and 6.75 ± 7.08 days, respectively. Hazards ratios (HR) of these three models were as follows: model 1, 1.061 (95%CI, 1.022–1.102; $p = 0.002$); model 2, 1.060 (95%CI, 1.015–1.105; $p = 0.007$) (adjusting for age, sofa score, systolic blood pressure, diastolic blood pressure, respiratory rate, temperature and SPO2); model 3, 1.076 (95%CI, 1.021–1.292; $p = 0.006$) (adjusting for age, systolic blood pressure, diastolic blood pressure, respiratory rate, temperature, SPO2, mild liver disease, serve liver disease, renal disease, malignant cancer, Charlson Comorbidity Index, SOFA score, bicarbonate, creatinine, glucose, hematocrit, hemoglobin, platelets, potassium, BUN, WBC count, and calcium) (Table 2). RCS curve showed that the risk of in-hospital mortality increased as the AG concentration increased (Figure 2A). With the Category 1 group as the reference, the HRs for all-cause in-hospital mortality were higher in the Category 2 (HR, 1.961; 95% CI, 1.157–3.324; $p = 0.012$) and Category 3 groups (HR, 2.151; 95% CI, 1.198–3.386; $p = 0.010$) (Table 3). Similar results were found for the association between AG concentration and ICU mortality (Figure 2B and Table 3). The Kaplan-Meier survival curve demonstrated significantly lower cumulative survival for patients with higher AG levels (log-rank $p < 0.001$) (Figure 3).

TABLE 2 Association between the anion gap and in-hospital and ICU mortalities.

Characteristics	HR (95% CI)	*P* value
In-hospital mortality		
Model 1[a]	1.061 (1.022–1.102)	0.002
Model 2[b]	1.060 (1.015–1.105)	0.007
Model 3[c]	1.076 (1.021–1.292)	0.006
ICU mortality[b]		
Model 1[a]	1.078 (1.032–1.125)	< 0.001
Model 2[b]	1.022 (1.007–1.037)	0.011
Model 3[d]	1.095 (1.030–1.164)	0.003

[a]Adjusting for nothing.
[b]Adjusting for age, heart rate, systolic blood pressure, diastolic blood pressure, respiratory rate, temperature, SPO2 and SOFA score.
[c]Adjusting for age, systolic blood pressure, diastolic blood pressure, respiratory rate, temperature, SPO2, mild liver disease, serve liver disease, renal disease, malignant cancer, Charlson Comorbidity Index, SOFA score, bicarbonate, creatinine, glucose, hematocrit, hemoglobin, platelets, potassium, BUN, WBC count, and calcium.
[d]Adjusting for age, heart rate, systolic blood pressure, diastolic blood pressure, respiratory rate, temperature, SPO2, mild liver disease, serve liver disease, myocardial infarction, paraplegia, renal disease, malignant cancer, Charlson Comorbidity Index, SOFA score, bicarbonate, creatinine, glucose, hemoglobin, platelets, potassium, BUN, WBC count, and calcium.

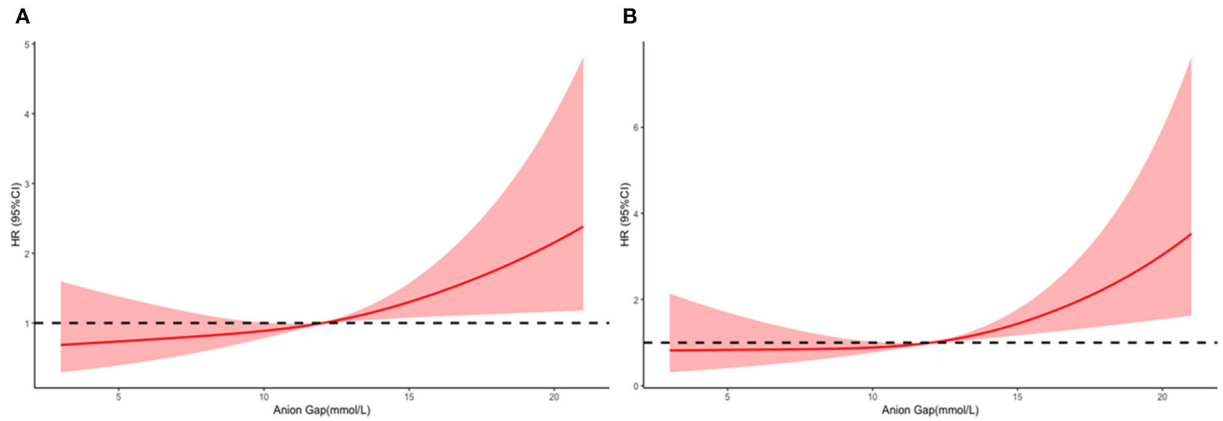

FIGURE 2
Multivariable adjusted hazard ratios (HRs) for all-cause mortality according to levels of anion gap (AG) on a continuous scale (mmol/L). Solid deep red lines are multivariable-adjusted HRs. Light red areas are the 95% confidence intervals derived from restricted cubic spline regressions with 3 knots. Dashed black lines are reference lines for no association at a hazard ratio of 1.0. In general, in-hospital **(A)** and ICU **(B)**, mortality are increased as AG concentration increased. In-hospital mortality analysis is adjusted for age, systolic blood pressure, diastolic blood pressure, respiratory rate, temperature, SPO2, mild liver disease, serve liver disease, renal disease, malignant cancer, Charlson Comorbidity Index, SOFA score, bicarbonate, creatinine, glucose, hematocrit, hemoglobin, platelets, potassium, BUN, WBC count, and calcium. ICU mortality analysis is adjusted for age, heart rate, systolic blood pressure, diastolic blood pressure, respiratory rate, temperature, SPO2, mild liver disease, serve liver disease, myocardial infarction, paraplegia, renal disease, malignant cancer, Charlson Comorbidity Index, SOFA score, bicarbonate, creatinine, glucose, hemoglobin, platelets, potassium, BUN, WBC count, and calcium.

TABLE 3 Multivariate Cox analysis for in-hospital and ICU mortalities across anion gap groups.

Characteristics	HR (95% CI)	*P* value
In-hospital mortality[a]		
Category 1	Ref	Ref
Category 2	1.961 (1.157–3.324)	0.012
Category 3	2.151 (1.198–3.864)	0.010
ICU mortality[b]		
Category 1	Ref	Ref
Category 2	1.869 (1.007–3.467)	0.047
Category 3	2.553 (1.279–5.10)	0.008

[a]Adjusting for age, systolic blood pressure, diastolic blood pressure, respiratory rate, temperature, SPO2, mild liver disease, serve liver disease, renal disease, malignant cancer, Charlson Comorbidity Index, SOFA score, bicarbonate, creatinine, glucose, hematocrit, hemoglobin, platelets, potassium, BUN, WBC count, and calcium.
[b]Adjusting for age, heart rate, systolic blood pressure, diastolic blood pressure, respiratory rate, temperature, SPO2, mild liver disease, serve liver disease, myocardial infarction, paraplegia, renal disease, malignant cancer, Charlson Comorbidity Index, SOFA score, bicarbonate, creatinine, glucose, hemoglobin, platelets, potassium, BUN, WBC count, and calcium.

Discussion

The relationship between serum AG and clinical outcomes in patients with spontaneous SAH is still unknown. This study found that patients with increased serum AG levels had worse clinical outcomes and a greater probability of in-hospital and ICU deaths.

Serum AG has been widely used to identify errors in measuring serum electrolytes or to detect and evaluate various acidotic conditions. Most recently, high AG levels were reported to be associated with decreased clinical outcomes in several diseases (5–13). Thus, serum AG, as a low-cost and easily available clinical index, may have great potential for evaluating prognosis. SAH accounts for 5–10% of strokes, but it occurs at a younger age, resulting in more loss of productive life years. The fatality rate ranges from 25 to 50%, owing to the consequences of either original bleeding or serious complications (16). In this study, the total in-hospital mortality rate was relatively low (17.7%). This explains why we only collected data on the first ICU admission, and this estimate did not fully account for patients who died before receiving medical attention (17).

Previous studies have also reported that case fatality rates have decreased with the introduction of improved management strategies (16). We found that the risk of mortality increased with increasing AG levels. This result may be partly explained by a decrease in bicarbonate levels. Increased plasma AG often reflected an acid imbalance due to inadequate tissue perfusion and renal excretion function disorders in the current study. Acid-base balance is critical for optimal physiological functions and cell metabolism (18). Categories 1, 2, and 3 had mean bicarbonate concentrations of 23.23 ± 3.32, 22.68 ± 3.31, and 20.57 ± 3.18 mmol/L ($p < 0.001$), respectively, and 5.3, 2.7, and 8.1% ($p = 0.008$) of patients had renal disorders, respectively. Cerebral vasospasm to extravascular blood cells and delayed cerebral ischemia are two of the most important and common complications in SAH (19).

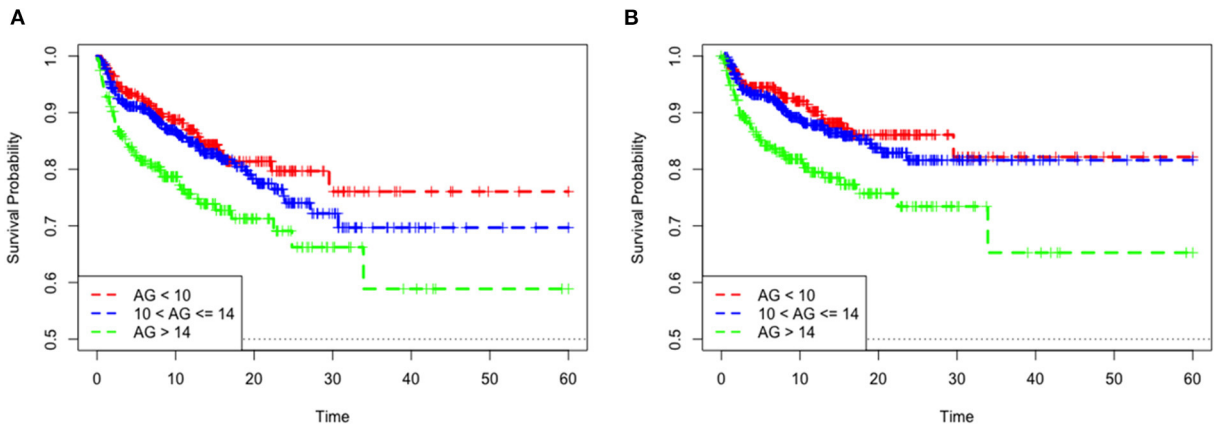

FIGURE 3
Kaplan–Meier survival curve of mortality among quartile groups of serum anion gap. In-hospital mortality **(A)**. ICU mortality **(B)**.

Cerebral vasospasm and delayed cerebral ischemia may lead to cerebral ischemia or brain hypoxia. These could be direct or indirectly cause by excess lactate production in the tissue, causing hyperventilation and hypocapnia (20) that could increase vasoconstriction and decrease intracranial pressure (21). Although the mechanism by which hypocapnia affects the prognosis of brain injury remains unclear, several studies have reported that hypocapnia is associated with poor outcomes in patients with brain injury. Solaiman et al. found that the duration of hypocapnia was associated with symptomatic vasospasm and unfavorable outcomes in aneurysmal SAH patients (22). Cai et al. also reported that both hypercapnia and hypocapnia were associated with a higher mortality risk in patients with SAH or other craniocerebral diseases (23). However, high AG levels may reflect renal excretion function disorders. In a large consecutive series of prospective cohort studies, Zacharia et al. found that renal dysfunction was an independent predictor of worse outcomes in patients with aneurysmal SAH patients (24).

Another explanation might be that the increase in AG levels partly reflects the increase in sodium concentration in SAH patients (25). Previous studies have reported that hypernatremia is associated with poor outcomes in SAH patients (26–32). In a clinical trial conducted at 54 neurosurgical centers in North America, Qureshi et al. found that although hypernatremia was not associated with the risk of symptomatic vasospasm, it was independently associated with poor outcomes after adjusting for previously identified outcome predictors (30). Fisher et al. found that hypernatremia was associated with adverse cardiovascular and neurological outcomes (26). Kumar et al. also found that hypernatremia was a significant risk factor for acute kidney injury in a patient with SAH (32). These results support that in addition to hypernatremia, AG levels may also further increase.

Compared to established indicators such as blood gas analysis or lactate, plasma AG is less costly and more frequently available in low-resource settings (33). As an alternative to assess acid-base imbalances, blood gas analysis may be used to predict prognosis in critically ill patients. However, blood gas analysis can indeed be influenced by compensatory respiratory alkalosis. Plasma AG is a sensitive tool for the treatment of metabolic diseases. Plasma AG is straightforward and does not require arterial puncture. In this study, plasma AG was found to be an independent predictor of in-hospital and ICU mortalities in patients with SAH.

Limitations

Our study has a few limitations that should be mentioned. First, the retrospective design may have introduced patient selection and analysis bias. However, we used real-world data from two large databases with patients from more than 200 hospitals to improve the generalizability of the results as much as possible. Second, patients with SAH were diagnosed using administrative diagnostic codes. Although we only selected the primary diagnosis sequence, there was still a chance of misclassification, leading to faulty connections. Third, hypoalbuminemia was prevalent in critically ill patients, which might have led to AG underestimation. Consequently, we only collected the minimum result for AG in the first 24 h to evaluate the relationship between AG increase and mortality. Third, the MIMIC-IV and eICU databases did not include long-term follow-up events. Thus, the association between long-term functional results and AG levels in patients was unclear. Given the small window of time for therapy following symptom onset of cerebral hemorrhage and the increase in cerebral hemorrhage severity, some patients with SAH may not have been referred to

other institutions. Therefore, our study may not have included high-risk patients. Furthermore, individuals with mild cerebral hemorrhage may have been admitted to the general ward and excluded from our study. Further research is needed to evaluate the external generalizability of our findings. Additional prospective case-control data are also needed to demonstrate the relevance of AG as a clinical marker for predicting the outcomes of SAH.

Conclusions

This large population-based study shows that the risk of mortality increases as AG concentration increases in patients with SAH. AG is an independent risk factor for all-cause in-hospital and ICU mortalities and is associated with poor clinical outcomes in these patients. Therefore, plasma AG could be a valuable marker for evaluating the prognosis of critically ill patients with SAH.

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

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

ZJ and AL conceived and designed the study. XT and KW collected the data. XT and LJ conceived of the project, analyzed the data, and wrote the paper. 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.

Publisher's note

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Venous stroke–a stroke subtype that should not be ignored

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Based on the etiology, stroke can be classified into ischemic or hemorrhagic subtypes, which ranks second among the leading causes of death. Stroke is caused not only by arterial thrombosis but also by cerebral venous thrombosis. Arterial stroke is currently the main subtype of stroke, and research on this type has gradually improved. Venous thrombosis, the particular type, accounts for 0.5–1% of all strokes. Due to the lack of a full understanding of venous thrombosis, as well as its diverse clinical manifestations and neuroimaging features, there are often delays in admission for it, and it is easy to misdiagnose. The purpose of this study was to review the pathophysiology mechanisms and clinical features of arterial and venous thrombosis and to provide guidance for further research on the pathophysiological mechanism, clinical diagnosis, and treatment of venous thrombosis. This review summarizes the pathophysiological mechanisms, etiology, epidemiology, symptomatology, diagnosis, and treatment heterogeneity of venous thrombosis and compares it with arterial stroke. The aim is to provide a reference for a comprehensive understanding of venous thrombosis and a scientific understanding of various pathophysiological mechanisms and clinical features related to venous thrombosis, which will contribute to understanding the pathogenesis of intravenous stroke and provide insight into diagnosis, treatment, and prevention.

KEYWORDS

cerebrovascular disease, arterial stroke, cerebral venous thrombosis, dural sinus thrombosis, venous stroke

Introduction

Stroke is a major cause of disability and mortality worldwide and the second leading cause of death in the United States (1, 2). The ischemic stroke accounts for the 87% of all cases, which results from the cerebral arteries occlusion due to thrombosis, atherosclerosis and platelets plug (3). Thrombosis also form in cerebral venous, which is termed as cerebral venous thrombosis(CVT), a particular type of cerebrovascular disease, characterized by intracerebral hemorrhage and infarction, associated with increased intracranial pressure due to cerebrospinal fluid absorption and cerebral venous

drainage, accounting for 0.5–1% of strokes (4). To date, there are more extensive and comprehensive studies on arterial thrombosis, with few clinical and basic studies on venous thrombosis, which greatly limits our understanding of venous thrombosis and the development of related drugs. In this review, we summarize the etiology, pathogenesis, symptomatology, diagnosis, and treatment heterogeneity of venous thrombosis based on current studies.

Molecular pathological hallmarks of ischemic stroke

Hypoxia-an essential aspect of arterial stroke and cerebral venous thrombosis

Hypoxia and ischemia of the brain are key pathophysiological mechanisms of ischemic stroke (5, 6). Hypoxia caused by impaired blood circulation can be referred to as circulatory hypoxia, which are classified as ischemic hypoxia and congestive hypoxia. Ischemic hypoxia is caused by an impaired arterial blood supply, whereas congestive hypoxia results from an impaired venous return. Hypoxia is caused by the sudden decrease in cerebral blood flow due to ischemic stroke (5), resulting in hypoxia-inducible factor-1 (HIF-1) production (7), oxidative and nitrative stress (8, 9), excitotoxicity (10, 11), metabolic abnormalities (12, 13), inflammation (14, 15), Ca^{2+} overload (16), cerebral edema and blood–brain barrier (BBB) disruption (17) (Figure 1).

HIF-1

HIF-1, including HIF-1α and HIF-1β, is an important regulator of hypoxia in stroke and participates in the pathological process of stroke by regulating glucose metabolism, angiogenesis, erythropoiesis and cell survival (18–20). Li et al. found that HIF-1α attenuates neuronal apoptosis by upregulating erythropoietin in rats with cerebral ischemia (21). Moreover, under hypoxic conditions, HIF-1 dynamically regulates reactive oxygen species (ROS) production *via* the glycolytic pathway and tricarboxylic acid cycles (22). Using a model of permanent middle cerebral artery occlusion (MCAO), Marti et al. demonstrated that hypoxia-induced upregulation of HIF-1 and HIF-2 increases expression of vascular endothelial growth factor (VEGF), thereby promoting neoangiogenesis (23). However, bidirectional roles of HIF-1 in different cells. After stroke, HIF-1 induces production and secretion of cytokines and chemokines, which in turn exacerbate inflammatory injury (19, 24). Moreover, Koh et al. verified that hypoxia-triggered neutrophil migration is decreased in HIF-1α-deficient mice, which is an important factor in regulating brain injury (25). Wang et al. found that inhibition of HIF-1 expression reduces BBB damage (26). In general, the beneficial or detrimental effects of HIF-1 on stroke depend on the duration and severity of hypoxia in arterial stroke and CVT.

Oxidative and nitrative stress

Brain ischemia and hypoxia can produce oxygen free radicals (ORFs), lipid radicals, and reactive nitrogen species

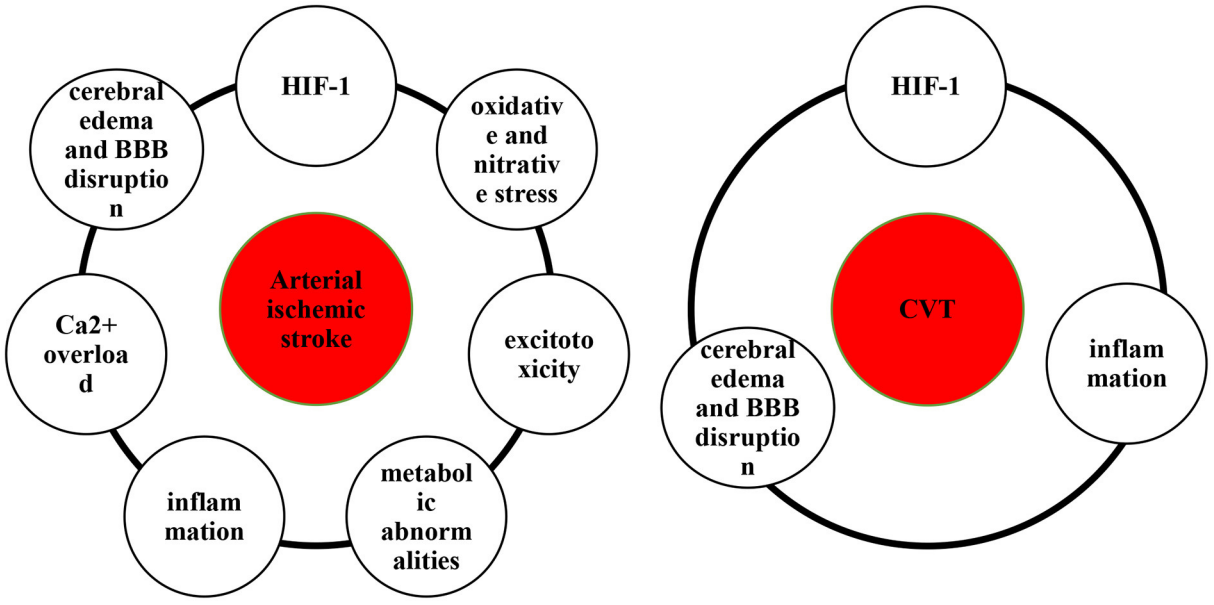

FIGURE 1
The molecular pathological hallmarks of arterial stroke and cerebral venous thrombosis.

(RNS). When these free radicals exceed the endogenous scavenging capacity, cells undergo oxidative stress and nitrative stress, resulting in apoptosis, autophagy and necrosis (27). ORFs, such as ROS and nitric oxide synthase (NOS), are affected by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (28), mitochondrial depolarization (29), nitric oxide synthase (30) and xanthine oxidase (31), thus triggering a ROS surge. ROS not only mediate cellular structural damage but also alter vascular permeability, dilate diastolic blood vessels, disrupt the BBB and lead to focal brain damage (32). It was demonstrated that accumulation of lipid ROS leads to intracellular oxidative stress and iron death after stroke, which is a pathway of nonapoptotic cell death mediated by iron (33, 34). Nitric oxide (NO) is a type of RNS generated by NOS (35). Serrano-Ponz et al. recorded and collected the data and clinical history of patients ($n = 76$) with acute ischemic stroke and monitored certain parameters. They found that an increase in nitric oxide metabolite (NOx) levels from Day 1 to Day 2 was beneficial (odds ratio (OR) = 0.91) but that a sharp increase in NOx levels from Day 2 to Day 7 was detrimental, and levels of NOx were associated with an increase in infarct volume (36). However, studies on stress responses to intravenous stroke are lacking overall.

Excitotoxicity

Excitotoxicity occurs when oxygen is insufficient to support aerobic respiration of mitochondria after cerebral ischemia (5). Disorders of energy metabolism inhibit the activity of sodium-potassium adenosine triphosphate (ATP)ase, resulting in decreased ATP synthesis and an imbalance of ionic gradients inside and outside nerve cells (37). According to Pietrogrande et al., low oxygen post-conditioning limits excitotoxicity-induced neuronal death and promote neuronal survival after secondary injury (38). In addition, ischemic stroke is associated with release of glutamate in the brain (39). During ischemia, excessive release of glutamate results in cell death. Using an animal model of MCAO, Campos et al. showed that activation of glutamate oxaloacetate transaminase inhibits the increase in glutamate after cerebral ischemia (40). Infarct size, edema volume, and sensorimotor deficits are significantly reduced as a result of the activation of glutamate oxaloacetate transaminase (40, 41). Fang et al. examined the effects of histamine on expression of glutamate transporter-1 (GLT-1) in an adult rat model of MCAO and found that inhibition of GLT-1 expression reduces excitatory toxicity (42). Notably, the role of excitotoxicity in CVT has not been proven and needs to be further investigated.

Metabolic abnormalities

Mitochondria are important organelles involved in energy metabolism (43). ATP produced by mitochondria cannot maintain the energy balance of neurocytes during ischemia and hypoxia after stroke, resulting in cell death (44). Moreover, mitochondrial homeostasis depends on mitophagy and the balance between mitochondrial fission and fusion (45). Grohm et al., reported that mitochondrial fission cause neuronal death after ischemic stroke and that inhibition of Drp1, a regulator of mitochondrial fission, protects neurons from glutamate excitotoxicity and reduces the infarct volume in a mouse model of transient focal ischemia (46). Importantly, on the basis of an MCAO rat model, peroxynitrite aggravates cerebral injury by recruiting Drp1 to damaged mitochondria to activate mitophagy (47). Therefore, mitochondria are potential therapeutic targets for treatment of ischemic stroke. For example, pramipexole restores neurological function though mitochondrial pathways in ischemia/reperfusion injury, such as reducing mitochondrial ROS and Ca^{2+} levels and improving mitochondrial oxidative phosphorylation (48). However, the tentative nature of impaired mitochondrial metabolism in CVT remains unknown and requires experimental confirmation. In general, there is still a lack of research on metabolic abnormalities in CVT.

Inflammation

Inflammation may be triggered by various factors after cerebral ischemia, including vascular obstruction, necrotic cells, and tissue injury (49–51). Different types of cells, cytokines and receptors are all involved in inflammatory processes (27). Neutrophils are among early infiltrators into the ischemic stroke brain, increasing within hours of onset and peaking after 1–3 days (52). Activated microglia can induce an inflammatory response. Hyperactivated microglia produce many toxic substances, such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), interferon-γ, IL-6 and ROS, promoting neuronal death (53). Microglia are targets for IGF-1, and the neuroprotective effects of IGF-1 may be mediated by the down-regulation of inflammatory mediators (54). In addition, studies have shown that expression of proinflammatory cytokines and chemokines is increased after stroke. IL-1 (15), TNF-α (55) and IL-6 (56) play important roles in stroke. It has been shown that Toll-like receptors (TLRs) play a role in the inflammatory response by initiating different downstream inflammatory cascades that cause tissue damage. Therefore, these receptors might mediate brain damage following ischemia (57, 58).

TLR2 and TLR4 overexpression is associated with poor outcome and inflammatory response in acute ischemic stroke, and TLR4 is also associated with infarct volume. Importantly, as TLR2- and/or TLR4-neutralizing antibodies impair the induced increase in expression of inflammatory markers in cultured serum cells, TLRs can be regarded as therapeutic targets for ischemic stroke (59). Similar results have been obtained in animal experiments (60).

Additionally, several inflammatory factors play an important role in the development of CVT. As demonstrated by van Aken et al., IL-8 concentrations in plasma above the 90th percentile lead to a 1.9-fold increased risk of venous thrombosis (61). Akbari et al. found that the plasma level of IL-6 in patients with cerebral venous sinus thrombosis was significantly higher than that in patients without thrombosis (62). In addition, proinflammatory cytokines such as IL-6 and IL-8 were found to be elevated in patients with idiopathic venous thrombosis (63). However, the role of inflammation in brain injury after CVT requires further research.

Ca^{2+} overload

Ca^{2+} overload has been proven to be involved in the neurotoxic effects of excitotoxicity (64) and oxidative stress (65). Zheng et al. have demonstrated that platelet-derived growth factors counteract the neuroprotective effects of oxidative stress by inhibiting Ca^{2+} overload (66). Moreover, hypoxia can alter intracellular Ca^{2+} channels, such as the Na^{+}/Ca^{2+} exchanger, L-type voltage-dependent Ca^{2+} channel, and inositol triphosphate receptor (IP3R) (16). Compensation with miR-132 attenuates the hypoxia-induced increase in Na^{+}-Ca^{2+} exchanger 1 (NCX1) expression and decreases apoptosis in cardiomyocytes by preventing Ca^{2+} overload (67). Li et al. reported the beneficial effects of IP_3R deletion in neuronal protection and reduction of cerebral dysfunction after stroke through disruption of Ca^{2+} signaling in astrocytes (68). The mechanism of Ca^{2+} overload in CVT remains elusive.

Cerebral edema and BBB disruption

Both arterial stroke and CVT are associated with disruption of the BBB and edema of the brain (69–71). Brain edema can be divided into three types based on severity: cytotoxic edema, ionic edema, and vasogenic edema (72, 73). Following ischemic injury, cytotoxic edema caused by adenosine triphosphate depletion disrupts Na^{+}/Ca^{+} and/or Na^{+}/K^{+} channels, resulting in intracellular cation accumulation to equalize ion concentrations without disrupting the BBB (72, 74, 75). Ionic edema, also known as iatrogenic cerebral edema, occurs before the BBB is damaged, and the main sources of edema in the peri-infarct region are the blood and cerebrospinal fluid (72, 76, 77). Furthermore, vascular-derived brain edema occurs at the end of the ischemic cascade. Neuronal death and/or damage caused by cerebral ischemia results in the production of reactive oxygen species, activation of immune cells and release of inflammatory factors, thereby breaking the BBB. After peripheral immune cells invade the brain parenchyma through the BBB, secretion of proinflammatory factors and permeability of the BBB increase, resulting in vasogenic edema (78, 79).

Molecular pathological hallmarks of hemorrhagic stroke

Hemorrhagic stroke (HS) presents more abruptly and induces more severe complications than ischemic stroke (1). The following discusses critical pathophysiology mechanisms in intracerebral hemorrhage after an arterial hemorrhagic stroke, such as oxidative stress (OS), inflammation, iron toxicity, and thrombin formation (80). Based on this, we speculate on the pathophysiology of venous hemorrhagic stroke.

Oxidative stress

Oxidative stress has been increasingly acknowledged as having an essential role in secondary brain injury following hemorrhagic strokes (80). Blood cell decomposition products, for instance, iron ions, heme, and thrombin can cause brain damage by producing free radicals (34, 81). Secondly, inflammatory cells, such as neutrophils and microglia, can produce free radicals after hemorrhagic strokes (82). During the inflammatory response triggered by hemorrhagic strokes, neutrophils become stimulated and activated, activating the respiratory chain, and releasing profound ROS, nitric oxide, and so on (83).

Inflammation

Physiologically, microglia and macrophages regulate the surrounding microenvironment and promote the stability of BBB, neurons, and matrix. When cerebral hemorrhage strokes, excessive microglia and macrophages release numerous inflammatory factors and trigger inflammatory cascades, resulting in pathological changes like BBB injury, edema, and cell death (80). Venous hemorrhagic stroke is not excluded. Following CVT, activated microglia release cytokines, resulting in brain injury, including disruption of the BBB, cerebral venous infarction, and brain edema (84). Immune cells are intensely activated, particularly microglia; macrophage activity increases are proven by Rashad et al. (71). Inflammation plays an essential role in venous hemorrhagic stroke injury, but further research is required.

Cytotoxicity of erythrocyte lysates

Within 24 hours of a cerebral hemorrhage, large amounts of hemoglobin-containing red blood cells leak into the brain's parenchyma, where they are broken down, which causes hemoglobin to disintegrate into heme and iron is a significant

contributor to brain injury affected by hemorrhagic stroke (81, 85). Inflammation, oxidation, nitric oxide scavenging, and edema are the primary mechanisms for brain injury caused by erythrocyte lysates (80). Firstly, HO-1, the critical enzyme for heme degradation, is expressed primarily in microglia after intracerebral hemorrhage and may further exacerbate brain damage by activating microglia and accumulating iron (85). Secondly, free radicals generated by iron may also cause tissue damage. Yeatts et al. confirmed that the iron chelator deferoxamine mesylate has multiple neuroprotective effects, including the reduction of perihematomal edema and neuronal damage, and enhances functional recovery after experimental intracerebral hemorrhage (86). Thirdly, hemoglobin depletes nitric oxide rapidly, triggering microthrombosis in subarachnoid hemorrhage and leading to brain damage (87). Finally, Wang et al. used the intracranial hemorrhage rat model to evidence that hemoglobin and its decomposition products are leading causes of edema (88). All in all, reducing iron accumulation and erythrocyte lysate toxicity is valuable in treating arterial hemorrhagic stroke; however, the same mechanism should be applicable for venous hemorrhagic stroke, but more research is needed to confirm it.

Thrombin formation

Earlier animal studies demonstrated that intracerebral injection of whole blood rendered brain damage, whereas injection of an inert substance did not produce this effect (89). Furthermore, whole blood injections induce brain injury within 24 hours, as opposed to concentrated blood cells, serum, or unclotted blood plasma (90). Similarly, intracerebral infusions of unheparinized blood results in perihematomal edema formation, while heparinized blood injections do not (91). These findings support the hypothesis that coagulation cascade and clotting may induce brain injury following HS. Thrombin, a prominent part of the coagulation cascade, produces immediately after ICH induction in the brain (92). Thrombin's poisonous or protective effects differ depending on its concentration; infusion of large amounts of thrombin directly into the brain produces inflammation, increased mesenchymal cells, brain edema, scar tissue, and seizures (93, 94). Brain impairments such as cerebral edema and BBB destruction may also occur in venous hemorrhagic strokes. We speculate that thrombin formation may also participate in CVT.

The clinical heterogeneity of cerebral venous thrombosis

CVT is a specific subtype of stroke with heterogeneous clinical manifestations. In the following sections, we describe the epidemiology, etiology, risk factors, pathological damage, clinical manifestations, diagnosis, treatment, and prognosis of CVT.

Epidemiological characteristics of CVT

Stroke is a significant cause of disability and vascular death worldwide (95), and ∼85% of strokes in adults are ischemic (96). According to a report from the American Heart Association published in 2021, the prevalence of stroke in adults in the United States is 3.4%; the global average lifetime stroke risk rose to 24.9% in 2016 and continues to rise (97). The incidences of CVT and ischemic stroke reported in several studies vary. CVT is an uncommon cerebrovascular event that accounts for 0.5–1% of all strokes in adults (98). At present, there are few epidemiological studies on CVT worldwide, and its true incidence is unknown. According to recent studies in the Netherlands and Australia, the incidence ranges from 13.2 to 15.7/1,000,000 annually (4, 99, 100).

Special etiology and risk factors for CVT

Risk factors for stroke can be classified as modifiable or nonmodifiable. In general, risk factors for CVT and ischemic stroke have different characteristics (101, 102). Numerous case–control and cohort studies have shown that age, sex, race/ethnicity, and genetics are unmodifiable risk factors for stroke. Thus, there are significant differences in the distribution of the affected population (103–105). Based on a retrospective cohort study of 162 patients, we conclude that CVT primarily affects young adults and children, with a mean age of onset of 42 (±17) years; 70% of patients were younger than 50 years, and 72% were female (106). American Heart Association data indicate that the incidence of ischemic stroke increases with age, and women have a greater lifetime stroke risk than men (107). Sex differences exist because women have specific risk factors, such as oral contraceptive use, pregnancy or puerperium, and hormone replacement therapy (108). A retrospective cohort study by Otite et al. indicated that the incidence of CVT differs by race (Blacks: 18.6–27.2; Whites: 14.3–18.5; Asians: 5.1–13.8) (105). In addition, among 3,298 Northern Manhattan Study participants, Blacks had the highest incidence of stroke, followed by Hispanics and Whites. Thus, stroke is more common in Blacks (hazard ratio (HR) = 1.51, 95% confidence interval (CI), 1.13–2.02) (109).Studies of genetic etiology provides important new insights into the pathophysiology of CVT (110). A genome-wide association study based on 882 patients with CVT, and 1,205 ethnicity-matched controls identified an association with 37 single nucleotide polymorphisms within the 9q34.2 region, this region more than doubled the likelihood of CVT, a greater risk than any previously identified genetic risk marker for thrombosis (111).

Different subtypes of stroke are influenced by different risk factors. Regarding risk factors for CVT, in a 12-year retrospective analysis of 83 patients with CVT, 24.1% had infection-associated CVT, with cavernous sinus thrombosis being the most common cause (112). As demonstrated in a case–control study involving 6278 controls and 594 patients with CVT, the risk of CVT in cancer patients was higher than that in those without cancer (odds ratio (OR) = 4.86; 95% CI = 3.46-6.81) (113). Additionally, patients with hematologic cancer have a significantly higher risk (114). The International Study of CVT (ISCVT) also identified lumbar puncture as a risk factor (115); head trauma and surgery are significant factors that should not be ignored (116). Bechchet's disease (9.4%), systemic lupus erythematosus (1.4%), antiphospholipid syndrome (0.6%), iron deficiency anemia (3.2%), ulcerative colitis ($n = 2$) and dehydration ($n = 3$) were risk factors for CVT in a multicenter study of 1,144 patients with cerebral venous thrombosis (117). A meta-analysis conducted by Dentali et al., reported odds ratios for Factor V Leiden mutation of 3.38 (95% CI, 2.27 to 5.05), mutation G20210A of 9.27 (95% CI, 5.85 to 14.67) and hyperhomocysteinemia of 4.07 (95% CI, 2.54 to 6.52) (118). Protein C deficiency increases the risk of CVT by 10.7-fold (3.1–37.7), protein S deficiency by 5.7-fold (1.4–22.4) and antithrombin deficiency by 3.8-fold (1.0–13.8) (119) (Figure 2).

Studies of risk factors associated with ischemic stroke are shown below. According to a worldwide meta-analysis that included 17,663 patients from 32 cohorts in 29 countries, the most important risk factor for stroke is hypertension (Blacks is 52.1%, Asian is 46.1%) (120). In the Northern Manhattan Study, the risk of ischemic stroke was associated with the duration of diabetes (adjusted HR = 1.03 per year with diabetes; 95% CI, 1.02–1.04), and patients with diabetes for more than 10 years had three times the risk compared with those without diabetes (121). The Oxford Vascular Study showed that the incidence of atrial fibrillation associated with ischemic stroke increased with age (122). In a prospective study conducted on individuals without a history of stroke, transient ischemic attack, or coronary heart disease, the researchers found that low-density lipoprotein cholesterol (LDL-C) was positively associated with ischemic stroke. Furthermore, lowering LDL-C to 1 mmol/L with statins may reduce the risk of ischemic stroke (123). The relative stroke risk for one cigarette a day is 1.25 (1.13–1.38) for men and 1.31 (1.13–1.52) for women (124).

Intracranial hypertension caused by venous thrombotic obstruction is characteristic pathological damage in CVT

Venous return obstruction may result from thrombosis of the cortical cerebral veins, deep cerebral veins, or dural venous sinuses. In contrast to isolated cerebral venous cortical thrombosis, most cortical venous thrombosis occurs in combination with dural venous sinus thrombosis (125). Deep cerebral vein thrombosis generally involves the intracerebral veins and the Galen veins. Approximately 60% of cerebral venous sinus thrombosis (CVST) patients have multiple dural venous sinuses (4). A multicenter clinical study of CVST in 624 patients found the superior sagittal sinus (62%), transverse sinus (41.2–44.7%), straight sinus (18%), and cavernous sinus (1.3%) to be the most commonly affected sites (126). The consequence of venous cerebral infarction is that venous pressure increases, capillary perfusion pressure decreases, cerebral blood volume increases, and intracranial pressure increases (127). Headache is a common symptom in the acute stage of cranial hypertension after cerebral vein occlusion. This is typically a sharp or pulsing pain through the head, both the forehead and top of the head. The headache can be aggravated by coughing, bending, the Valsalva maneuver and elevated cranial hypertension after exertion or even lying down (70). In addition to headaches, visual impairment can manifest, including visual field defects and optic papilledema (128). Symptoms of visual impairment may include swelling, elevation, and blurring of the optic disk, followed by bruising, hemorrhage and even retina infarction, which are due to increased pressure of cerebrospinal fluid in the optic nerve sheath and stagnation of the axoplasmic flow of nerve fibers (129).

It is also worth noting that infarction and hemorrhage are the most significant determinants of neuronal damage and patient prognosis (130). Compared with arterial thrombosis, venous thrombosis is associated with a tendency toward more frequent bleeding due to increased venous and capillary pressure after venous obstruction. Approximately 10–50% of patients with venous occlusion have combined infarction and hemorrhage, mostly at the gray–white matter-cortical junction (131). There is a direct relationship between arterial cerebral occlusion and thrombosis. Arterial cerebral occlusion causes irreversible damage, and imaging typically reveals a small penumbra, whereas venous cerebral occlusion involves unbalanced thrombosis and thrombolysis, yet most regions of the brain are only functionally or metabolically affected and not permanently damaged (132).

Vascular malformation as cause of venous hypertension (133). Dural arteriovenous fistula (DAVF) is a kind of vascular malformation characterized by an abnormal connection between an artery and vein within the dura (134). A reopening of preexisting physiological arteriovenous channels or hypoxia-induced stimulation of neoangiogenesis by venous hypertension has been proposed as pathogeneses of DAVF resulting from CVT (133, 135, 136). Lindgren et al. demonstrated that DAVF occurred in ∼2% of CVT patients and was correlated with chronic CVT onset, aging, and male gender according to the data from the international cerebral venous thrombosis consortium (137). Arteriovenous malformations (AVM)' pathogenesis

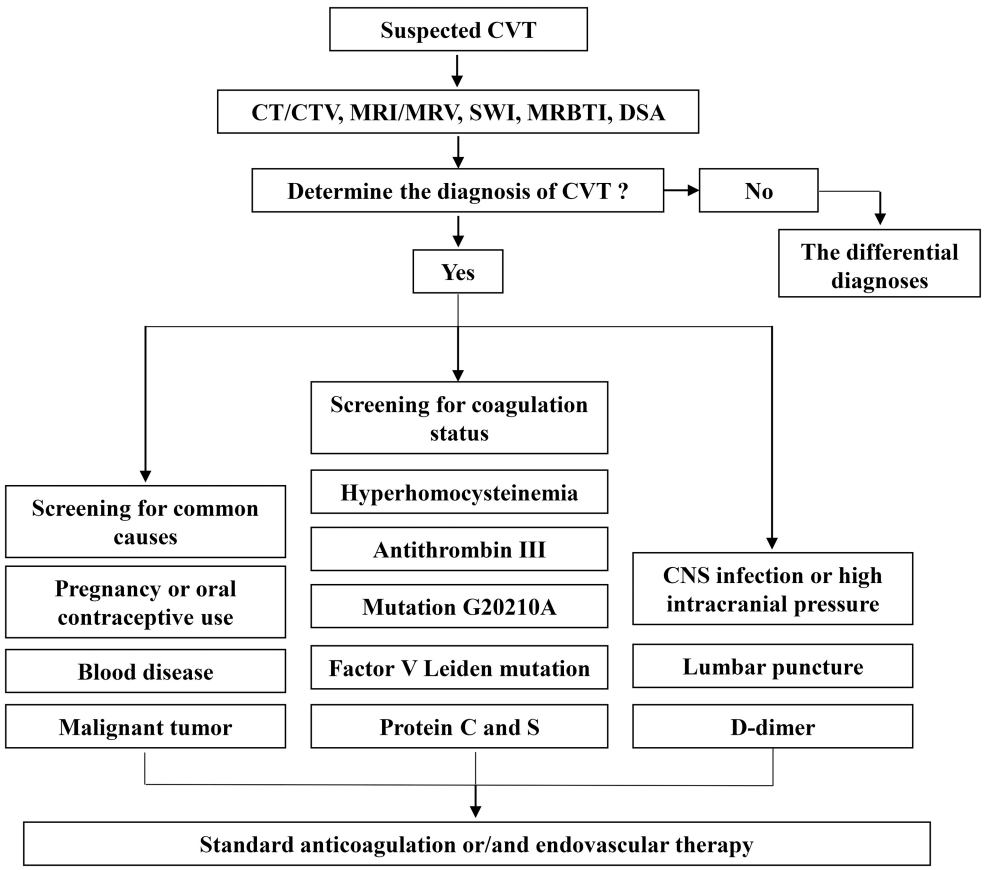

FIGURE 2
Clinical diagnosis and treatment of CVT.

resembles that of DAVF, the relationship of CVT with AVM scarcely has been reported (138).

Intracerebral hemorrhage and infarction of CVT

Cerebral edema and increased intracranial pressure may develop; thus, hemorrhagic, and ischemic lesions cannot be avoided (139). Venous hemorrhagic stroke-related intracranial hemorrhage appears inhomogeneous surrounded by irregular margins and occurs frequently in the parietal and parietooccipital brain regions adjacent to the cortical and subcortical layers (140). In the VENOST study, of 1,193 patients with CVT, 198 patients had hemorrhagic infarction, 43 patients had intracerebral hemorrhages, acute mode of onset was prominent, neurological symptoms included epileptic seizures (46.9%), altered consciousness (36.5%), nausea and vomiting (36.5%), and focal neurological deficits (33.6%) ($p \leq 0.001$) (141). In approximately one-third of CVT patients, intracerebral hemorrhages are associated with poor prognoses and severe presentation (141, 142).

Clinical manifestations of CVT

There are also differences in the clinical manifestations of arterial stroke and CVT (143, 144), with the clinical manifestation of ischemic stroke depending on the site of thrombosis. For example, lesions in the anterior cerebral artery involve symptoms of urinary incontinence, apraxia of gait and motor mutism; lesions in the middle cerebral artery may include hemianopia, impaired movement of arms and legs, aphasia and inattention; lesions in the vertebrobasilar artery are associated with hemianopia, brainstem cranial nerve palsy, ataxia, nystagmus and hemiplegia; and lesions in the small blood vessels are related to lacunar stroke syndrome (145).

In addition to the clinical manifestations of stroke similar to those of arterial stroke, CVT involves high cranial pressure and specific clinical manifestations (117). The most common clinical

manifestation of CVT is cranial hypertension, as represented by headache and visual impairment (146). Headache is associated with CVT in at least 85% of patients (147). It usually presents as acute or pulsating pain in the holocranial, forehead, or vertex, which may be isolated or accompanied by other signs or symptoms (148). Visual impairment, including visual acuity impairment, visual field defects and optic papillary edema (129). Acute optic papillary edema was present in 28% of patients during the ISCVST study (126). In the VENOPORT study, 13% of patients had visual impairment, and 2% had significant vision loss (149). Epilepsy (150), psychological and cognitive impairment (151), and dural arteriovenous fistula (152) are also specific clinical manifestations of CVT.

Challenges in the diagnosis of CVT

The clinical and radiological characteristics of CVT are nonspecific, which delays diagnosis and subsequent treatment (153). Therefore, cases of CVT have a high rate of under- and misdiagnosis, and the median time from onset to diagnosis is ∼7 days (144, 154). On the contrary, as ischemic stroke is a condition with a narrow treatment window, rapid diagnosis and prioritization are necessary. Computerized tomography (CT)/computerized tomography venography (CTV) and magnetic resonance imaging (MRI)/magnetic resonance venous imaging (MRV) can be used as the preferred examination methods for arterial and CVT. Digital subtraction angiography (DSA) is the gold standard for both diagnosis (4, 155) (Figure 2). Aside from imaging studies, the necessary hematology, coagulation, and biochemical tests should be performed (4) (Figure 2). In a prospective study of 34 patients with acute CVT, other auxiliary tests, such as D-dimer levels, had a sensitivity of 94.1% and specificity of 97.5% for the diagnosis of stroke (156).

Cranial computed tomography/computed tomography venography

The direct signs of CVT on noncontrast CT are often referred to as the "dense clot sign" or "cord or string sign," that is, the high-density shadow of thrombi in the cerebral sinus and veins (157). Within two weeks, the density of the thrombus gradually declines to the average level (158). Contrast-enhanced CT help assess the venous sinuses and cortical veins filling defects, changes in collateral venous drainage, and the vein (sinus) walls (159, 160). The specific sign on contrast-enhanced CT is called the "empty delta sign," indicating superior sagittal sinus thrombosis (161). Hemorrhagic infarction, brain edema and mass effect are common indirect CT signs, which are more common than direct signs (162). A meta-analysis indicated a sensitivity of 0.79 and specificity of 0.90 for CT (163). CTV can diagnose cerebral sinus thrombosis accurately, but its use in the diagnosis of cortical vein thrombosis is limited (164). The preferred diagnostic modalities for ischemia are CT and MRI, CT with sensitivities of 57–71% in the first 24 h compare to MRI (165). In addition to assessing acute ischemic stroke, noncontrast CT can be used to evaluate acute infarct size. The method for quantifying the size of the infarct is the Alberta Stroke Programme Early CT Score (166, 167). CT angiography is the first choice to detect intracranial large vessel occlusion, with a sensitivity approaching 100% (168).

Cranial magnetic resonance imaging /magnetic resonance venous imaging

MRI and CT can show the same direct and indirect signs, but MRI has advantages in comparison to CT in detecting parenchymal lesions and cerebral edema (169). Thrombus appearances on different MRI sequences are dependent on the time of evolution (170). On T1-weighted images, it appears isointense within 5 days; on T2-weighted images, it seems hypointense. The thrombus becomes hyperintense on both T1 and T2 sequences within 6–15 days. After 15 days, they turn isointense on T1 and iso- or hyperintense on T2 sequences. Upon examination of T1 and T2 sequences four months later, no abnormalities were detected (170). Diffusion-weighted imaging can distinguish between vasogenic and cytotoxic oedemas (152). A study of 23 patients with cerebral venous thrombosis confirmed by the novel magnetic resonance black-blood thrombus imaging (MRBTI) method showed that MRBTI can be successfully used as first-line diagnostic imaging (171, 172). Time-of-flight MRV (TOF-MRV) and contrast-enhanced MRV(CE-MRV) are two of the most frequently used MRV techniques. CE-MRV provides better visualization of cerebral venous anatomy without being dependent on blood flow signals, therefore, more sensitive than TOF-MRV, but less sensitive to isolated cortical venous thrombosis (161, 173). Compared with CT, MRI has a sensitivity of 73–92% within 3 h and close to 100% within 6 h (168). Based on a prospective study of 267 patients, MRI was more sensitive than CT in diagnosing acute ischemic stroke with large vessel occlusion (174).

Digital subtraction angiography

Ultimately, DSA is the gold standard for diagnosis (175). Nevertheless, with the development and widespread application of imaging technology, invasive DSA is rarely required to diagnose CVT. DSA is recommended if the non-invasive imaging examination is uncertain, endovascular treatment is considered, or DAVF is suspected (4).

Treatment of CVT

General treatment

The first step in the treatment of CVT is to actively treat the primary disease. If anticoagulation is not contraindicated in patients with CVT, it should be performed as soon as possible, and low molecular weight heparin should be used in the acute phase (4) (Figure 2). This view has been confirmed by a meta-analysis involving 79 patients (176). The oral anticoagulant warfarin should be taken after the acute phase. Direct oral anticoagulants (DOACs) like dabigatran are most likely to provide benefits in treating CVT (177). Among 845 CVT patients in a multicenter international retrospective study, 33.0% received DOACs alone, 51.8% received warfarin alone, and 15.1% received both treatments simultaneously (178). Compared with warfarin treatment, DOACs were linked with an analogous risk of recurrent venous thrombosis (aHR, 0.94; $P = 0.84$) but a lower risk of significant hemorrhage (aHR, 0.35; $P = 0.02$) (178). Another international retrospective cohort study of 766 patients with CVT, showed an overall incidence of 35.1 recurrences per 1,000 patient-years (95% CI, 27.7–40.4) after discontinuation of anticoagulant therapy, indicating that oral anticoagulants are effective at reducing the recurrence and mortality of CVT (179). Nevertheless, the efficacy of new oral anticoagulants remains to be further observed.

Endovascular therapy

Endovascular therapy has therapeutic value for both venous and arterial stroke (180, 181). Anticoagulation is not always effective in patients with CVT, so endovascular treatment (EVT) may be beneficial for these patients (182) (Figure 2). In a systematic review of 26 patients, local thrombolysis was found to be beneficial but associated with a certain risk of bleeding (183). Thrombolysis or Anticoagulation for Cerebral Venous Thrombosis (TO-ACT) studied the use of neuro intervention vs. conventional treatment in patients with severe CVT (characterized by deep venous involvement, intracranial hemorrhage, Glasgow coma score (GCS) < 9), 67 participants were randomized, the trial was terminated early for futility due to no difference in the modified Rankin Score(mRS) at 12 months (67 vs. 68%; RR 0.99, 95% CI 0.71–1.38) (184). EVT has not yet been proven effective in patients with CVT based on the available evidence, the data of EVT in CVT are derived from some small retrospective studies, patients weren't assigned randomly, and the study was probably influenced by disease severity, thus prone to bias (184–186).

Prognosis of CVT

The overall prognosis of CVT is favorable. The VENOST study of 1,144 patients with CVST showed that 78.4% had a modified ranking scale (mRS) of 0–1, 11.7% had an mRS of 2, and 10.0% had an mRS of 3–5 (117). The ISCVST study followed CVST patients for 6 months after discharge and found that 78.1% recovered completely, with an mRS of 0–1, 8.0% had a partial recovery, with an mRS of 2, and 14.0% had functional disability or died, with an mRS of 3–6 (187). Ischemic stroke is incurable, has a poor prognosis and is often accompanied by complications. A total of 76.9% of patients had at least one complication, and 20% experienced three or more (188). A cohort study of 1,075 patients who underwent rehabilitation after stroke in Poland confirmed that the most common complication of ischemic stroke is urinary tract infection (23.2%), followed by depression (18.9%), falls (17.9%), unstable hypertension (17.6%) and shoulder pain (14.9%) (188).

Conclusion

As a particular type of stroke, CVT is usually considered a disease with favorable outcomes, mostly occurring in young and middle-aged patients; however, at least 13% of all patients die or are severely handicapped. The traditional pathophysiological mechanisms of strokes have focused on the results due to artery thrombosis and fail to deeply explore the process and results of cerebral venous thrombosis. Compared with arterial stroke, cerebral venous thrombosis characterized by intracerebral hemorrhage with infarction, which might be termed as venous stroke needs to be further studied.

Author contributions

YZ and HJ: design the article structure and write the paper. XJ and CZ: propose research ideas, design the article structure, check the overall situation, and approve the paper. HW and LL: research and organize the literature and verify 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.

Publisher's note

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The difference in red blood cell distribution width from before to after thrombolysis as a prognostic factor in acute ischemic stroke patients: A 2-year follow-up

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Purpose: The aim of our study was to determine whether delta red blood cell distribution (ΔRDW) improves neurological outcomes in acute ischemic stroke (AIS) patients 2 years after intravenous thrombolysis (IVT) therapy.

Methods: AIS patients who received IVT between January 2013 and December 2019 were retrospectively analyzed. In accordance with their mRS scores, the patients were divided into two groups. A binary logistic regression analysis was conducted to determine the influencing factors of adverse functional outcomes. It was decided to evaluate the variables' the predictive ability by using the area under the receiver operating characteristic. For the poor neurological recovery risk model, features were selected using the LASSO regression model. We also developed a predictive model based on logistic regression analysis, which combined the features selected in the minimum absolute contraction and selection operator regression models. An evaluation of the discrimination, calibration, and clinical applicability of the predictive model was conducted using the C index, calibration chart, and decision curve analysis. Internal validation was evaluated via bootstrapping.

Results: Binary logistic regression analysis showed that ΔRDW was an independent influencing factor for poor neurofunctional outcomes. The most appropriate ΔRDW cut-off value for predicting the recovery of poor neurological outcomes was 18.9% (sensitivity: 89.9%, specificity: 78.6%, $p < 0.001$). The predictive factors included in the nomogram were age, the occurrence of CHD, stroke, AF, ΔRDW, NIHSS score at onset, interval time from onset to IVT, and whether there were indwelling urine catheters and gastric tubes. The model has not only a good discrimination ability, which was indicated by an overall C index of 0.891 (95% confidence interval: 0.829–0.953), but also a considerable calibration ability. Decision curve analysis showed that the nomogram of adverse neurological outcomes recovery was useful in the clinical practice when intervention was implemented above the threshold of 1% possibility of adverse neurological outcomes recovery.

Conclusion: In patients with AIS after thrombolysis, the ΔRDW is a potential influencing factor that can be readily used to predict the likelihood of poor neurological function recovery.

KEYWORDS

acute ischemic stroke, red blood cell distribution width, recombinant tissue plasminogen activator, predictive factor, 2-year follow-up

Introduction

Acute ischemic stroke (AIS) is a condition caused by blood circulation disorders and ischemia-hypoxia of the brain that causes tissue necrosis. One of the most common forms of stroke is an ischemic stroke, which is characterized by a focal brain infarction, along with sudden and persistent neurological deficits. Globally, a stroke, whether ischemic or hemorrhagic, is the most common cause of serious adverse events, like deaths and disability (1, 2). AIS is a severe acute disease of the nervous system that accounts for over 65% of all stroke cases (3). Intravenous thrombolysis rapidly restores blood flow (perfusion), which is vital for neuronal survival and recovery, and improves clinical outcomes. However, it is estimated that only 2–5% of stroke patients have been treated with intravenous recombinant tissue plasminogen activator (rt-PA) (4), although patients suffering from AIS responded well to intravenous rt-PA treatment (5). Furthermore, the effective therapeutic time window for cerebral reperfusion via thrombolysis is limited to approximately 4.5 h after ischemia (6, 7). Due to its complications of ischemic stroke, which worsened patients' prognosis, treatment with rt-PA should be carefully considered, both for risks and benefits. Hence, better decision strategies and effective implementation are of urgent need to mitigate the disease burden of ischemic stroke in China (8).

Red blood cell distribution width (RDW) could be obtained from a complete blood count (CBC), which is a convenient and inexpensive way to measure erythrocyte size variability. Anemia and inflammation are commonly assessed by RDW clinically. The normal range of RDW is 11.0 to 16.0%, and it can rise under some pathological or even physiological conditions (9, 10). Anemia could be diagnosed, classified, and treated guided by analyzing the size of erythrocytes using RDW. Previously, Higher RDW used to be closely related to increased mortality in patients with cardiovascular and cerebrovascular events, such as ischemic stroke, coronary disease, and peripheral artery disease (11–13). Nowadays, clinical diagnosis of AIS relies on history, neurological examinations and neuroimaging, while several scoring systems and RDW are adopted to assess the severity of stroke (14). There is still no surrogate biomarker to diagnose stroke. RDW by flow cytometry, on the other hand, is one of the most promising optional markers for predicting the occurrence of stroke and prognosis after rt-PA therapy in ischemic stroke.

Studies showed that RDW change from day 1 to day 4 was an independent predictor of mortality in patients with community-acquired pneumonia (15). However, there is no literature reporting the association between the difference in RDW from before to after thrombolysis (ΔRDW) and AIS, especially neurological recovery after rt-PA therapy. It is hypothesized that changes in ΔRDW may affect neurological outcome in acute stroke patients. In this study, we sought to evaluate the prognostic effect of RDW difference on mRS score in patients with AIS 2 years after rt-PA therapy, which may be. It is of great significance for the prognostic analysis and treatment of acute ischemic cerebral infarction.

Abbreviations: AIS, Acute ischemic stroke; rt-PA, recombinant tissue plasminogen activator; IVT, intravenous thrombolysis; CBC, complete blood count; ΔRDW, the third day after IVT RDW – on admission RDW; NIHSS, NIH Stroke Scale; mRS, Modified Rankin Scale; CT, computed tomography; 3.0T MR, 3.0 Tesla magnetic resonance; EDTA, The ethylene-diamine-tetra-acetic acid; lasso, The least absolute shrinkage and selection operator; AUC, The area under the curve; BP, blood pressure; BG, blood glucose; CHD, coronary heart disease; AF, atrial fibrillation.

Subjects, materials and methods

Subjects and designs

We got a formal ethical approval from the Shanghai East Hospital Ethics Committee prior to implementation. Clinical, laboratory, and neuroimaging data of 361 AIS patients who received reperfusion therapy with intravenous thrombolysis (IVT) consecutively were retrospectively analyzed at Shanghai East Hospital between January 2013 and December 2019. Stroke-specialized neurologist diagnosed AIS based on radiographic and clinical findings from a brain imaging study.

Criteria for inclusion and exclusion

Including criteria: (1) As defined by Guidelines for the Diagnosis and Treatment of AIS (16). (2) Rt-PA intravenous thrombolysis was performed based on the latest guidelines of

the American Heart Association (ASA) (17). (3) All patients and family members signed informed consent forms.

Excluding criteria

(1) Patients with malignant tumors and severe heart, liver, or kidney dysfunction; (2) Patients with cerebral hemorrhage, imaging changes of early large-scale cerebral infarction by craniocerebral computed tomography (CT) examination or epilepsy; (3) Patient with contraindications to thrombolysis; (4) Patients that was treated with anticoagulant. (5) Patients under the age of 18, or with incomplete clinical or laboratory data.

Neurological deficits were graded from 0 to 42 using the NIH Stroke Scale (NIHSS) score (18) and a higher score indicates a more severe condition. In addition to collecting demographic information, neurologists trained and certified in neurology collected modified Rankin Scale (mRS) scores. We grouped the patients according to the mRS score system (19), which was used to measure the outcome of functional recovery after stroke by the following: 0–2 being favorable and 3–6 being unfavorable. We recorded the NIHSS and mRS scores of patients on admission, discharge, and 2 years after hospital discharge.

Observation of indexes

We documented the baseline characteristics, including gender, age, cerebrovascular risk factors (hypertension, diabetes mellitus, heart disease, current smoking and drinking, blood glucose (BG) and blood lipid on admission. The ethylene-diamine-tetra-acetic acid (EDTA) tubes were used for collecting blood samples on admission and the third day after IVT, respectively. Hematology automated analyzer XE-2100 (Sysmex Company, Japan) was used to conduct the CBC within 20 min (min) after the sample was collected. ΔRDW values were calculated as the difference between the RDW value measured upon the third day after IVT and on admission (i.e., [the third day after IVT hospitalization RDW] - [on admission RDW]). All the enrolled subjects underwent CT imaging using an 64-detector row CT scanner (Philips brilliance, Japan) and MR imaging using a 3.0 T scanner (Discovery MR750, GE Healthcare, Milwaukee, WI, USA) on admission or in outpatient in this study.

Follow-up and study endpoints

A 2-year follow-up was performed on all patients to track their neurological recovery. A favorable functional outcome was defined as a mRS score of 0–2 and an unfavorable functional outcome as 3–6 based on the mRS score system. 2-year mortality was the endpoint. Outpatient visits and telephone follow-ups were used to collect follow-up information.

TABLE 1 Baseline characteristics.

	Values
No. of patients	361
Age (median [IQR]), years	66.00 [59.00, 75.00]
Gender, female, *n* (%)	129 (35.7)
Onset to thrombolysis (median [IQR]), minutes	180.00 [140.00, 220.00]
ΔRDW (median [IQR]), *n* (%)	0.00 [−0.20, 0.30]
LDL (median [IQR]), mmol/L	2.75 [2.22, 3.44]
BG (median [IQR]), mmol/L	7.07 [5.99, 8.87]
Hypertension, *n* (%)	248 (68.7)
DM, *n* (%)	98 (27.1)
Smoking, *n* (%)	142 (39.3)
Drinking, *n* (%)	86 (23.8)
CHD, *n* (%)	56 (15.5)
Stroke, *n* (%)	42 (11.6)
AF, *n* (%)	80 (22.2)
AF drug, *n* (%)	7 (1.9)
Intravascular thrombectomy, *n* (%)	25 (6.9)
Cerebral infarction	
Large-artery atherosclerosis, *n* (%)	121 (33.5)
Small-vessel disease, *n* (%)	178 (49.3)
Cardioembolic, *n* (%)	57 (15.8)
Other cause, *n* (%)	4 (1.1)
Unknown cause, *n* (%)	1 (0.3)
Hemorrhage transformation, *n* (%)	55 (15.2)
Urine tube placed, *n* (%)	78 (21.6)
Stomach tube placed, *n* (%)	81 (22.4)
NIHSS at onset (median [IQR])	4.00 [2.00, 10.00]
NIHSS at discharge (median [IQR])	3.00 [1.00, 9.00]

RDW, red blood cell distribution width; LDL, low density lipoprotein; CHD, coronary heart disease; BG, blood glucose; DM, diabetes mellitus; AF, atrial fibrillation; NIHSS, the national institutes of health stroke scale; ΔRDW is equal to [the third day after thrombolysis RDW -on admission RDW].

Statistical analysis

The patients' baseline characteristics were presented as the total percentage and the mean ± SD in the categorical variables or median and interquartile range (IQR) in the continuous variables based on the normality of the distribution. Pearson's χ test or Fisher's exact test Student's *t*-test and Mann-Whitney U test were selected to calculate the differences for numerical and categorical variables, as appropriate.

The least absolute shrinkage and selection operator (LASSO) regression technique was adopted for the selection of data dimension and predictors. Models incorporating multivariate logistic regression were used to determine risk levels and develop a nomogram for poor prognoses. The probability of each variate contributing to the outcomes was assigned based on the regression coefficient value for each variate in our study. To further assess the accuracy of the nomogram in predicting

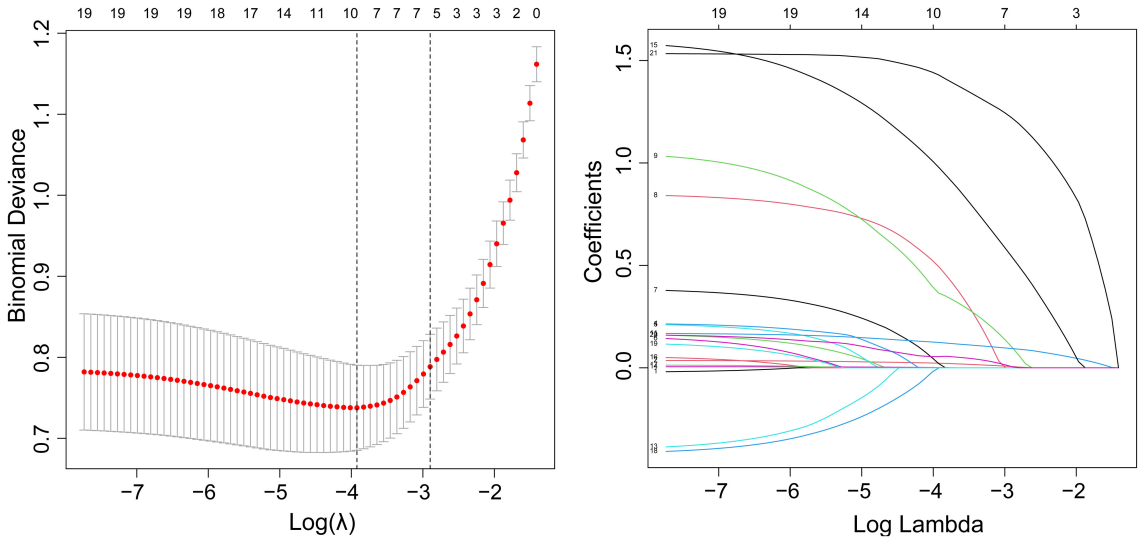

FIGURE 1
Demographic and clinical feature selection using the LASSO binary logistic regression model. **(A)** Fivefold cross-validation of the LASSO model's optimal parameter (lambda) was used. Plotted against log (lambda) is the partial likelihood deviance (binomial deviance). A minimum criteria and a 1 SE of the minimum criteria (the 1-SE criteria) were used to draw the dots representing the optimal values. **(B)** Profiles of the 21 features calculated by LASSO coefficients. This function plots coefficients vs. log (lambda) sequence. When lambda was optimal, there were nine features with non-zero coefficients, which was drawn as a vertical line. SE stands for standard error, and LASSO stands for least absolute shrinkage and selection operator.

TABLE 2 Univariate and multivariate analysis of risk factors for poor neurological outcomes recovery.

Variables	Univariate		Multivariate	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age	0.951 (0.929–0.973)	<0.001	0.963 (0.932–0.994)	0.022
CHD	0.522 (0.288–0.947)	0.032	0.669 (0.281–1.593)	0.364
History of stroke	0.405 (0.21–0.783)	0.007	0.385 (0.154–0.964)	0.041
AF	0.247 (0.146–0.418)	<0.001	0.616 (0.293–1.295)	0.201
NIHSS at onset	0.807 (0.768–0.848)	<0.001	0.858 (0.805–0.916)	< 0.001
ΔRDW	0.153 (0.083–0.283)	<0.001	0.212 (0.100–0.448)	< 0.001
Urine tube placed	0.084 (0.047–0.15)	<0.001	1.017 (0.372–2.780)	0.973
Stomach tube placed	0.062 (0.034–0.112)	<0.001	0.181 (0.071–0.46)	< 0.001
Interval time from onset to IVT	1 (0.996–1.005)	0.919	-	-

RDW, red blood cell distribution width; CHD, coronary heart disease; AF, atrial fibrillation; ΔRDW is equal to (the third day after IVT RDW – on admission RDW).

patient prognoses, plotted the receiver operating characteristic (ROC) curve and calculated the area under curve of ROC (AUC) for receiver operating characteristics. The calibration curve was then introduced to evaluate the consistency of prediction and observation. In order to evaluate the clinical applicability of the nomogram, the decision curve analysis (DCA) was conducted by quantifying the net benefit across all threshold probabilities. Internal validation completed by the bootstrapping method (Resampling = 1,000).

The statistical analysis in this article was performed using R software (version 4.2.0, https://www.r-project.org). The nomogram was generated using the "rms" package of R software. Statistical significance levels reported were all two-way, with $P < 0.05$ considered statistically significant.

Results

Patients' characteristics

A total of 361 patients visited our clinic from January 2013 to December 2019. All participants were presented in Table 1 with their baseline characteristics. A mean age of 66 years was found among the patients, including the 129 women (35.7%). The cohort consisted of 248 participants with hypertension, 56

with CHD, 80 with AF, and 42 with stroke (previous stroke history). One hundred and forty two patients (39.3%) had a history of tobacco smoking and alcohol consumption was mentioned in 86 patients (23.8%). The median ΔRDW, LDL, and BG concentrations were 0, 2.75, and 7.07 mmol/L. The mean interval time from stroke onset to IVT was 180.00 min. All patients were divided into good and poor neurological outcomes recovery groups based on mRS scores. Table 1 provided all the clinical data regarding demographics, disease features, and treatment in each two groups.

Feature selection

Among all the demographics and clinical parameters, nine out of 21 features were thought to be potential predictors based on 361 patients in the cohort (Figures 1A,B) and showed non-zero coefficients in the LASSO regression model. These features included age, the occurrence of CHD, AF, stroke, ΔRDW, NIHSS score at onset, interval time from onset to IVT, and whether there were indwelling urine catheters and gastric tubes (Table 2).

Development of an individualized and applicable predictive model

Uni-variable binary logistic regression analysis showed that each additional unit of age ($p < 0.001$), the incidence of CHD ($p = 0.032$), stroke (previous history of stroke) (0.007), and AF ($p < 0.001$), NIHSS score at onset ($p < 0.001$), ΔRDW ($p < 0.001$), and indwelling urine catheters ($p < 0.001$) and gastric tubes ($p < 0.001$) were significantly associated with patients' neurological outcomes recovery after thrombolysis for acute ischemic stroke (Table 2). AUC (ΔRDW) = 0.905 with a 95% confidence interval of AUC = 0.869 to 0.934 was obtained from and the statistically significant difference ($p < 0.001$) made by this model using ROC analysis (endpoint: poor neurological outcomes recovery) (see Figure 2). The optimal critical value for ΔRDW was 18.9%, while at this point with a sensitivity of 89.9% and specificity of 78.6% to calculated the maximum approximate index. Furthermore, in view of clinical parameters, multivariable binary logistic regression model demonstrated that every one unit increase in age (OR: 0.963; 95%CI: 0.932–0.994; $p = 0.022$), RDW (OR: 0.212; 95%CI: 0.100–0.448; $p < 0.001$), NIHSS score at onset (OR: 0.858; 95%CI: 0.805–0.916; $p < 0.001$), indwelling gastric tubes (OR: 0.181; 95%CI: 0.071–0.460; $p < 0.001$), and the occurrence of stroke (OR: 0.385; 95% CI: 0.154–0.964; $p = 0.041$) were considered to be independent predictors of patients' neurological outcomes recovery after stroke thrombolysis for AIS. An integrated model incorporating the aforementioned statistically independent predictors in multivariable binary logistic regression was designed and presented in the form of a nomogram in Figure 3.

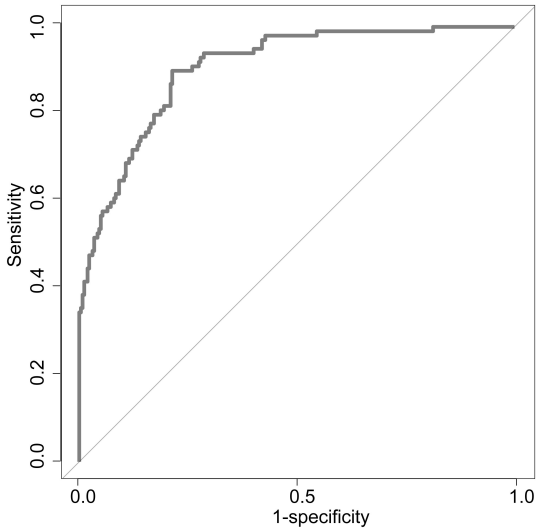

FIGURE 2
ROC curve to determine the predictive performance of ΔRDW for poor neurological outcomes recovery. ROC, Receiver operating characteristic; ΔRDW, the third day after IVT red blood cell distribution width − on admission red blood cell distribution width.

Apparent performance of the nonadherence risk nomogram and clinical application

The calibration curve of risk nomogram for predicting poor neurological outcomes recovery risk in patients after thrombolysis for AIS demonstrated a good fit (Figure 4). The C-index of the prediction nomogram was 0.891 (95% CI: 0.829–0.953) for the cohort and was confirmed through 1,000 bootstrapping validation, which suggested the model's good discrimination (Figure 5). The decision curve analysis for the poor neurological outcomes recovery nomogram was presented in Figure 6 and indicated that ΔRDW was a negative independent risk factor of poor neurological outcomes with clinical net benefit in a range of risk thresholds (threshold >1%) where the net benefit was comparable within this range, though there were several overlaps in the poor function recovery risk nomogram.

Discussion

Table 1 and Supplementary materials showed that, compared to favorable functional outcome group after IVT, the unfavorable outcome group varied in not only ΔRDW,

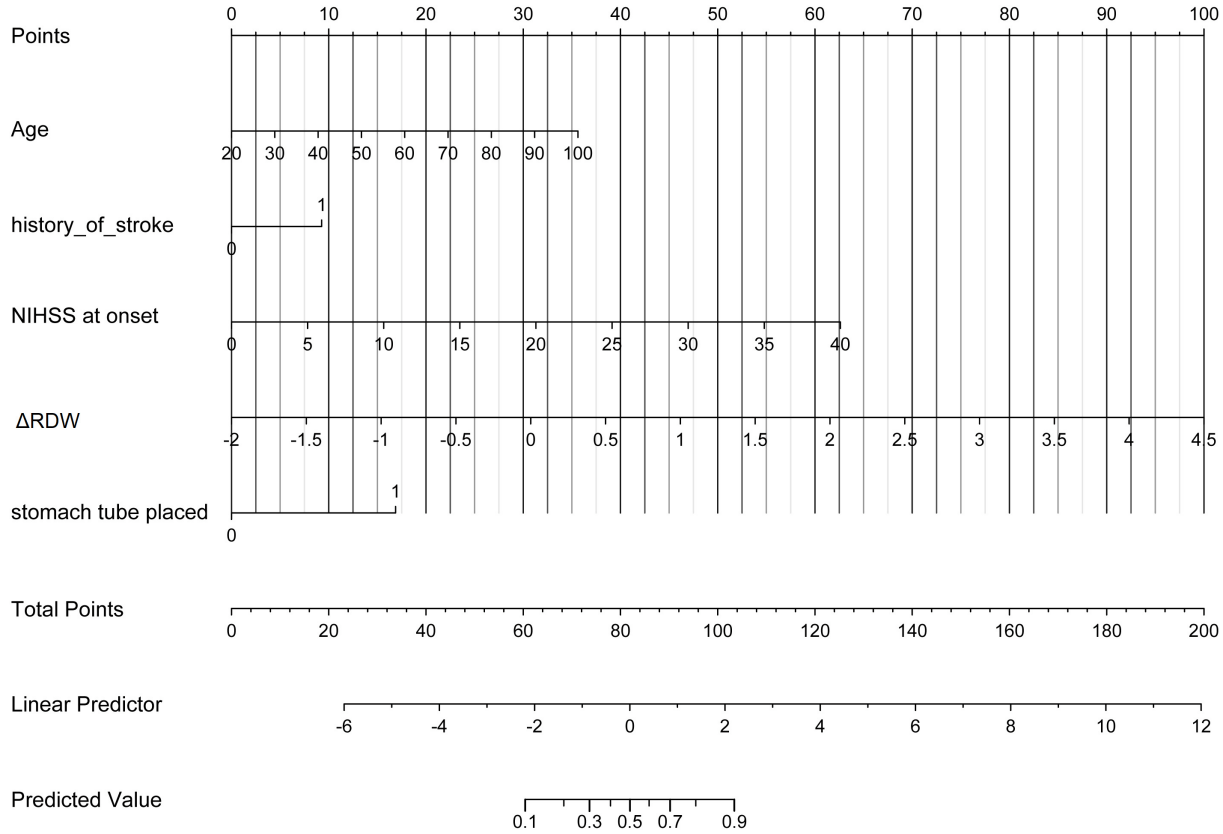

FIGURE 3
Developed poor neurological outcomes recovery nomogram. With the age, the history of stroke, NIHSS score at onset, ΔRDW, and indwelling gastric tubes in the cohort, a poor neurological outcomes recovery nomogram was developed. In our research, depending on the size of the regression coefficient, the nomogram scored the value of each variable. By converting each patient's score into a probability, the probability of different outcomes for each patient could be calculated. ΔRDW, the third day after IVT red blood cell distribution width − on admission red blood cell distribution width.

but also seven other features, including age, NIHSS score at onset or over, the occurrence of CHD, previous history of stroke, hemorrhagic transformation, incidence of thrombectomy and whether there were indwelling urine catheters or gastric tubes, which was in accordance with previous studies. Previous studies have shown that age identified as an unmodifiable risk factor for stroke (20), NIHSS score strongly affects stroke outcomes (21), hemorrhagic transformation after thrombolysis will affect prognosis (22), etc. It has been shown that thrombolytic therapy can improve the outcome of neurological injury and death in AIS patients when the indications for thrombolytic therapy are met, and contraindications are not present (16). In nursing and managing patients with AIS, proper and timely management plays an important role (23, 24), including an accurate diagnosis and prognosis. It is therefore necessary to determine the severity of neurological impairment and cognitive impairment on admission when treating AIS patients and to identify the risk factors that are associated with adverse outcomes following thrombolysis. Among many examinations, including physical examination and imaging examination, the value of routine laboratory parameters (such as RDW) is often underestimated. There has been an increase in the use of simple and inexpensive RDW tests in whole blood over the past few years to help predict many disease (25–27). As we all know, there are still a small portion of patients with cerebral infarction who suffer from serious conditions such as post-thrombotic hemorrhage after receiving thrombolytic therapy. Studies have shown that high RDW levels increase the risk of hemorrhagic transformation and stroke recurrence in patients with cerebral infarction receiving thrombolytic therapy (28, 29). Another research shows that high levels of RDW before thrombolysis can predict poor neurological recovery in patients with cerebral infarction at 1 year after IVT therapy (30) which means that RDW, as a fast, simple, and cheap method, is capable of distinguishing poor recovery of nerve function following thrombolysis. According to the ROC curve, the appropriate cutoff value for detection was 0.189, with 0.786 specificity and 0.899 sensitivity in our study. Furthermore, a logistic regression model was developed using several clinical and demographic parameters, including age, CHD, history of stroke, AF, ΔRDW,

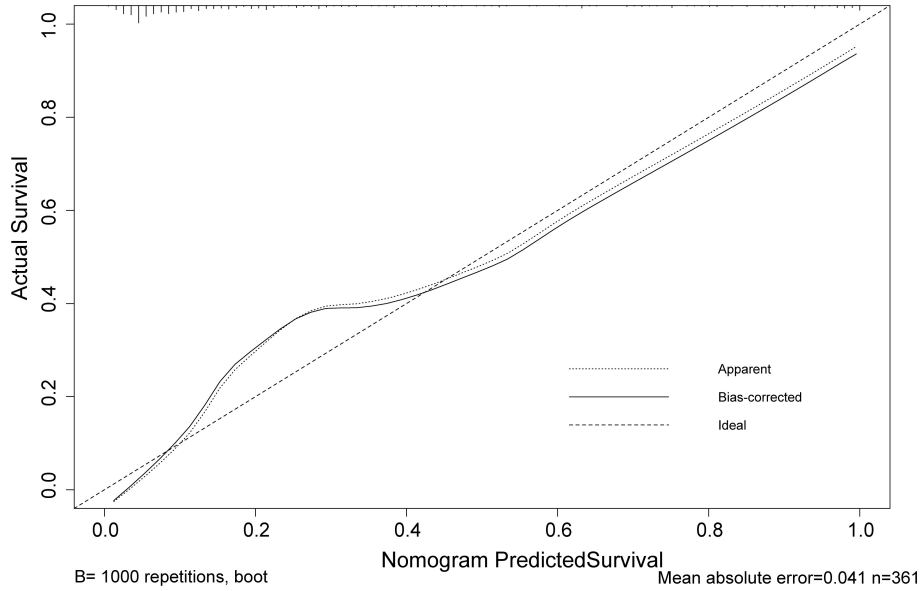

FIGURE 4
Calibration curve of the poor neurological outcomes recovery nomogram prediction in the cohort. Predicted poor neurological outcomes recovery risk is represented by the x-axis. The y-axis represents the actual diagnosed poor neurological outcomes recovery. Ideal model predictions are represented by the diagonal dotted line. The solid line represents the predictive performance of our nomogram, and a closer fit to the diagonal dotted line indicates a better prediction.

indwelling catheters, and the NIHSS score at the onset and after thrombolysis and was proven to have good discrimination and calibration capabilities. The results showed that the changes in RDW at the time of admission and that on the third day after thrombolysis were associated with poor prognoses for neurological outcomes. The decision curve analysis showed that when the intervention is implemented above the threshold of 1% of the possibility of poor neurological recovery, the nomogram of poor neurological recovery had a certain practical value in clinical practice. Previous studies have shown that high RDW levels are closely related to complications and death risks of various diseases (31–33). For example, RDW can be used as a prognostic factor for the severity and progression of AIS after antithrombotic therapy (34). Other studies have shown that RDW may become a biomarker of a high inflammatory response (35). Nevertheless, the biological mechanism that underlies the changes in RDW before and after thrombolysis and the recovery of function following thrombolysis in AIS remains unclear, inflammation and oxidative stress have been suggested as potential mechanisms.

The increase in RDW level can be attributed to the following reasons: (1) It is possible that metabolic pathway disorders contribute to the increase in RDW level (36, 37). (2) Since the increase in RDW levels is directly related to hypertension, a risk factor for cerebrovascular disease, a relationship between RDW and inflammation is highlighted, which supports the hypothesis that chronic inflammation contributes to elevated RDW levels (38). (3) As a result of large red blood cells occluding the carotid arteries, RDW may promote the progression of ischemic stroke (39). (4) An increase in RDW levels is associated with oxidative stress and the production of free radicals, which can further contribute to the occurrence of atherosclerosis following an ischemic stroke (40). (5) Studies have suggested that malnutrition may be a contributing factor to the increase in RDW level (41). (6) There is a correlation between an increase in RDW level and cerebrovascular diseases, suggesting that it may play a role in the changes in cerebral hemodynamics (42). Erythropoietin-induced erythrocyte maturation is inhibited by proinflammatory cytokines (43), which is partially reflected in an increase in RDW. Then, an inflammatory response to the body can affect bone marrow function and iron metabolism (44, 45). Moreover, oxidative stress has been implicated in increased RDW by shortened RBC survival and increasing the number of large premature RBC (46). Hence, resolution of inflammation and oxidative stress after thrombolysis in acute cerebral infarction may reduce RDW level.

It appears that cerebrovascular events may be a trigger for red blood cell abnormalities in AIS events as well as after thrombolysis. Likewise, regression analysis found a significant correlation between the changes in RDW before and after thrombolysis and the recovery of neurological function. Consequently, RDW represents a valuable diagnostic biomarker in these patients. In this

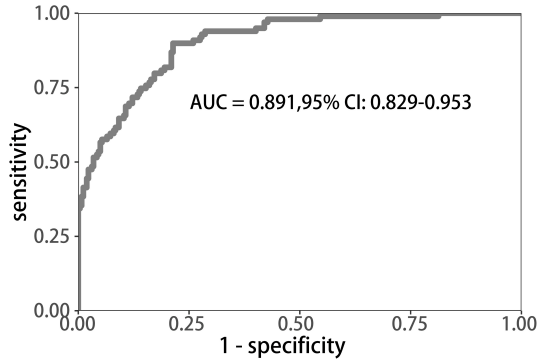

FIGURE 5
C-index analysis for the sensitivity and specificity of the nomogram.

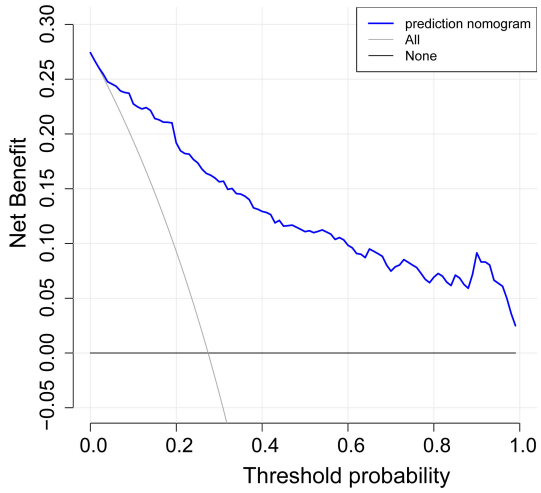

FIGURE 6
Decision curve analysis for the poor neurological outcomes recovery nomogram. The y-axis measures the net benefit. The blue line represents the poor neurological outcomes recovery risk nomogram. The light gray line represents the assumption that all patients suffer from poor neurological outcomes recovery. The black solid line represents the assumption that no patients suffer from poor neurological outcomes recovery.

regard, abnormal erythropoiesis and metabolic processes might lead to acute cerebrovascular disease or even exacerbate it. It is well-known that red blood cells, as oxygen carriers in the blood, can increase RDW level when their structures are abnormal, thus promoting thrombosis (47). Secondly, anisocytosis can cause a rise in nitric oxide, decreasing blood flow-dependent arterial dilatation, triggering ischemic injury events, or aggravating previously existing ischemic injuries.

It is true that this study has some limitations. First, patients' compliance varies after discharge. It is possible for some patients to die as a result of other major diseases or accidents. Additionally, we did not perform a stratified analysis of risk factors. Meanwhile, we only investigated the relationship between ΔRDW value and poor neurological outcomes. Considering that other factors may be influenced RDW level, continuous monitoring may be necessary. Finally, this clinical study focused more on correlation than causality, without exploring the mechanism behind the relationship. The conclusion needs to be supported and verified by further animal experiments. We will also conduct multicenter research in our next experiment to obtain more reliable results. Moreover, larger sample sizes and multicenter studies are required to evaluate our results, primarily to determine whether ΔRDW affected the results and if the results are clinically significant or not.

Conclusion

Our study showed that ΔRDW is an independent risk factor for poor neurological recovery after thrombolysis in AIS patients. It is a reference value for treatment strategy for those patients with poor neurological recovery within 2 years of thrombolysis based on ΔRDW prediction.

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 Shanghai East Hospital Ethics Committee. 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

Project administration, supervision, and resources: DH. Conceptualization, investigation, and resources: WY. Writing—review and editing: YW. Data curation and validation: AA. Methodology, funding acquisition, and visualization: CR. Formal analysis, writing—original draft, and software: YJ. All authors

have read and agreed to the published version of 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

Supplementary material

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The impact of COVID-19 pandemic on treatment delay and short-term neurological functional prognosis for acute ischemic stroke during the lockdown period

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Background: Preventive strategies implemented during the COVID-19 pandemic may negatively influence the management of patients with acute ischemic stroke (AIS). Nowadays, studies have demonstrated that the pandemic has led to a delay in treatment among patients with AIS. Whether this delay contributes to meaningful short-term outcome differences warranted further exploration.

Objective: The objective of this study was to evaluate the impacts of the COVID-19 pandemic on treatment delay and short-term outcomes of patients with AIS treated with IVT and MT.

Methods: Patients admitted before (from 11/1/2019 to 1/31/2020) and during the COVID-19 pandemic (from 2/1/2020 to 3/31/2020) were screened for collecting sociodemographic data, medical history information, and symptom onset status, and comparing the effect of treatment delay. The patients treated with IVT or MT were compared for delay time and neurological outcomes. Multivariable logistic regression was used to estimate the effect of treatment delay on short-term neurological prognosis.

Results: In this study, 358 patients receiving IVT were included. DTN time increased from 50 min (IQR 40–75) before to 65 min (IQR 48–84), $p = 0.048$. 266 patients receiving MT were included. The DTP was 120 (112–148) min vs. 160 (125-199) min before and during the pandemic, $p = 0.002$. Patients with stroke during the pandemic had delays in treatment due to the need for additional PPE ($p < 0.001$), COVID-19 screening processes ($p < 0.001$), multidisciplinary consultation ($p < 0.001$), and chest CT scans ($p < 0.001$). Compared with pre-COVID-19, during the pandemic, patients had a higher likelihood of spontaneous intracranial hemorrhage after IVT (OR: 1.10;

95% CI, 1.03–1.30) and a lower likelihood of mRS scores 0–2 at discharge (OR: 0.90; 95% CI, 0.78–0.99). In logistic regression analysis, high NIHSS score at admission, increasing age, worse pre-admission mRS, large vessel occlusion, admission during the lockdown period, and low mTICI grade after MT were associated with an mRS $\geq$ 3.

Conclusion: The COVID-19 pandemic has had remarkable impacts on the management of AIS. The pandemic might exacerbate certain time delays and play a significant role in early adverse outcomes in patients with AIS.

KEYWORDS

acute stroke, COVID-19, intravenous thrombolysis, mechanical thrombectomy, treatment delay

Introduction

The likelihood of acute ischemic stroke (AIS) patients with large vessel occlusion (LVO) to receive emergency care, such as endovascular mechanical thrombectomy (MT) or intravenous thrombolysis (IVT), is extremely time-dependent (1). This is partially influenced by the effectiveness of pre-hospital care and the sufficiency of hospital resources. However, the impact of the pandemic is perhaps inevitable. Since the pandemic breakout, there has been a noticeably lower rate of thrombolysis and thrombectomy in patients with AIS, as documented by various facilities (2–5). Meanwhile, several articles have reported that the COVID-19 outbreak was linked to delays in the treatment of patients with AIS (6–8). Speculative explanations for these delays include the disruption of medical services cause by the pandemic, the anxiety of patients over contracting SARS-CoV-2, psychological stress brought on by the pandemic, and associated lockdowns (8, 9).

Hospitals must take the required precautions due to the outbreak escalation to prevent the simultaneous spread of the SARS-CoV-2 virus to patients and medical staff (10). According to reports, some medical facilities increased their precautionary procedures in responding to the pandemic, thus leading to longer delays in diagnosis and treatment (11), resulting in poor outcomes. Even during the pandemic, thrombolysis and thrombectomy should be administered to patients with AIS without any delay to reduce mortality and morbidity (12). With the escalation of the pandemic, the preventive measures around the world have also been upgraded accordingly. During the pandemic, China had launched several control measures to gradually reduce COVID-19 transmission. Recent studies have also shown a decrease in stroke admissions during the pandemic, but data on emergency stroke management and treatment outcomes are still limited (13). Therefore, we performed a multicenter retrospective study to compare treatment processes and clinical outcomes of patients with AIS who underwent IVT and MT before and after the pandemic outbreak to evaluate the impact of the pandemic on the processes and outcomes of IVT and MT performed in patients with AIS.

Methods

Study design and patient population

This study was part of an ongoing program for analyzing the COVID-19 pandemic in managing patients with stroke. The current study was a retrospective analysis of prospectively collected data. A total of six tertiary hospitals with comprehensive stroke centers were included in this study, four of which are in Jiangsu Province, namely, Jinling Clinical College of Nanjing Medical University, Affiliated Yixing Hospital of Jiangsu University, The Fourth Affiliated Hospital of Nanjing Medical University, and The Affiliated Hospital of Yangzhou University. The First Affiliated Hospital and College of Clinical Medicine of Henan University of Science and Technology is located in Henan Province, and NO 984 Hospital of PLA is in Beijing. On 31 January 2020, the Chinese government announced several nationwide strategies for preventing the COVID-19 pandemic. Patients with AIS diagnosed from 1 December 2019 to 31 January 2020 (pre-COVID-19), and those diagnosed from 1 February 2020 to 31 March 2020 (post-COVID-19) were compared in this study. AIS was diagnosed based on clinical symptoms and computed tomography or magnetic resonance imaging. Patients who reached the hospitals within 7 days after stroke onset were included. Socioeconomic status, medical history, stroke symptoms, National Institutes of Health Stroke Scale (NIHSS) score, Alberta Stroke Program Early CT Score (ASPECTS), modified thrombolysis in cerebral infarction (mTICI) score before discharge, onset-to-door (OTD) time defined as the time from onset to hospital arrival, door-to-needle (DTN) time defined as the time from hospital arrival to initiation of thrombolysis, door-to-puncture (DTP) time defined as the time from hospital arrival to groin puncture, and post-treatment NIHSS scores were reviewed

and analyzed. All participants and their relatives provided written informed consent, and the study was approved by the ethics committees of the participating hospitals. The reporting of this study conformed to the STROBE statement (14). The emergency department staff were equipped with adequate personal protective equipment (PPE). Nucleic acid tests, body temperature measurements, inquiries about recent travel, complete blood count checks, and chest CT scans were all part of the COVID-19 screening process. Patients with definite fever or respiratory symptoms, as well as those whose routine chest CT scans suggested COVID-19 imaging, would need to undergo in-hospital multidisciplinary consultation. According to an expert consensus on the stroke emergency map during the epidemic of coronavirus disease 2019 (15), hospitals involved in the study had a 24/7 on-call COVID-19 expert group (associate chief physician and above), including respiratory physicians, infection physicians, critical care physicians, imaging physicians, emergency medicine physicians, respiratory nurses, and critical care nurses, to closely coordinate with the stroke green channel, responsible for the consultation of patients with suspected COVID-19. We would consult patients with suspected COVID-19 acute stroke (mainly by video consultation) to clarify the diagnosis and guide the clinical treatment and protection strategy. Patients were triaged by multidisciplinary consultation based on the results of these screenings. In brief, COVID-19 nucleic acid-negative patients requiring MT were treated in routine standard operating procedures. Suspected positive patients were treated in a specialized operating room with the highest level of protection and transferred to a specialized isolation ward after surgery. Patients with AIS undergoing MT procedures were generally recommended to undergo local anesthesia, unless the patients were irritable and uncooperative.

The primary outcome of this trial was the mRS score at discharge after IVT or MT. Safety outcomes included intracerebral hemorrhage (ICH), arterial perforation, subarachnoid hemorrhage (SAH), and arterial entrapment (a complication of entrapment of the internal carotid artery, the vertebrobasilar artery entrapment, and the large intracranial vessels).

All variables with a $p < 0.1$ were then entered into the multivariable logistic regression model—influencing factors for short-term clinical poor outcomes. For patients treated with IVT, variables such as age, sex, stroke etiology, pre-admission mRS, NIHSS at admission, stroke history, hypertension, diabetes, hyperlipidemia, atrial fibrillation, and coronary heart disease were included in the full model. For patients with MT treatment, mTICI 2b-3 was also included in the model. In this study, variables such as smoking, alcohol drinking, anterior circulation, solitary, daytime onset, and residence were removed from the full to final models.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation or median and interquartile range (IQR), as appropriate. Categorical variables were presented as frequency and percentage. Continuous variables with normal distribution were compared using Student's *t*-test. The $\chi 2$ and Fisher's exact tests were used for comparing categorical values. Multivariable stepwise logistic regression was used to determine influencing factors for short-term clinical poor outcomes (mRS $\geq$ 3) among all the patients with AIS enrolled in this study. A two-sided *p*-value of < 0.05 was deemed statistically significant. All statistical analyses were performed using SPSS 25.

Results

In this study, a total of 1,286 patients with AIS were enrolled, among which 928 were ultimately not treated with thrombolysis due to contraindications, out-of-time window, personal preferences, and either too high or too low NIHSS ratings (Figure 1). Finally, 358 patients receiving IVT were included (Table 1), with 230 (64.2%) in the pre-COVID-19 group and 128 (35.7%) in the post-COVID-19 group. No discernible baseline differences were found between the two groups. All the post-COVID-19 group patients underwent additional screening, such as a chest CT scan and a multidisciplinary evaluation. In the pre-COVID-19 group, the median admission NIHSS score (IQR) was 9 (6–13), but in the post-COVID-19 group, it was 11 (7–15). According to expectations, the patients admitted during the COVID-19 period exhibited more severe symptoms ($p = 0.020$). OTD time increased from 68 min (interquartile range [IQR] 40–111) before to 85 min (IQR 45–127) after the COVID-19 pandemic ($p = 0.001$). DTN time increased from 50 min (IQR 40–75) before to 65 min (IQR 48–84) after the COVID-19 pandemic ($p = 0.048$). Compared with the pre-COVID-19 group, post-COVID-19 patients with IVT treatment had a higher rate of symptomatic intracranial hemorrhage (sICH) ($p = 0.043$). No significant differences were found in early neurological improvement, in-hospital mortality, mRS score (0-2) pre-admission, and at discharge between the two groups (Table 1).

Of the patients enrolled, 1020 of them were excluded for out-of-time window, low NIHSS scores, high pre-stroke mRS scores, low ASPECTS, no evidence of large artery occlusion, or refusal to undergo MT (Figure 1). A total of 266 patients with intracranial large artery occlusion were finally included (Table 2); of these, 168 (63.2%) were in the pre-COVID-19 group and 98 (36.8%) in the post-COVID-19 group. Compared with the pre-COVID-19 group, post-COVID-19 group patients had a higher NIHSS score [12 (9–17) vs. 10 (7–15), $p = 0.042$], and a lower percentage of pre-admission mRS (0–2) [68 (37.5%) vs. 29

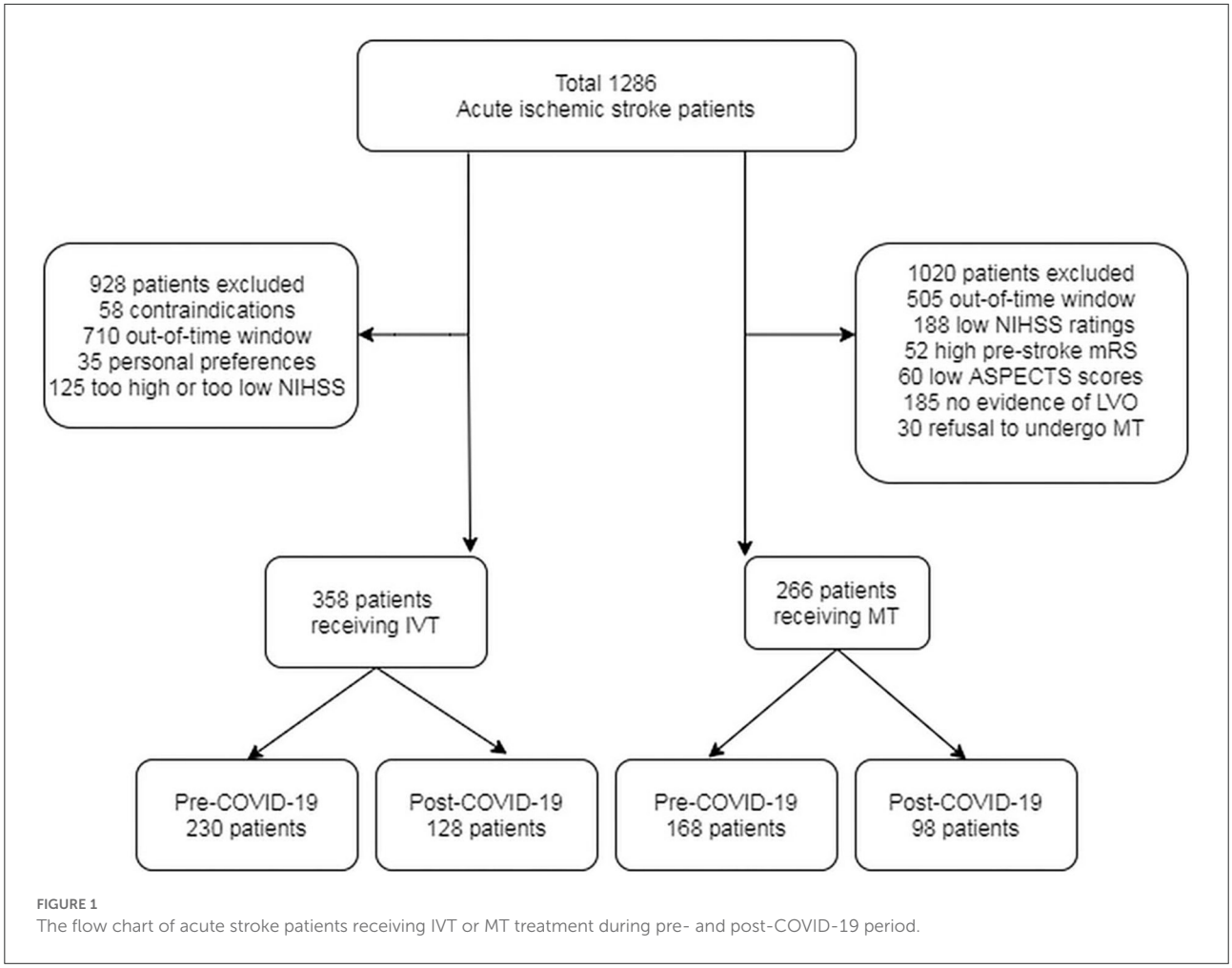

FIGURE 1
The flow chart of acute stroke patients receiving IVT or MT treatment during pre- and post-COVID-19 period.

(29.6%), $p = 0.011$], but no significant differences were found in the remaining baseline characteristics, including ASPECTS and the percentage of anterior circulation. The OTD time was 72 (38–118) min vs. 87 (41–136) min in the pre- and post-COVID-19 groups, $p = 0.012$. The DTP time was 120 (112–148) min vs. 160 (125–199) min before and after the pandemic, $p = 0.002$. No significant difference was found in the mTICI 2b-3 scores of the two groups (86.2 vs. 82.4 %, $p = 0.088$). Adverse events, including sICH, were not significantly different between the two groups (Table 2).

The post-COVID-19 group underwent a substantial delay for patients receiving IVT or MT due to the need for additional PPE ($p < 0.001$), COVID-19 screening processes ($p < 0.001$), multidisciplinary consultation ($p < 0.001$), and chest CT scans ($p < 0.001$). No significant differences were found in the proportion of patients receiving intravenous antihypertensive medication, family hesitancy about therapy, or hypoglycemia between the two groups before IVT or MT (Tables 3, 4).

Table 5 presents the potential influencing factors for the prognosis of AIS by multivariate logistic regression analysis. Compared with the patients before the pandemic, the patients during the COVID-19 lockdown period had an odds ratio (OR) of 1.10 (95% confidence interval [CI], 1.03–1.30) for spontaneous intracranial hemorrhage, and an OR of 0.90 (95% CI, 0.78–0.99) for mRS scores 0–2 at discharge, whereas no significant differences were found in the proportion of lower extremity venous thromboembolism (VTE) or pulmonary embolism (PE) during hospitalization and discharge disposition (home, inpatient rehabilitation).

In the current study, we found increasing age, OR: 1.81 (1.18–2.92), $p = 0.005$; worse pre-admission mRS, OR: 1.30 (1.15–1.48), $p = 0.010$; higher NIHSS score at admission, OR: 2.84 (1.45–4.8), $p < 0.001$; large vessel occlusion, OR: 2.02 (1.32–3.05), $p < 0.001$; admission during the lockdown period, OR: 1.22 (1.02–1.34), $p = 0.050$; mTICI grade 2b-3 after MT, OR: 0.44 (0.25–0.67), $p < 0.001$, to be significantly associated with poor outcomes in AIS (mRS $\geq$ 3) by logistic regression, whereas sex, history of stroke, hypertension, diabetes, hyperlipidemia, atrial fibrillation, and coronary artery disease were not (Table 6).

TABLE 1 Characteristics of acute ischemic stroke patients with intravenous thrombolysis treatment before and during the COVID-19 pandemic.

Characteristics	Pre-COVID-19 ($n = 230$)	Post-COVID-19 ($n = 128$)	p value
Age, year, mean	71.5 ± 10.1	70.9 ± 10.9	0.422
Male, sex, *n* (%)	138 (60.0)	75 (58.6)	0.737
Education, *n* (%)			0.511
Elementary education	69 (30.0)	35 (27.3)	
Secondary education	121 (52.6)	76 (59.4)	
Higher education	40 (17.4)	17 (13.3)	
Solitary, *n* (%)	75 (32.6)	46 (35.9)	0.110
Residence, *n* (%)			0.128
Urban	142 (61.7)	80 (62.5)	
Rural	88 (38.3)	48 (37.5)	
Daytime onset, *n* (%)	188 (81.7)	110 (85.9)	**0.033**
Stroke etiology *n* (%)			0.540
Large artery atherosclerosis	147 (63.8)	84 (65.6)	
Small vessel disease	31 (13.5)	17 (13.3)	
Cardioembolism	48 (20.9)	25 (19.5)	
Other demonstrated cause	2 (0.9)	1 (0.8)	
Undetermined cause	2 (0.9)	1 (0.8)	
NIHSS, median (IQR)	9 (6-13)	11 (7-15)	**0.020**
Stroke history, *n* (%)	46 (20.0)	29 (22.7)	0.179
Hypertension, *n* (%)	155 (67.4)	88 (68.8)	0.208
Diabetes, *n* (%)	95 (41.3)	55 (42.9)	0.225
Hyperlipidemia, *n* (%)	71 (30.9)	41 (32.0)	0.750
Atrial fibrillation, *n* (%)	35 (15.2)	18 (14.1)	0.209
Coronary heart disease, *n* (%)	47 (20.4)	28 (21.9)	0.177
Smoking, *n* (%)	104 (45.2)	56 (43.8)	0.353
Alcohol drinking, *n* (%)	88 (38.3)	48 (37.5)	0.501
OTD, min, median (IQR)	68 (40–111)	85 (45–127)	**0.050**
DTN, median (IQR), min, *n* (%)	50 (40–75)	65 (48–84)	**0.046**
In-hospital mortality, *n* (%)	11 (4.8)	7 (5.5)	0.220
sICH, *n* (%)	15 (6.5)	11 (8.6)	**0.043**
Pre-admission mRS (0–2), *n* (%)	125 (54.3)	62 (47.4)	0.072
mRS (0–2) at discharge, *n* (%)	138 (60.0)	72 (56.3)	0.180

IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; sICH, symptomatic intracranial hemorrhage. Bold values are significant at $p < 0.05$.

TABLE 2 Characteristics of acute ischemic stroke patients with mechanical thrombectomy treatment before and after the COVID-19 pandemic.

Characteristics	Pre-COVID-19 ($n = 168$)	Post-COVID-19 ($n = 98$)	p value
Age, year, mean	71.7 ± 10.4	70.6 ± 10.6	0.320
Male, sex, *n* (%)	86 (51.2)	52 (52.0)	0.107
Education, *n* (%)			0.721
Elementary education	50 (29.7)	27 (27.5)	
Secondary education	92 (54.8)	56 (57.1)	
Higher education	26 (15.5)	15 (15.3)	
Solitary, *n* (%)	42 (25.0)	26 (26.5)	0.310
Residence, *n* (%)			0.225
Urban	101 (60.1)	61 (62.2)	
Rural	67 (39.9)	37 (37.8)	
Daytime onset, *n* (%)	142 (84.5)	82 (83.7)	0.110
Stroke etiology *n* (%)			0.788
Large artery atherosclerosis	152 (90.5)	90 (91.8)	
Small vessel disease	0	0	
Cardioembolism	16 (9.5)	8 (8.2)	
Other demonstrated cause	0	0	
Undetermined cause	0	0	
NIHSS, median (IQR)	10 (7–15)	12 (9–17)	**0.042**
Stroke history, *n* (%)	35 (20.8)	22 (22.4)	0.189
Hypertension, *n* (%)	116 (69.0)	66 (67.3)	0.207
Diabetes, *n* (%)	68 (40.5)	41 (41.8)	0.525
Hyperlipidemia, *n* (%)	52 (31.0)	32 (32.6)	0.750
Atrial fibrillation, *n* (%)	29 (17.2)	17 (17.4)	0.929
Coronary heart disease, *n* (%)	36 (21.4)	24 (24.5)	0.097
Smoking, *n* (%)	84 (50.0)	51 (52.0)	0.251
Alcohol drinking, *n* (%)	75 (44.6)	47 (47.9)	0.081
OTD, min, median (IQR)	72 (38–118)	87 (41–136)	**0.012**
DTP, min, median (IQR)	120 (112–148)	160 (125–199)	**0.002**
Puncture to reperfusion time, min, median (IQR)	41 (29–54)	35 (27–47)	0.120
Onset to reperfusion time, min, median (IQR)	250 (178–330)	288 (190–385)	**0.045**
ASPECTS, (IQR)	9 (8–10)	9 (8–10)	1.000
Anterior circulation, *n* (%)	121 (72.0)	70 (71.4)	0.504
mTICI2b-3, *n* (%)	142 (84.5)	81 (82.7)	0.188
Adverse events, *n* (%)	46 (27.4)	29 (29.6)	0.220
Pre-admission mRS (0–2), *n* (%)	68 (37.5)	29 (29.6)	0.011
mRS score 0–2 at discharge, *n* (%)	98 (58.3)	52 (53.1)	**0.050**

IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; ASPECTS, Alberta Stroke Program Early CT Score; mTICI, modified thrombolysis in cerebral infarction. Bold values are significant at $p < 0.05$.

Discussion

In this retrospective study with focus on the COVID-19 lockdown period, we evaluated the impact of the pandemic on treatment delays and short-term clinical outcomes in patients with AIS. In this study, DTN time and DTP time were significantly longer during the COVID-19 period than during the pre-COVID-19 period. In our previous study, we found that the onset-to-door time was significantly prolonged during the COVID-19 pandemic compared with that in the pre-pandemic period (16). In this study, the need for additional PPE, viral

TABLE 3 Influencing factors for delayed intravenous thrombolysis treatment.

Variables	Pre-COVID-19 ($n = 230$)	Post-COVID-19 ($n = 128$)	p value
Need for additional PPE, *n* (%)	0	47 (36.7)	**<0.001**
COVID-19 screening processes, *n* (%)	0	128 (100)	**<0.001**
Multidisciplinary consultation, *n* (%)	13 (5.7)	24 (18.8)	**<0.001**
Hypertension requiring aggressive control with IV medications, *n* (%)	18 (7.8)	12 (9.3)	0.133
Initial refuse, *n* (%)	45 (19.6)	24 (18.8)	0.067
Blood glucose <50 mg/dl, seizures or major metabolic disorders, *n* (%)	11 (4.8)	5 (3.9)	0.115
Equipment-Related Delay, *n* (%)	6 (2.6)	6 (4.7)	**<0.001**
Need for chest CT scans, *n* (%)	87 (37.8)	128 (100)	**<0.001**
Other, *n* (%)	23 (10.0)	12 (9.4)	0.335

PPE, personal protective equipment. Bold values are significant at $p < 0.05$.

TABLE 4 Influencing factors for delayed mechanical thrombectomy treatment.

Variables	Pre-COVID-19 (n = 168)	Post-COVID-19 (n = 98)	p value
Need for additional PPE, *n* (%)	0	36 (36.7)	**<0.001**
COVID-19 screening processes, *n* (%)	0	98 (100)	**<0.001**
Multidisciplinary consultation, *n* (%)	11 (6.5)	19 (19.4)	**<0.001**
Hypertension requiring aggressive control with IV medications, *n* (%)	30 (17.8)	20 (20.4)	**0.013**
Initial refuse, *n* (%)	29 (17.2)	16 (16.3)	0.564
Need for chest CT scans, *n* (%)	52 (31.0)	98 (100)	**<0.001**
Care team unable to determine eligibility, *n* (%)	10 (5.9)	5 (5.1)	0.079
Equipment-Related Delay, *n* (%)	10 (5.9)	6 (5.1)	0.079
Need for additional imaging, *n* (%)	52 (31.0)	29 (29.6)	0.106
Catheter Lab Not Available, *n* (%)	18 (10.7)	11 (11.2)	0.098
Other, *n* (%)	29 (17.2)	18 (18.3)	0.260

PPE, personal protective equipment. Bold values are significant at $p < 0.05$.

TABLE 5 Association of the COVID-19 pandemic with outcomes among patients with AIS.

	OR (95% CI)	p value*
sICH among IV alteplase patients	1.10 (1.03–1.30)	**0.050**
VTE or PE during hospitalization	1.21 (0.83–2.10)	0.202
Discharge mRS 0–2	0.90 (0.78–0.99)	**0.028**
Discharge to inpatient rehabilitation facility	0.88 (0.73–1.39)	0.321
Discharge to home	2.10 (0.73–3.00)	0.520

*Regression models compare outcomes in patients during the COVID-19 period to those before the pandemic. Models are adjusted for patient demographics, clinical characteristics, medical history, and hospital characteristics.
OR, odds ratio; CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; sICH, symptomatic intracerebral hemorrhage within 36 h of thrombolysis; VTE, venous thromboembolism; PE, pulmonary embolism. Bold values are significant at $p < 0.05$.

nucleic acid testing, and chest CT scans were the main causes of in-hospital delay during the pandemic. PPE contributed to the delay in treatment times mainly due to the shortage or unavailability including the filtering facepiece respirators and gowns in the early lockdown period. In addition, the pandemic in itself was an independent risk factor for treatment delay and short-term unfavorable outcomes in patients with treated stroke. A large registry study involving 55,296 patients with AIS showed that in-hospital mortality was higher in patients with delayed thrombolytic therapy and that treatment delay was associated with poor clinical outcomes (17). According to a previous study, patients admitted during the COVID-19 pandemic experienced pre- and in-hospital treatment delays to varying extents (16).

Recent research has shown that even minor delays might have a negative impact on clinical outcomes in the short term (18). In the current study, the COVID-19 pandemic played a significant role in early adverse outcomes in patients with AIS. This effect is mainly attributed to the pandemic, which exacerbated certain time delays. The proportion of VTE or PE during hospitalization was not significantly associated with the COVID-19 pandemic partly due to the relatively short hospital stay in this study. In addition, a marginal increase in in-hospital mortality was noted among patients with AIS during the pandemic, which may have been due to the greater severity of stroke (19). Many patients with mild to moderate stroke avoided hospital admissions during the lockdown period, as indicated by the reports from some countries, which showed a 50–80% reduction in acute stroke admissions (20). The increased NIHSS score in the post-COVID-19 period partially supports this proposition. A similar pattern of delay in seeking medical care due to the fear of being infected within the hospital was observed during the Ebola epidemic in West Africa (21).

Our study also indicated that admission during the pandemic was an independent risk factor for short-term mortality and other adverse outcomes. It is crucial to identify and examine specific stroke workflows to improve stroke reperfusion rates and clinical outcomes. According to a previous study, inadequate imaging techniques may result in delays (22). However, this finding was unnoticed in our study and some other studies (23, 24). Kansagra et al. (25) reported a 39% decrease in the use of stroke imaging during the early COVID-19 pandemic period. Among these phases, delays from imaging to thrombolysis were the main factor responsible for the overall

TABLE 6 Multivariate logistic regression analysis of influencing factors for short-term clinical poor outcomes (mRS ≥ 3).

Variables	OR	95% CI	*p* value
Increasing age	1.81	1.18-2.92	**0.005**
Sex (female)	0.90	0.81-1.22	0.330
pre-admission mRS	1.30	1.15-1.48	**0.010**
Higher NIHSS at admission	2.84	1.45-4.88	**<0.001**
Large vessel occlusion	2.02	1.32-3.05	**<0.001**
COVID-19 pandemic	1.22	1.02-1.34	**0.050**
Stroke history	1.31	0.88-2.01	0.434
Hypertension	1.01	0.92-1.24	0.886
Diabetes	1.28	0.96-1.20	0.132
Hyperlipidemia	1.19	0.90-1.18	0.675
Atrial fibrillation	1.29	0.85-1.45	0.366
Coronary heart disease	0.99	0.87-1.25	0.278
mTICI 2b-3	0.44	0.25-0.67	**<0.001**

OR, odds ratio; CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; ASPECTS, Alberta Stroke Program Early CT Score; mTICI, modified thrombolysis in cerebral infarction. Bold values are significant at $p < 0.05$.

delay. Therefore, future research concentrating on imaging flow may be crucial to achieving a reduction in treatment delays in the ongoing pandemic. The findings of the current investigation (26) indicate that the prolongation of DTN time and DTP time can have a negative impact on short-term outcomes.

The strength of this study was that it included a large number of patients with non-COVID strokes based on real-world data. While previous studies from China for the most part focused on Wuhan, which was the initial area of the COVID-19 outbreak, we collected data from three provinces away from Wuhan, which could reflect the impact of the pandemic on stroke management outside the epicenter. Another strength of our study was that we examined how the pandemic affected treatment delays and clinical outcomes in patients with treated strokes and also identified the pandemic as an independent risk factor for poor short-term prognosis in AIS.

Limitation

A main limitation of this study was its retrospective, observational design, which made it prone to selection bias. Second, because COVID-19 -infected patients were not included in this study, we could not analyze any potential detrimental effects of the virus on patient prognosis. Third was the lack of data on the other changes that could have contributed to the delay such as adjustments made to triage protocols and availability of staff due to extremely busy emergency personnel during the earliest stages of the pandemic. Furthermore, the time of treatment delay in bridging therapy, which means patients received both thrombolysis and MT treatment, was not specifically collected by some centers, so the potential detrimental effects on the bridging therapy were not analyzed. In addition, the long-term effects of treatment delay on the 90-day functional outcomes of patients with AIS were not examined in the current study. Therefore, prospective multicenter studies with sizable sample numbers are needed to clarify the aforementioned findings.

Conclusion

In-hospital delays during the COVID-19 pandemic negatively impacted the treatment of non-COVID strokes in China. Given that anti-COVID-19 measures are evolving into medical norms, stroke centers need to evaluate local practice patterns to optimize the management processes and lessen the impact of the pandemic on clinical outcomes in patients with AIS.

Data availability statement

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

Author contributions

SG, JL, and ZD: study design, interpretation of results, and manuscript drafting. SG, YB, JL, HZ, and HS: study design and interpretation of results. YY, ZD, YB, JL, HS, HZ, and SZ: data collection. WT, YY, SZ, JL, YB, ZD, HS, and SG: study design, statistical analysis, and critical revision of the manuscript. SG and WT: interpretation of results, critical revision of the manuscript, has full access to all of the data in the study, took responsibility for the integrity of the data, and the accuracy of the data analysis. 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

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Outcomes after endovascular thrombectomy for acute ischemic stroke patients with active cancer: A systematic review and meta-analysis

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Background: Active cancer (AC) is a known risk factor for stroke and a common comorbidity among patients being considered for treatment with endovascular thrombectomy (EVT). This systematic review and meta-analysis aimed to evaluate the current evidence for the feasibility, efficacy, and safety of EVT for patients with AC.

Methods: MEDLINE, EMBASE, and the Cochrane Library were searched for relevant randomized controlled trials (RCTs) and observational studies which met the inclusion criteria for EVT in patients with AC. Studies were excluded due to the mismatch of data format, article type, and group design. The risk of bias was assessed through different scales according to the study design. I^2 statistics were used to evaluate the heterogeneity. Funnel plots were used to evaluate publication bias.

Results: A total of six studies and 3,657 patients were included. Compared to without active cancer (WC) patients, patients with AC had a significantly higher proportion of in-hospital mortality (OR 3.24; 95% CI, 1.03–10.15). The estimated rate of favorable outcome of six studies was lower in patients with AC than in patients with WC (OR 0.47; 95% CI, 0.35–0.65). For 90-day mortality of four studies, the AC group had a higher proportion when compared with the WC group (OR 3.87; 95% CI, 2.64–5.68). There was no difference between rate of six studies of successful recanalization (OR 1.24; 95% CI, 0.90–1.72) and four studies of symptomatic ICH (OR 1.09; 95% CI, 0.61–1.97) comparing AC and WC.

Conclusion: Patients with AC are less likely to have a favorable outcome and have a higher risk of mortality after EVT. Further studies are warranted for this unique patient population.

KEYWORDS

acute ischemic stroke, endovascular thrombectomy, active cancer, meta-analysis, systematic review

Introduction

Cancer is a widely known risk factor of acute ischemic stroke (AIS), especially among patients with active cancer (AC) which was diagnosed within 6 months or during the admission period, requires chemotherapy or surgical treatment within 6 months, or was recurrent, metastatic, or inoperable (1). Several mechanisms related to malignancy theoretically increase the risk of AIS, such as hypercoagulation state, migratory thrombosis, and tumor embolus (2–4). Also, AC-related stroke is associated with a higher morbidity in several studies (5–8). Indeed, about 10% of hospitalized patients with AIS had AC (9–11). Unfortunately, patients with AC are often ineligible for intravenous thrombolysis (IVT) due to various reasons, such as bleeding tendency and recent prior surgery (12, 13).

Endovascular thrombectomy (EVT) has revolutionized acute stroke care and is recommended as the first-line treatment for AIS due to large vessel occlusion (LVO) (14, 15). Whether EVT benefits AC-related stroke patients to a similar degree remains uncertain. In a previous meta-analysis including a relatively limited number of studies, the AC group had a comparable rate of successful recanalization and symptomatic intracerebral hemorrhage (sICH) compared to the control group, but a lower rate of favorable outcome (modified Rankin Scale $\leq$ 2) and a higher rate of mortality (16). However, some recent clinical studies indicated that rate of favorable outcome may be similar between the two groups, which differs from the aforementioned meta-analysis (4, 5). Other studies suggested that patients with active cancer are more likely to have any cerebral hemorrhage (4, 6). Thus, this article aims to investigate

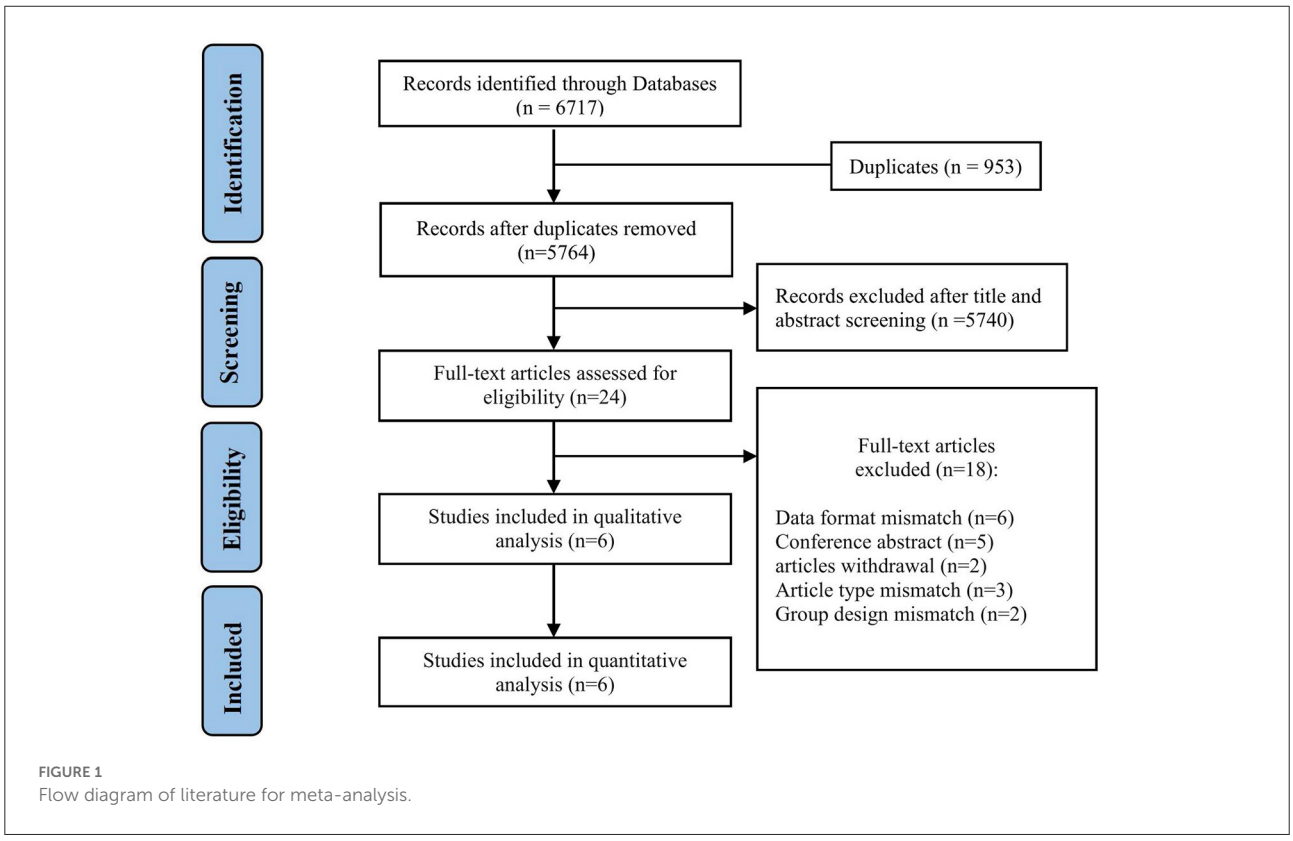

FIGURE 1
Flow diagram of literature for meta-analysis.

TABLE 1 Baseline characteristics.

	Bang-Hoon Cho et al. (3)		Verschoof et al. (20)		Fabrizio Sallustio et al. (21)		Lee et al. (6)		Joshi et al. (4)		Ciolli et al. (5)	
Basic information												
Publication time	2019		2022		2019		2019		2021		2021	
Country	South Korea		United States		Italy		South Korea		United States		Italy	
Type of studies	Single center		Muticenter		Single center		Single center		Single center		Single center	
NOS score												
Demographic characteristics												
Number	AC ($n = 27$)	WC ($n = 351$)	AC ($n = 124$)	WC ($n = 2459$)	AC ($n = 24$)	WC ($n = 24$)	AC ($n = 26$)	WC ($n = 227$)	AC ($n = 19$)	WC ($n = 95$)	AC ($n = 14$)	WC ($n = 267$)
Gender, male, n (%)	20 (74.1)	185 (52.7)	58 (46.8)	1277 (51.9)	8 (33.3)	8 (33.3)	18 (69.2)	137 (60.4)	2 (10.5)	10 (10.5)	8 (57)	135 (51)
Age (years) mean ± SD median [IQR]	69.04 ± 9.95	70.12 ± 11.46	69 ± 11	70 ± 14	69 ± 10.1	70.7 ± 9.3	63.2 ± 11.6	68.8 ± 11.3	70.9 (11.16)	70.7 (11.4)	73 (61–78)	72 (60–79)
Baseline NIHSS mean ± SD median [IQR]	11 (7–14)	12 (9–15)	16 (12–19)	16 (11–19)	14.2 ± 5.2	14.1 ± 4.9	14 (10–18)	13 (9–17)	22 (7.5)	22 (9.5)	20 (10–23)	16 (10–21)
Baseline ASPECTS mean ± SD median [IQR]	8 (6–8.25)	8 (6–9)	9 (8–10)	9 (7–10)	9.1 ± 0.9	8.8 ± 1	NR	NR	NR	NR	NR	NR
Cancer type												
Digestive tract, *n* (%)	7 (25.9)	0	41 (33.1)	0	5 (20.8)	0	NR	NR	2 (10.5)	0	NR	NR
Hepatobiliary, *n* (%)	7 (25.9)	0	0	0	0	0	NR	NR	1 (5.3)	0	NR	NR
Lung, *n* (%)	6 (22.2)	0	31 (25.0)	0	8 (33.3)	0	NR	NR	6 (31.6)	0	NR	NR
Urogenital, *n* (%)	3 (11.1)	0	26 (21.0)	0	4 (16.6)	0	NR	NR	5 (26.3)	0	NR	NR
Breast, *n* (%)	1 (3.7)	0	16 (12.9)	0	4 (16.6)	0	NR	NR	3 (15.8)	0	NR	NR
Hematological, *n* (%)	3 (11.1)	0	3 (2.4)	0	1 (4.1)	0	NR	NR	2 (10.5)	0	NR	NR
Other[a], *n* (%)	0	0	7 (5.6)	0	2 (8.2)	0	NR	NR	0	0	NR	NR
Medical history												
Hypertension, *n* (%)	14 (51.9)	208 (59.3)	50 (41.3)	1262 (52.3)	15 (62.5)	17 (70.8)	14 (53.8)	147 (64.8)	NR	NR	9 (64)	179 (72)
Admission SBP, mmHg mean ± SD median [IQR]	130.37 ± 21.75	137.35 ± 22.84	145 ± 25	150 ± 25	135.1 ± 22.2	145 ± 19.4	NR	NR	NR	NR	143 (135–173)	151 (138–170)
Admission DBP, mmHg mean ± SD median [IQR]	81.11 ± 12.51	85.56 ± 14.88	80 ± 16	82 ± 16	78.2 ± 13	81.1 ± 13.6	NR	NR	NR	NR	86 (76–100)	80 (70–90)
Diabetes mellitus, *n* (%)	7 (25.9)	73 (20.8)	25 (20.5)	391 (16)	8 (33.3)	5 (20.8)	7 (26.9)	56 (24.7)	NR	NR	3 (23)	51 (21)
Current smoking, *n* (%)	3 (11.1)	50 (14.2)	36 (29.8)	531 (21.8)	8 (33.3)	6 (25)	NR	NR	NR	NR	NR	NR
Dyslipidemia, *n* (%)	2 (7.4)	30 (8.5)	32 (26.7)	696 (29.5)	NR	NR	3 (11.5)	61 (26.9)	NR	NR	3 (23)	115 (48)
Atrial fibrillation, *n* (%)	6 (22.2)	117 (33.3)	32 (26.2)	582 (23.9)	7 (29.1)	9 (37.5)	6 (23.1)	118 (52.0)	NR	NR	NR	NR
Previous stroke, *n* (%)	3 (11.1)	60 (17.1)	17 (13.9)	408 (16.7)	NR	NR	3 (11.5)	47 (20.7)	NR	NR	NR	NR
Laboratory results												
Glucose, mean ± SD median [IQR]	NR	NR	7.4 ± 2.5	7.5 ± 2.3	142.9 ± 58.1	131.3 ± 57.8	NR	NR	NR	NR	NR	NR
Thrombocyte count, mean ± SD median [IQR]	NR	NR	272 ± 120	249 ± 83	NR	NR	212.35 ± 134.25	218.37 ± 63.52	NR	NR	NR	NR

(Continued)

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TABLE 1 (Continued)

	Bang-Hoon Cho et al. (3)		Verschoof et al. (20)		Fabrizio Sallustio et al. (21)		Lee et al. (6)		Joshi et al. (4)		Ciolli et al. (5)	
Loction of the occlusion												
MCA, *n* (%)	NR	NR	NR	NR	14 (58.3)	15 (62.5)	14 (53.7)	123 (54.2)	14 (73.7)	68 (71.5)	NR	NR
M1, *n* (%)	16 (59.3)	172 (49.0)	73 (62.4)	1350 (57.5)	7 (29.2)	8 (33.3)	12 (46.2)	105 (46.3)	NR	NR	NR	NR
M2, *n* (%)	2 (7.4)	55 (15.7)	12 (10.3)	364 (15.5)	NR	NR	2 (7.7)	19 (8.4)	NR	NR	NR	NR
ICA, *n* (%)	9 (33.3)	124 (35.3)	31 (26.5)	617 (26.3)	NR	NR	15 (57.7)	67 (29.5)	5 (26.3)	19 (20)	NR	NR
Other, *n* (%)	NR	NR	1 (0.9)	17 (0.7)	NR	NR	2 (7.7)	59 (26.0)	NR	NR	NR	NR
Intervention characteristics												
IV thrombolysis, *n* (%)	17 (63)	203 (57.8)	69 (56.6)	1862 (75.8)	11 (45.8)	11 (45.8)	5 (19.2)	79 (35.0)	NR	8 (42)	5 (36)	127 (48)
Onset to puncture, Min mean ± SD median [IQR]	NR	NR	203 (155–258)	200 (153–260)	NR	NR	NR	NR	NR	NR	205 (168–368)	268 (195–494)
Onset to recanalization, Min mean ± SD median [IQR]	351.81 ± 170.59	341.05 ± 170.35	255 (203–335)	256 (204–320)	NR	NR	NR	NR	NR	NR	335 (260–515)	380 (290–615)

[a]Metastases from unknown primary tumor, malignant tumor lower leg (histopathological findings not reported), sarcoma central pulmonary artery, melanoma, Non-Hodgkin lymphoma and pancreas.

AC, active cancer; WC, without cancer; AC is defined as being diagnosed with tumor and receiving or refusing relevant therapy.

SD, standard deviation; IQR, interquartile range; IV, intravenous; ICA, intracranial carotid artery; MCA, middle cerebral artery; M1, segment of the MCA; M2, segment of the MCA; NR, not report; NIHSS, national institutes of health stroke scale; ASPECTS, Alberta stroke program early CT score.

the safety and effectiveness of EVT in AC-related stroked patients, so as to provide clinicians with the most comprehensive and updated evidence for decision-making in clinical practice.

Methods

This study was conducted according to the statement of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (17).

Search strategy

Studies for inclusion were identified by two independent reviewers (CS and ZS) from the three databases: MEDLINE, EMBASE, and the Cochrane Library. Eligible studies were restricted from database inception until 24 January 2022 in the English language. The terms "ischemic stroke", "brain ischemia", "cancer", "neoplasm", "embolectomy", "mechanical thrombectomy", and "endovascular thrombectomy" were applied in our search strategy for potentially relevant studies. The detailed search strategy is presented in the online Supplementary material (Supplement File 1).

Study eligibility

The criteria for study design were specified according to the Population, Intervention, Comparison, Outcome (PICO) model.

Patient selection criteria

Inclusion criteria included adult patients (age ≥ 18 years) with AIS due to LVO, including anterior or posterior circulation occlusions, undergoing EVT. These were divided into active cancer (AC) group and without active cancer (WC) group according to the presence of AC. Active cancer was defined as cancer that was diagnosed within 6 months or during the admission period, requires chemotherapy or surgical treatment within 6 months, or was recurrent, metastatic, or inoperable (1). Arterial occlusion is confirmed by either computed tomographic angiography (CTA), magnetic resonance angiography (MRA), or digital subtraction angiography (DSA). We did not collect any primary data from patients, so ethics approval was deemed unnecessary by our IRB given there was a minimal patient risk.

Intervention

Mechanical thrombectomy with modern devices, such as stent retrievers or aspiration catheters, for patients is available to additional intravenous thrombolysis.

Outcomes

At least one of the following items was reported:

Primary outcomes:

1. Favorable outcome defined as modified Rankin Score (mRS) of 0–2 or equal to pre-stroke score at 90 days.

TABLE 2 Inclusion and exclusion criteria of patients in included studies.

Study	Verschoof et al.	Lee et al.	Cho et al.	Sallustio et al.	Joshi et al.	Ciolli et al.
Time window	ND	Presentation within 24 h of stroke onset	Presentation within 8 h of stroke onset	ND	ND	ND
Active cancer	Cancer diagnosis within 12 months prior to stroke, metastatic disease, or cancer treatment in the last 30 days	Patients with any metastatic disease, were undergoing current treatment for a malignancy offered treatment for a malignancy, but declined	Current or previous metastatic disease; patients undergoing current treatment for malignancy; patients refused treatment for current cancer; initial diagnosis of malignancy was made during hospitalization after the onset of stroke	ND	Patients who were diagnosed with cancer and were either receiving treatment or were treated conservatively, or those who were diagnosed with but refused treatment for cancer	Cancer was considered active if the diagnosis had occurred within six months before stroke, if patients had been treated for cancer within the previous six months, or in the presence of recurrent or metastatic cancer.
Baseline neurologic evaluation	ND	ND	mRS $\leq$ 2	ND	ND	ND
Imaging evaluation	ND	On-enhanced cranial CT scan and multimodal MRI; DWI–perfusion-weighted imaging mismatch	No evidence of ICH on CT or MRI; major arterial occlusion on MRA or CTA; a target mismatch pattern on multimodal MRI according to visual estimation; infarct volume of less than one-third of the MCA territory on DWI or non-enhanced CT	CTA for assessment of collaterals; CT quantified by ASPECTS	A non-contrast head CT and CTA of the head and neck with or without perfusion.	CT; CTA; CTP
Exclusion criteria	Patients with a history of cancer but not fulfilling the definition of active cancer.	AIS without occlusion of the relevant artery; arterial reperfusion performed > 24 h after symptom onset; intracranial neoplasm or metastasis; other causes of AIS; failure of IAT due to technical reasons and if clinical follow-up with a modified Rankin scale (mRS) value at 90 days was unavailable	ND	ND	ND	Patients were excluded if endovascular treatment, oncologic or follow-up data were missing

ND, no documentation; CT, computed tomography; MRI, magnetic resonance imaging; DWI, Diffusion Weighted Imaging; CTA, computed tomography angiography; mRS, modified Rankin Scale; MRA, MR angiography; ASPECTS, Alberta stroke program early CT score; NIHSS, national institute of health stroke scale; CTP, CT perfusion; ICH, intracranial hemorrhage; FLAIR, fluid attenuated inversion recovery.

2. Symptomatic intracranial hemorrhage (SICH) was diagnosed if a new intracranial hemorrhage was associated with any of the following conditions: (1) NIHSS score increased >4 points than that immediately before worsening; (2) NIHSS score increased >2 points in one category; (3) deterioration of neurological status led to intubation, hemicraniectomy, external ventricular drain placement, or other major medical or surgical intervention, according to the second European Australasian Acute Stroke Study classification (ECASS II) (18).

Secondary outcomes:

1. Successful recanalization (MTICI 2b-3) determined by post-interventional DSA.
2. Mortality at 90-day follow-up.
3. In-hospital mortality.

TABLE 3 The outcomes of comparison between AC and WC of primary and safety outcomes.

Outcomes	OR (95% CI)	I^2	*P*-value
Favorable outcome at 90-day	0.47 (0.35, 0.65)	0.0%	0.547
Rate of successful recanalization	1.24 (0.90, 1.72)	0.0%	0.828
Symptomatic ICH	1.09 (0.61, 1.97)	0.0%	0.386
90-day mortality	3.87 (2.64, 5.68)	19.3%	0.287
In-hospital mortality	3.24 (1.03, 10.15)	66.6%	0.030

CI, confidence interval; I2, the variation attributable to heterogeneity; mTICI, modified Thrombolysis in Cerebral Infarction; mRS, modified Rankin Score; ICH, Intracerebral hemorrhage.

Comparison

The comparator was patients of the WC group, who also received EVT without limitation of additional intravenous thrombolysis.

Studies

We included RCTs and observational studies, including cohort studies and case-controlled studies. Other types of articles such as abstracts, conference reports, and case reports were excluded. Studies which did not report the above outcomes or extractable complications were also excluded.

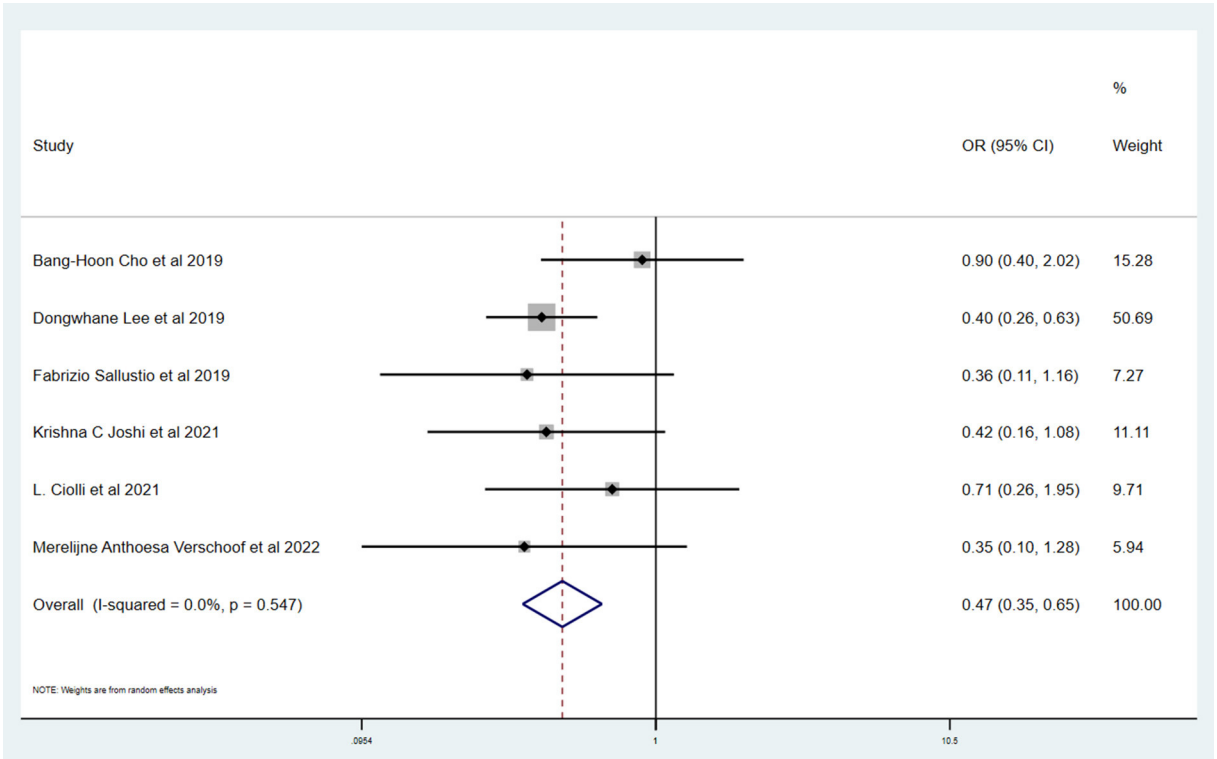

FIGURE 2
Forest plots of meta-analyses of favorable outcome at 90 days.

Selection of studies and data extraction

Two reviewers (ZF and ZS) independently searched the databases to include eligible studies. In the initial stage of screening, titles, keywords, and abstracts were reviewed, and irrelevant studies were excluded. Subsequently, full articles of all the remaining studies were obtained and carefully reviewed to assess eligibility, and reasons for inclusion or exclusion of studies were documented in detail. Conflicts in study selection between two reviewers were resolved by a third reviewer (HZ).

The extraction of data from included studies was conducted by two independent reviewers (LD and HZ) using a standardized data extraction form. The extracted information of included studies was as follows: (1) characteristics of the study, such as publication time, country, and the number of patients; (2) demographic characteristics, such as age, gender, cancer type, medical history, laboratory results, site of occlusion by angiography, and intervention characteristics; (3) aforementioned outcomes such as sICH and favorable outcome. The resolution of disagreement regarding data extraction was achieved through the assistance of another reviewer (XB). For missing or ambiguous data in included studies, clarification of data through direct contact with the corresponding authors by e-mail was attempted.

Assessment of risk bias and heterogeneity

Two reviewers (QT and XW) independently assessed the risk of bias for each included study. The Cochrane Collaboration criteria were applied in the process of selection of RCTs (Supplementary File 2). The Newcastle–Ottawa scale was used for observational studies, such as cohort studies and case-control studies (Supplementary File 3) (19). The heterogeneity of pooled outcomes was evaluated by I^2 statistic. If I^2 is <20%, the heterogeneity was considered acceptable. The Mantel–Haenszel method for fixed-effects estimation was applied if heterogeneity was mild or moderate. For substantial heterogeneity of outcomes, we conducted meta-regression and sensitivity analyses to explore the potential source of heterogeneity.

Measures of treatment effect

A meta-analysis on a specific result was performed only when there were at least two suitable studies for analysis. If there were insufficient suitable studies for meta-analysis, the results were described in the narrative. We adapted OR with 95% CIs for dichotomous data and the mean differences (MD) with 95%

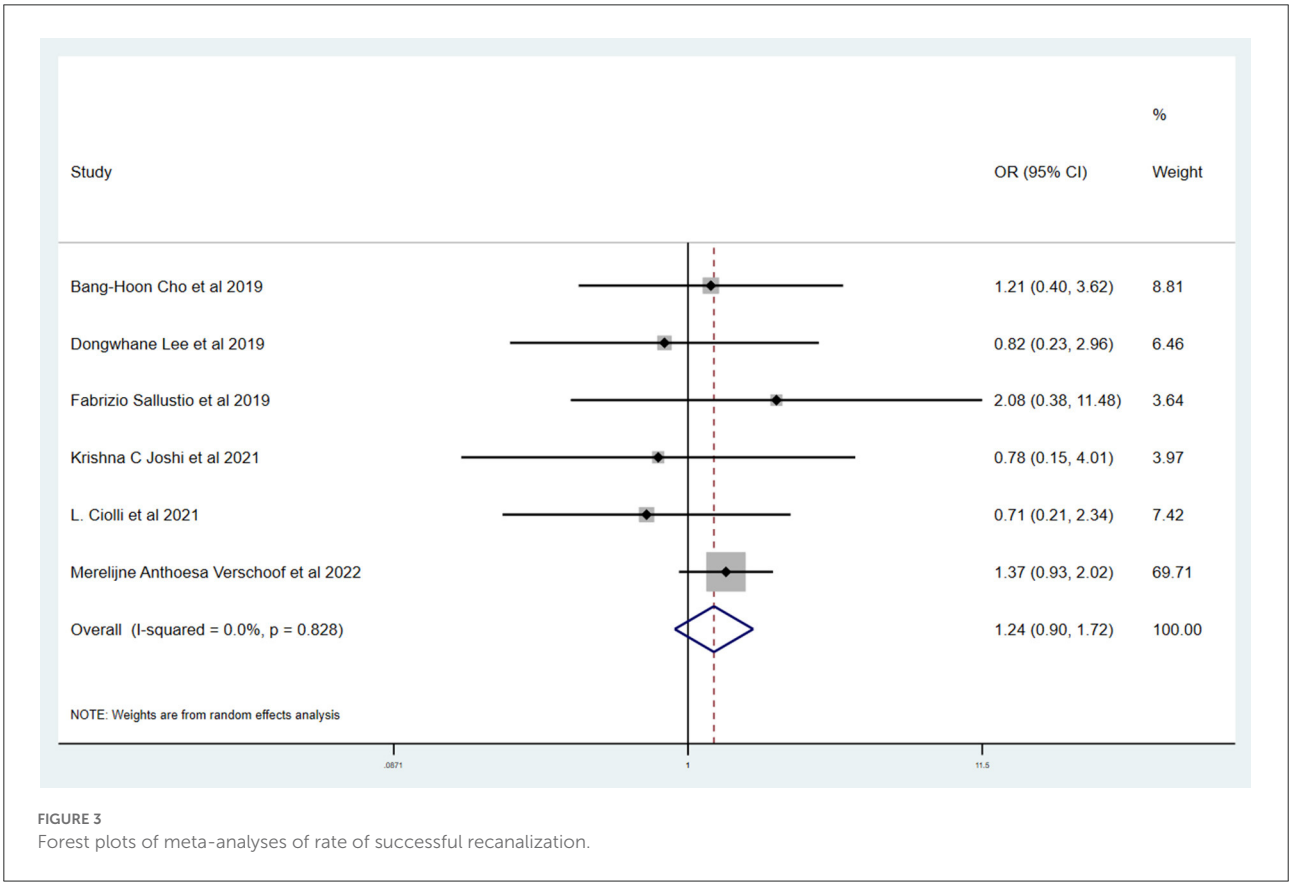

FIGURE 3
Forest plots of meta-analyses of rate of successful recanalization.

CIs for continuous data. The standard of p-value < 0.05 was considered statistically significant. The Stata statistical software (version 15.0, Stata Corp, College Station, Texas, USA) was used for data analysis and heterogeneity assessment.

Results

Study selection and study characteristics

We found 5,764 references, abstracts, and related clinical trials from the three electronic databases and clinical trial registries. Among the results, 24 full-text articles were retrieved after initial checks, and six studies were finally eligible for inclusion in the qualitative and quantitative analysis. The process of study selection and reasons for exclusion are summarized in Figure 1. Table 1 shows the characteristics of included studies and patients. A total of six studies and 3,657 patients met inclusion criteria (3–6, 20). All studies were published after 2019, two were conducted in Italy, two were conducted in the USA, and two were performed in South Korea. There was one multicenter study, and the remaining were single-center studies. The inclusion and exclusion criteria for patients included in the individual studies are summarized in Table 2. The inclusion criteria consisted of the time window, cancer diagnosis, and baseline neurological and imaging evaluations. The number of patients in each included study ranged from 48 to 2,583, and the ratio of men to women was equal. The site of occlusion as determined by angiography was mostly located at anterior circulation, especially the internal carotid artery and M1 segment of the middle cerebral artery. The estimated times of onset to groin puncture and onset to revascularization ranged from 153–494 to 203–615 min, respectively.

Meta-analysis of primary and secondary outcomes

Table 3 shows the primary and secondary outcomes for patients with AC and WC. In four studies and 3,140 patients, compared with patients with WC, patients with AC had a significantly higher proportion of in-hospital mortality (OR, 3.24; 95% CI, 1.03–10.15, $p = 0.030$; $I^2 = 66.6\%$). The estimated rate of favorable outcome of six studies and 3,657 patients was lower in patients with AC than in patients with WC (OR, 0.47; 95% CI, 0.35–0.65, $p = 0.547$; $I^2 = 0.0\%$). The outcome of four studies and 3,345 patients of 90-day mortality showed that the AC group had a higher proportion when compared with the WC group (OR, 3.87; 95% CI, 2.64–5.68; $p = 0.287$; $I^2 = 19.3\%$). There was no difference between rate of successful recanalization (OR, 1.24; 95% CI, 0.90–1.72; $p = 0.828$; $I^2 = 0.0\%$) in six

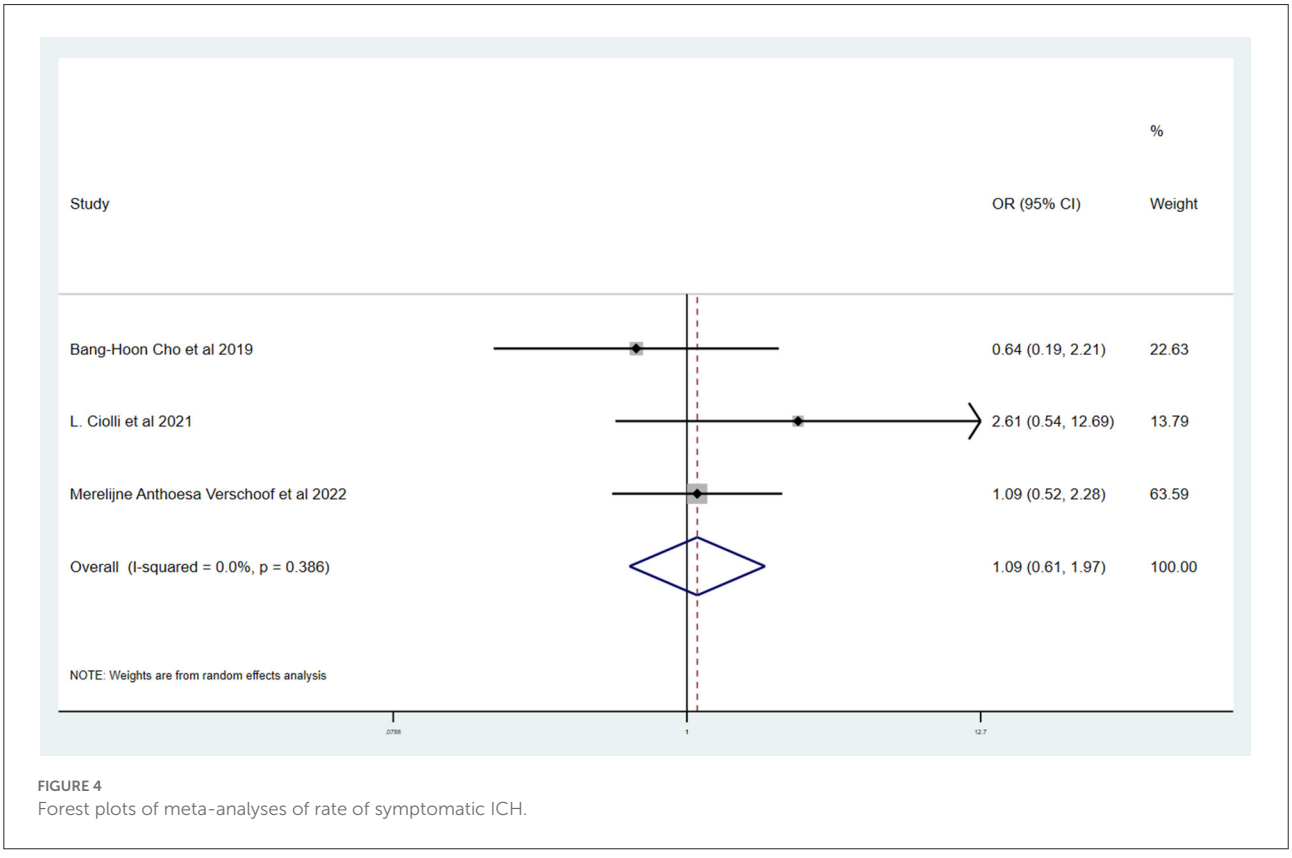

FIGURE 4
Forest plots of meta-analyses of rate of symptomatic ICH.

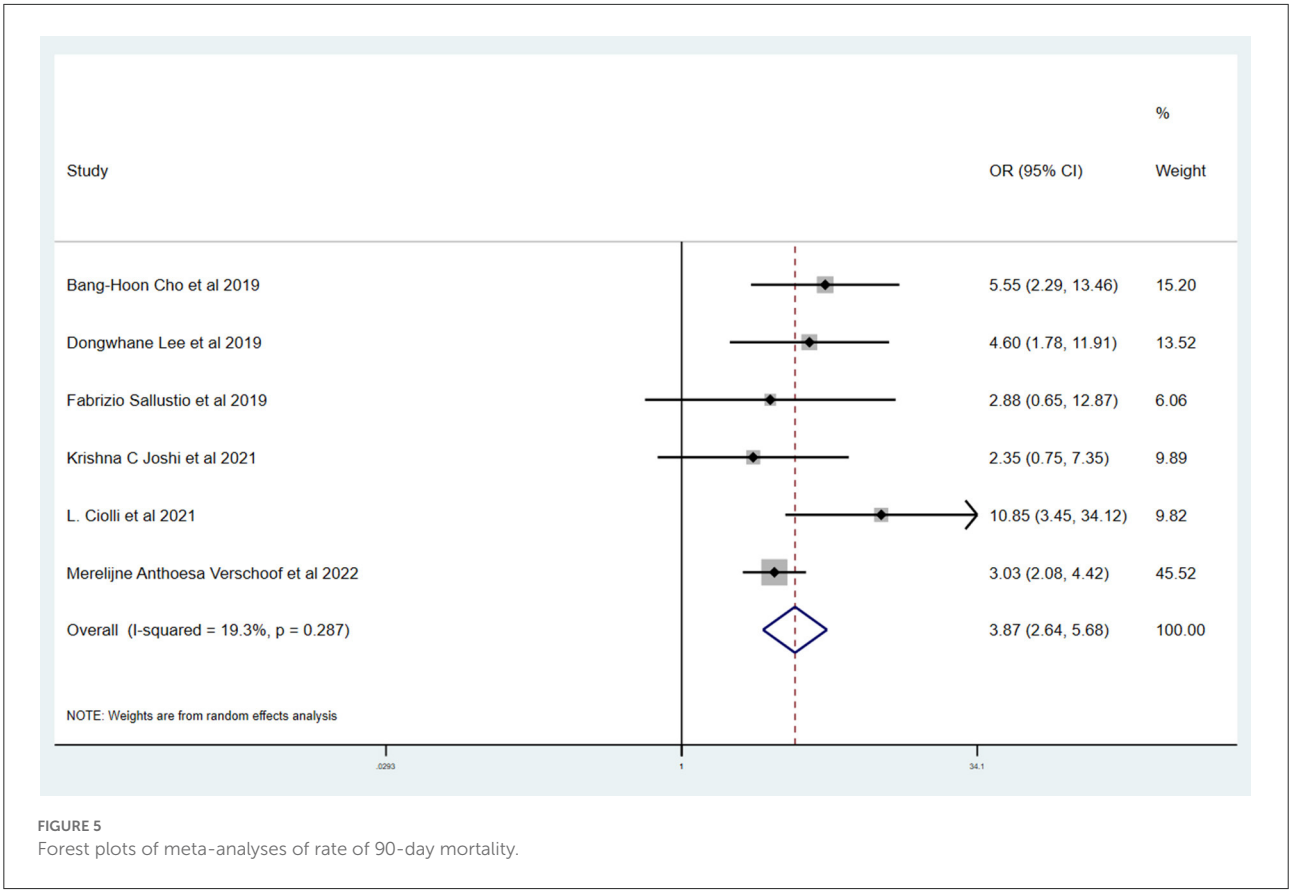

FIGURE 5
Forest plots of meta-analyses of rate of 90-day mortality.

studies and 3,657 patients and symptomatic ICH (OR, 1.09; 95% CI, 0.61–1.97; $p = 0.386$; $I^2 = 0.0\%$) in four studies and 3,140 patients between groups AC and WC.

Risk of bias

The Newcastle–Ottawa scale was used to assess the bias risk of observational studies, such as case-control studies, with the majority of included studies being low-risk. Funnel plots were used to explore the publication bias, with the results demonstrating no evident reporting bias. The outcomes of the above analyses are presented in Figures 2–6.

Discussion

This systematic review and meta-analysis summarized the safety and efficacy of EVT for AIS patients with AC. The successful recanalization proportion (OR, 1.24; 95% CI, 0.90–1.72; $p = 0.828$; $I^2 = 0.0\%$) and rate of symptomatic ICH (OR, 1.09; 95% CI, 0.61–1.97; $p = 0.386$; $I^2 = 0.0\%$) in patients with AC were comparable with patients with WC. However, patients with AC had a significantly lower rate of favorable outcome (OR, 0.47; 95% CI, 0.35–0.65, $p = 0.547$; $I^2 = 0.0\%$). We also showed a tendency for a higher proportion of in-hospital mortality (OR, 3.24; 95% CI, 1.03–10.15, $p = 0.030$; $I^2 = 66.6\%$) and 90-day mortality (OR, 3.87; 95% CI, 2.64–5.68; $p = 0.287$; $I^2 = 19.3\%$) in patients with AC.

The similar rate of successful recanalization and symptomatic ICH in patients with AC and WC supports the safety of EVT in patients with AC. However, our study found that there was a significant difference in 90-day favorable outcome and mortality after EVT in patients with and without active cancer. The presence of analytical abnormalities or hemostatic abnormalities is common in cancer patients, and it may be a major cause for the poorer prognosis of these patients. The level of platelet in blood of cancer patients often decreases, which may result in the tendency of bleeding and a higher rate of complications. What is more, the growth of cancer consumes a large amount of body's energy and may bring to malnutrition, thus giving rise to the poor prognosis. In addition, EVT will inevitably lead to the injury of vessels and plaque and trigger the inner repairing mechanism. Accumulation of platelet is a reaction of inflammation, which is a stimulation to malignant cancer and is likely to worsen the post-surgery prognosis. It should be noticed that, according to recent articles, under certain circumstances, thrombosis is a physiological process that

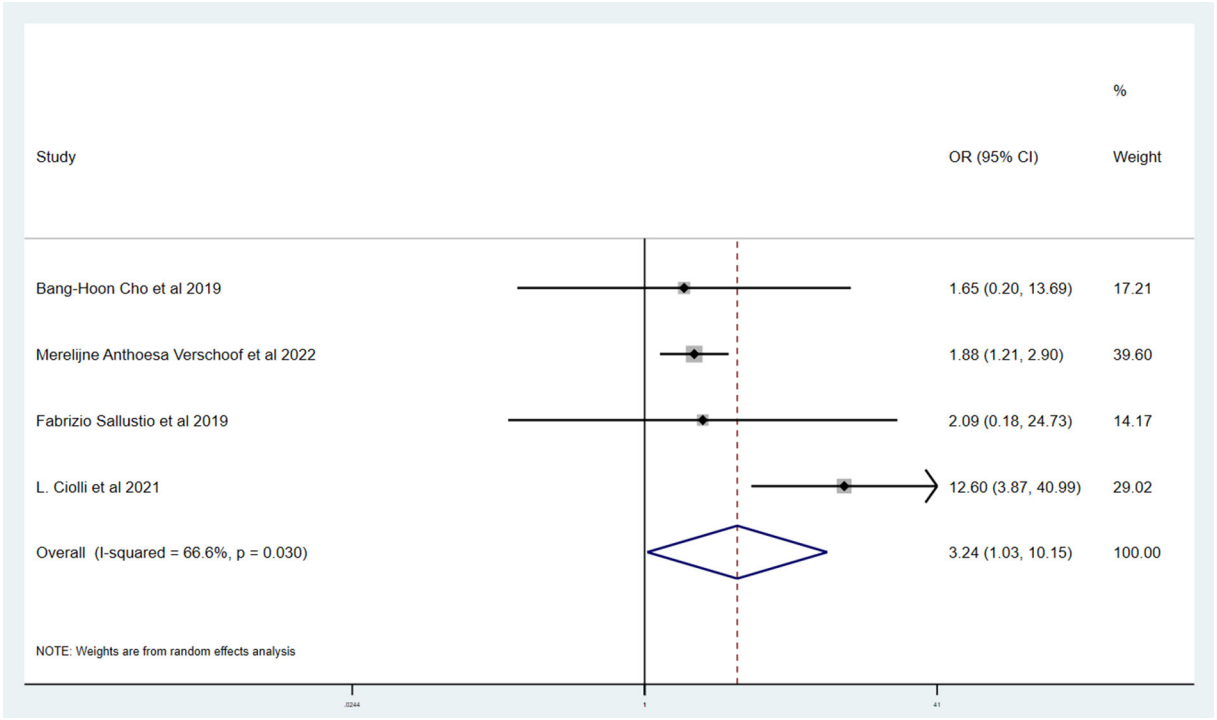

FIGURE 6
Forest plots of meta-analyses of rate of in-hospital mortality.

constitutes an intrinsic effector mechanism of innate immunity, whereas the rapid update of cancer cells will result in blood internal environment disorder and disable the defense system by thrombosis (22).

Intravenous thrombolysis (IVT), given to many patients in our meta-analysis, may be associated with higher risks in patients with AC. However, we did not observe a difference in hemorrhage rates and other studies have reported that IVT using alteplase is safe and effective for patients with AC (23, 24). The condition of cancer itself may also contribute to a higher rate of mortality. Stroke symptoms and related disability may influence decisions regarding cancer treatment, such as tumor resection and chemotherapy (5). Therefore, the death of patients in the AC group may be related to cancer and tumor progression. It is necessary to carry out more research on the stage, grade, and type of tumor to further analyze the mortality due to cancer.

Our study has several limitations. First, we could not evaluate the effects of cancer-related factors, including the cancer staging, brain metastasis, treatment status, and life expectancy due to insufficient data. Second, selection bias is inevitable in our study selection process. Furthermore, several included studies had small sample sizes, and there were inconsistent outcome measures. All of the included studies are retrospective, and only one was multicenter. More data are needed to confirm our conclusion.

Conclusion

AC is likely to influence patient outcomes after EVT and may hold a higher risk of mortality. While there were similar rates of reperfusion and hemorrhage, further high-quality studies are warranted to better understand long-term outcomes.

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.

Author contributions

LD contributed to the initial idea for this study. CS, QT, and XW developed and revised the search strategy. LD and HZ finished the study design. BY was consulted about the clinical issues. LD, ZF, and HZ contributed to the original draft. LD, ZF, HZ, CS, QT, AD, RR, ZS, XG, XW, and BY were responsible for the revision of the draft. All authors approved the final work 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.2022.992825/full#supplementary-material

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Which is the most effective rescue treatment after the failure of mechanical thrombectomy for acute basilar artery occlusion?

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Objective: The aim of this study was to evaluate the effectiveness and safety of rescue therapy, a therapy in which rescue devices such as balloon angioplasty, Apollo stent, Wingspan stent, Solitaire stent, or other self-expanding stents are used after the failure of mechanical thrombectomy (MT) and to determine the most effective rescue measure for acute basilar artery occlusion (BAO) after the failure of MT.

Methods: For this study, we recruited patients from the BASILAR registry. All participants were divided into three groups: the recanalized with rescue therapy group, the recanalized without rescue therapy group, and the non-recanalized group. Clinical outcomes at 90 days and 1 year were compared. The association of rescue measures with favorable outcomes (modified Rankin Scale [mRS] score of 0–3) in patients achieving successful recanalization *via* rescue therapy was estimated using multivariate logistic regression analyses.

Results: Among the participants, recanalization failure was found in 112 patients and successful recanalization in 473 patients, with 218 patients receiving rescue therapy and 255 patients without rescue therapy. Of these, 111 (43.5%) patients in the recanalized without rescue therapy group, 65 (29.8%) patients in the recanalized with rescue therapy group, and nine (8.0%) patients in the non-recanalized group achieved favorable outcomes at 90 days. Both the recanalization with rescue therapy and the recanalization without rescue therapy groups were associated with favorable outcomes at 90 days and 1 year compared with the non-recanalized group. Moreover, in patients receiving rescue therapy, Wingspan stents, Apollo stents, and balloon angioplasty were associated with higher rates of favorable outcomes at 90 days and 1 year than Solitaire stents.

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Conclusion: Whether rescue therapy is administered or not, recanalization leads to favorable outcomes in patients with acute BAO. For acute BAO after MT failure, balloon angioplasty, Wingspan stenting, and Apollo stenting could be considered effective and safe rescue options but not Solitaire stenting.

KEYWORDS

basilar artery occlusion, mechanical thrombectomy, rescue therapy, stroke, recanalization

Introduction

Acute basilar artery occlusion (BAO) is one of the fatal diseases, with a mortality of 90% and extremely high risk of disability if left untreated. Endovascular treatment (EVT) is an important treatment for acute BAO in clinical practice. Recently, some prospective or retrospective cohort studies suggested that EVT could improve the prognosis in patients with acute BAO (1–3).

For recanalization, both stent retrievers and contact aspiration are highly effective in removing emboli; however, 20–40% of patients with BAO still require rescue treatment to sustain recanalization (4, 5). In pursuit of successful reperfusion, multiple stent retriever passes or prolonged contact aspiration is often practiced, which is associated with worse outcomes and longer procedure duration as rescue treatment may lead to perforator occlusion and reperfusion delay (6, 7). Hence, it is essential to achieve successful reperfusion with fewer numbers of stent retriever passes. Whether it is safe to use rescue treatment after recanalization failure is unclear. Various devices are available for rescue treatment, including balloon angioplasty, Apollo stent, Wingspan stent, Solitaire stent, and other off-label self-expanding stents (Neuroform EZ and Enterprise stent). However, the effect of these devices on the prognosis of patients with acute BAO remains unascertained.

Using the data from the Endovascular Treatment for Acute Basilar Artery Occlusion (BASILAR) study, we aimed to evaluate the impact of rescue therapy on short- and long-term outcomes of EVT in patients with acute BAO. We also aimed to determine the most effective rescue treatment for acute BAO after the failure of mechanical thrombectomy (MT).

Methods

Study design and participants

The BASILAR study was a nationwide prospective registry conducted in 47 comprehensive stroke centers in China between January 2014 and May 2019. The study protocol was approved by the ethics committee of the Xinqiao Hospital, Army Medical University, Chongqing, China as well as that of each subcenter. The BASILAR study was registered with the Chinese Clinical Trial Registry (http://www.chictr.org.cn; ChiCTR1800014759).

Patients were eligible for inclusion if they were 18 years or older with acute ischemic stroke caused by BAO within 24 h of estimated occlusion time, confirmed by computed tomographic angiography, magnetic resonance angiography, or digital subtraction angiography. Patients with cerebral hemorrhage, a premorbid modified Rankin Scale (mRS) score greater than 2, current pregnancy or lactation, or a serious, advanced, or terminal illness were excluded. Details of the study design have been published previously (1). We obtained written informed consent from patients or their legally authorized representatives according to the Declaration of Helsinki.

Treatments and data collection

All eligible patients received EVT in combination with SMT. Treatment modalities of EVT, including mechanical thrombectomy with stent retrievers and/or thrombo-aspiration, balloon angioplasty, stenting, intra-arterial thrombolysis, or a combination of these approaches, were chosen at the discretion of neurointerventionalists. Rescue treatment can be defined as the use of rescue devices, such as balloon angioplasty, Apollo stent, Wingspan stent, Solitaire stent, and other self-expanding stents after the failure of MT. Based on recanalization with or without rescue therapy, the participants were divided into three groups: the non-recanalized group, the recanalized without a rescue therapy group, and the recanalized with a rescue therapy group.

The demographic characteristics, stroke risk factors, the premorbid modified Rankin Scale (mRS) score, the baseline National Institutes of Health Stroke Scale (NIHSS) score, and the baseline posterior circulation–Acute Stroke Program Early CT Score (pc-ASPECTS) were graded, as described earlier (8). The posterior circulation collateral score (PC-CS) represents the collateral circulation status based on the presence of potential collateral pathways on computed tomography angiography (9), the trial of Org 10172 in Acute Stroke Treatment (TOAST) classification, the location of the occlusion,

intravenous thrombolysis, important time metrics, and the thrombolysis in cerebral infarction (TICI) score. A TICI score ≥2b was defined as successful recanalization (10).

Outcome measures

The clinical outcomes we measured included short- and long-term outcomes. The primary outcome was a favorable functional outcome defined as an mRS score of 0–3 at 90 days. Secondary outcomes were functional independence (defined as an mRS score of 0–2) at 90 days, favorable outcomes at 1 year, mortality within 90 days or 1 year, re-occlusion within 24 h, and symptomatic intracerebral hemorrhage (sICH) within 48 h based on the Heidelberg Bleeding Classification.

Statistical analysis

Categorical and binary variables were compared using χ^2 tests (or Fisher exact tests), while continuous variables were compared using Student's *t*-test (mean comparison) and the Mann–Whitney *U* test for normal distribution variables. The Bonferroni test was used for multiple comparisons.

For baseline characteristics and outcomes, normally distributed continuous variables were presented as means and standard deviations, non-normally distributed continuous variables and ordinal variables were indicated as medians and interquartile ranges (IQRs), and categorical variables were indicated as absolute numbers and percentages. The effects of recanalization or rescue therapy on clinical outcomes were assessed using multivariable logistic regression, adjusting for age, history of diabetes, the baseline NIHSS score, baseline pc-ASPECTS, and occlusion site.

The second part only included patients achieving recanalization after rescue therapy. The clinical outcomes of different rescue measures were compared using the χ^2 test or the Fisher exact test, with Bonferroni correction. Using the means of other variables, favorable outcomes for different rescue measures were predicted.

We plotted the probabilities of favorable functional outcomes, and then we presented adjusted odds ratios with 95% confidence intervals. Probabilities of predicted outcomes were presented as three-dimensional distribution surface diagrams generated by SigmaPlot 14 and assessed using the R^2 correlation metric.

We performed statistical analyses using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and STATA version 16.0 (StataCorp LLC, TX, USA). The key variables in this study had low missingness and were analyzed with complete cases, but missing outcomes (sICH, 11 [1.9%]; mRS 0–2, mRS 0–3, and mortality at 1 year, 28 [4.8%]) were imputed using multiple imputations in the multivariate regression models. In the two-tailed test, a *P*-value < 0.05 was considered statistically significant.

Results

Patient characteristics

In the BASILAR registry involving 829 patients, we included 585 patients treated with EVT and with available mTICI scores. Among them, recanalization failure occurred in 112 patients and successful recanalization in 473 patients, with 218 patients receiving rescue therapy and 255 patients not receiving rescue therapy. The median (IQR) age and baseline NIHSS score of the entire cohort were 64 (56–74) years and 27 (7–33) years, respectively. A total of 147 patients (25.1%) were women and 438 patients (74.9%) were men.

A comparison of baseline characteristics of patients with endovascular thrombectomy in the recanalized with or without rescue therapy and non-recanalized groups is provided in Table 1. The patients in the recanalized without rescue therapy group had higher baseline pc-ASPECTS, more occlusion of the distal basilar artery (BA), less occlusion of the middle BA, higher frequency of atrial fibrillation, and shorter puncture-to-recanalization time. The patients in the non-recanalized group had higher baseline glucose.

Clinical outcomes

mRS distributions at 90 days in the non-recanalized group, the recanalized without rescue therapy group, and the recanalized with rescue therapy group are shown in Figure 1. Among the patients treated with EVT, nine patients (8.0%) in the non-recanalized group, 111 patients (43.5%) in the recanalized without rescue therapy group, and 65 patients (29.8%) in the recanalized with rescue therapy group achieved favorable functional outcomes at 90 days (Table 2). Whether rescue therapy was administered or not, the patients with successful recanalization had higher rates of functional independence and favorable outcomes, lower mortality at 90 days and 1 year, and lower rates of sICH within 48 h than the patients in the non-recanalized group (all adjusted *P*-value < 0.05). Moreover, compared with the recanalized without rescue therapy group, the recanalized with rescue therapy group showed lower rates of functional independence and favorable outcomes at 90 days, and functional independence at 1 year (all adjusted *P*-value < 0.05).

In the multivariable analyses, compared with the non-recanalized group, the recanalized without rescue therapy group resulted in a 6.7-fold increased probability of 90-day favorable functional outcomes (adjusted OR, 7.7, 95% CI, 3.05 to 19.44, $P < 0.001$), and the recanalized with rescue therapy group resulted in a 5.85-fold increased probability of 90-day favorable

TABLE 1 Baseline characteristics of the patients treated with endovascular thrombectomy in the recanalized with or without rescue therapy and the non-recanalized groups.

	Recanalized without rescue therapy group ($n = 255$)	Recanalized with rescue therapy group ($n = 218$)	Non-recanalized group ($n = 112$)	*P*-value
Age, y, median (IQR)	64 (56–74)	64 (57–72)	63 (53–73)	0.174
Women, n/total *n* (%)	74/255 (29.0)	42/218 (19.3)	31/112 (27.7)	0.040
Baseline NIHSS, median (IQR)	27 (17–33)	25 (15–32)	29 (19–35)	0.089
Baseline pc-ASPECTS, median (IQR)	8 (7–10)	8 (7–9)	7 (6–8)	0.000
GLU, median (IQR)	7.2 (5.9–9.1)	7.5 (6.1–9.7)	8.2 (6.6–10.9)	0.001
SBP, median (IQR)	148 (130–162)	150 (135–170)	150 (135–167)	0.123
Premorbid mRS, n/total *n* (%)				0.562
0	222 (87.1)	182 (83.5)	91 (81.3)	
1	22 (8.6)	25 (11.5)	16 (14.3)	
2	11 (4.3)	11 (5.0)	5 (4.5)	
TOAST, n/total *n* (%)				0.000
LAA	108 (42.4)	193 (88.5)	70 (62.5)	
CE	118 (46.3)	17 (7.8)	29 (25.9)	
Others	29 (11.4)	8 (3.7)	13 (11.6)	
History, n/total *n* (%)				
Ischemic stroke	44. (17.3)	57 (26.1)	24 (21.4)	0.063
Hypertension	162. (63.5)	160 (73.4)	82 (73.2)	0.039
Diabetes	51. (20.0)	55 (25.2)	28 (25.0)	0.339
Hyperlipidemia	81. (31.8)	73 (33.5)	42 (37.5)	0.563
Atrial fibrillation	93 (36.5)	14 (6.4)	22 (19.6)	0.000
Intravenous thrombolysis, n/total *n* (%)	55 (21.6)	43 (19.7)	28 (25.0)	0.544
Location of occlusion, n/total *n* (%)				0.000
Distal BA	138 (54.1%)	25 (11.5)	27 (24.1)	
Middle BA	57 (22.4)	76 (34.9)	45 (40.2)	
Proximal BA	28 (11.0)	54 (24.8)	21 (18.8)	
VA-V4	32 (12.5)	63 (28.9)	19 (17.0)	
Time metrics, min, median (IQR)				
Onset to puncture time	315 (218–457)	360 (233–541)	340 (215–496)	0.488
Puncture-to-recanalization time	79 (60–114)	128 (93–168)	120 (85–164)	0.000

IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; pc-ASPECTS, posterior circulation–Acute Stroke Prognosis Early Computed Tomography Score; GLU, glucose; SBP, systolic blood pressure; mRS, modified Rankin Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment; LAA, large-artery atherosclerosis; CE, cardioembolism; BA, basilar artery; VA-V4, V4 of the vertebral artery.

functional outcomes (adjusted OR, 6.85, 95% CI, 2.68 to 17.50, $P < 0.001$). Compared with the non-recanalized group, the recanalized without rescue therapy and the recanalized with rescue therapy groups had other better short- and long-term outcomes. However, compared with the recanalized without rescue therapy group, the recanalized with rescue therapy group did not show worse short- and long-term outcomes (Table 3). The probability of favorable functional outcomes declined with the increase in the NIHSS score and puncture-to-recanalization time in the recanalized with and without rescue therapy groups, although there was no interaction between favorable outcomes, NIHSS, and puncture-to-recanalization time (Supplementary Figures 1A,B).

Rescue measures and clinical outcomes

According to the rescue devices used, the patients treated with rescue therapy were divided into four subgroups: balloon angioplasty, Apollo stent, Solitaire stents, and other self-expanding stents. No difference was found among the characteristics of patients using different rescue devices (Supplementary Table 1).

As shown in Table 4, the Solitaire stent had a significantly lower rate of favorable outcomes at 90 days than balloon angioplasty and other self-expanding stents. Also, the Solitaire stent had a significantly higher rate of mortality at 90 days than the Apollo stent and other self-expanding stents. Although

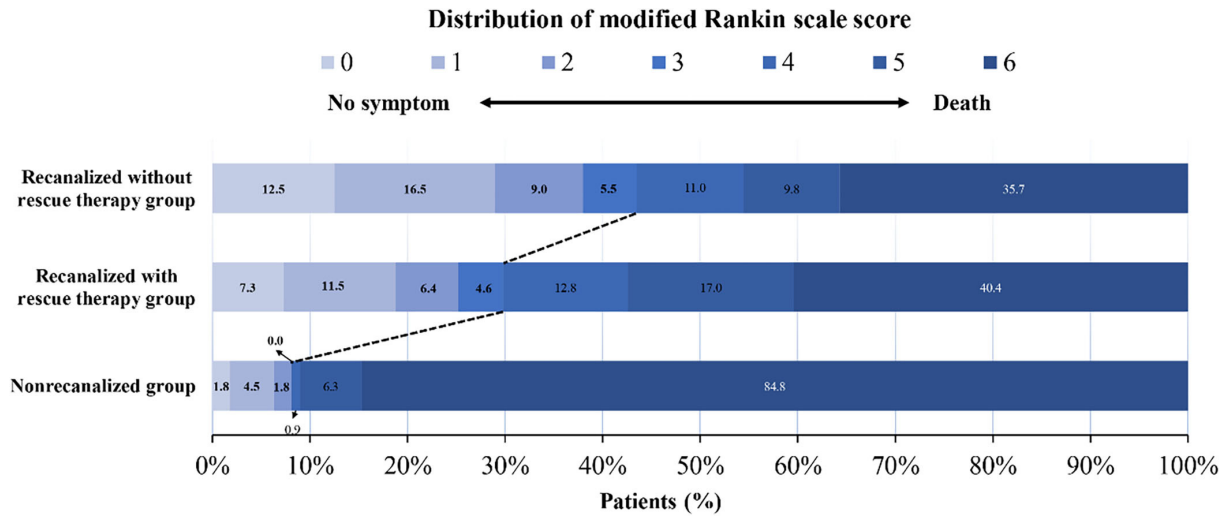

FIGURE 1
Distribution of scores on the modified Rankin Scale (mRS) at 90 days. The distribution of the mRS scores for favorable functional outcomes and mortality among patients in the non-recanalized group and in the recanalized with or without rescue therapy group. The numbers in the charts are percentages of patients who had each score, and the percentages may not sum to 100% owing to rounding.

the Solitaire stent had numerically less favorable outcomes and higher mortality rates at 1 year than other rescue devices, the differences were not statistically significant. The comparisons of re-occlusion within 24 h and sICH within 48 h among the four rescue devices were not significant (Table 4). Similar trends were observed in analyses of the occlusion of the middle BA and in analyses of the occlusion of the proximal BA or segment 4 of the vertebral artery, but trends were not significant due to small sample sizes (Supplementary Tables 2, 3). Different rescue measures have a direct effect on the probability of favorable functional outcomes. The estimated marginal effects of favorable outcome probability on the Wingspan and Apollo stents were higher than those of other rescue measures in 1 year, but not in 90 days. Furthermore, a lower risk of mortality and reocclusion was found in rescue therapy with Wingspan and Apollo stents (Figure 2).

Discussion

This study demonstrated that recanalization with or without rescue therapy led to better clinical outcomes in patients with acute BAO. Recanalization with rescue therapy showed clinical outcomes similar to recanalization without rescue therapy. Based on these outcomes, it seemed reasonable and necessary to use rescue therapy for acute BAO after the failure of MT. Moreover, rescue therapy with balloon angioplasty, Wingspan stenting, or Apollo stenting might be effective and safe rescue options, but not with the Solitaire stent, for acute BAO after MT failure.

Our findings confirmed the safety of using rescue therapy after the failure of MT for acute BAO, which was considered clinically meaningful. To our knowledge, the frequency of recanalization was considered a practical and useful clinical marker in the delivery of EVT. As reported in previous studies, a strong correlation was found between successful recanalization and favorable outcomes at 3 months in non-recanalized patients with acute ischemic stroke (11, 12). Moreover, as reported in previous studies, early recanalization was confirmed as a strong predictor of good outcomes in patients undergoing either EVT or SMT after acute stroke with large-vessel occlusion (13, 14). However, the relationship between rescue therapy and good outcomes remains controversial. Lazzaro et al. demonstrated that rescue therapy was associated with poor outcomes due to a longer recanalization time and a lower percentage of successful recanalization in patients undergoing rescue therapy (7). Nevertheless, Jia et al. reported that mechanical thrombectomy in conjunction with a standard rescue therapy could achieve favorable outcomes in patients with intracranial large-artery occlusion (ILAO) with underlying intracranial atherosclerosis (ICAS) (15). Moreover, the data from observational studies indicated that rescue therapy with intracranial angioplasty and/or stenting was safe and efficacious in patients with emergent LAO with underlying ICAS (16). Our study demonstrated that patients with acute BAO could benefit from rescue therapy after the failure of MT, corroborating the clinical application of rescue therapy for acute BAO.

Our study indicated that rescuing with balloon angioplasty alone after the failure of MT achieved a good outcome at 90 days, which was consistent with a previous report (17).

TABLE 2 Comparisons of clinical outcomes among recanalized patients with or without rescue therapy and non-recanalized patients.

	Overall	Non-recanalized group	Recanalized without rescue therapy group	Recanalized with rescue therapy group	*P*-value[a]	*P*-value[b]	*P*-value[c]	*P*-value[d]
Clinical outcomes in the short term, n/total *n* (%)								
mRS 0–3 at 90 days	185/585 (31.6)	9/112 (8.0)	111/255 (43.5)	65/218 (29.8)	< 0.001	< 0.001	< 0.001	0.003
mRS 0–2 at 90 days	161/585 (27.5)	9/112 (8.0)	97/255 (38.0)	55/218 (25.2)	< 0.001	< 0.001	< 0.001	0.002
Mortality at 90 days	274/585 (46.8)	95/112 (84.8)	91/255 (35.7)	88/218 (40.4)	< 0.001	< 0.001	< 0.001	0.342
SICH within 48 hours	38/574 (6.6)	15/106 (14.2)	14/255 (5.5)	9/213 (4.2)	0.002	**0.011**	**0.003**	0.678
Clinical outcomes in the long term, n/total *n* (%)	174/557 (31.2)	7/106 (6.6)	106/249 (42.6)	61/202 (30.2)	< 0.001	< 0.001	< 0.001	0.003
mRS 0–2 at 1 year								
mRS 0–3 at 1 year	201/557 (36.1)	9/106 (8.5)	115/249 (46.2)	77/202 (38.1)	< 0.001	< 0.001	< 0.001	0.039
Mortality at 1 year	304/557 (54.6)	95/106 (89.6)	107/249 (43.0)	102/202 (50.5)	< 0.001	< 0.001	< 0.001	0.134
Severe adverse events, n/total *n* (%)								
Pulmonary infection	439/585 (75.0)	83/112 (74.1)	187/255 (73.3)	169/218 (77.5)	0.558	0.979	0.579	0.344
Respiratory Failure	241/585 (41.2)	71/112 (63.4)	81/255 (31.8)	89/218 (40.8)	< 0.001	< 0.001	< 0.001	0.051
Circulatory failure	140/585 (23.9)	47/112 (42.0)	46/255 (18.0)	47/218 (21.6)	< 0.001	< 0.001	< 0.001	0.399
Gastrointestinal hemorrhage	104/585 (17.8)	26/112 (23.2)	34/255 (13.3)	44/218 (20.2)	0.037	0.028	0.620	0.061

Comparisons were made using the chi-square test with Bonferroni correction for multiple comparisons.
mRS, modified Rankin Scale; sICH, symptomatic intracranial hemorrhage.
[a] *P*-value for comparison among three groups.
[b] Adjusted *P*-value for the recanalized without rescue therapy group vs. non-recanalized group.
[c] Adjusted *P*-value for the recanalized with rescue therapy group vs. non-recanalized group.
[d] Adjusted *P*-value for the recanalized with rescue therapy group vs. recanalized without rescue therapy group.

TABLE 3 Multivariable regression analyses of clinical outcomes at 90 days and 1 year.

	Recanalized without rescue therapy group vs. Non-recanalized group		Recanalized with rescue therapy group vs. Non-recanalized group		Recanalized with rescue therapy group vs. Recanalized without rescue therapy group	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
mRS 0–3 at 90 days	7.70 (3.05–19.44)	<0.001	6.85 (2.68–17.50)	<0.001	0.89 (0.52–1.52)	0.669
mRS 0–2 at 90 days	6.03 (2.36–15.39)	<0.001	5.28 (2.04–13.67)	0.001	0.88 (0.50–1.53)	0.642
Mortality at 90 days	0.10 (0.05–0.21)	<0.001	0.10 (0.05–0.21)	<0.001	1.01 (0.59–1.71)	0.980
SICH within 48 hours	0.37 (0.14–0.97)	0.043	0.28 (0.11–0.72)	0.008	0.74 (0.27–2.02)	0.558
mRS 0–3 at 1 year	8.75 (3.58–21.37)	<0.001	7.77 (3.18–18.99)	<0.001	0.89 (0.52–1.53)	0.888
mRS 0–2 at 1 year	10.02 (3.67–27.39)	<0.001	8.14 (2.96–22.42)	<0.001	0.81 (0.47–1.41)	0.462
Mortality at 1 year	0.10 (0.04–0.22)	<0.001	0.11 (0.05–0.25)	<0.001	1.13 (0.66–1.94)	0.663

Adjusted for sex, pc-ASPECTS, GLU, TOAST, hypertension, Atrial fibrillation, location of occlusion, and puncture-to-recanalization time.
CI, confidence interval; mRS, modified Rankin Scale; SICH, symptomatic intracranial hemorrhage; pc-ASPECTS, posterior circulation–Acute Stroke Prognosis Early Computed Tomography Score; GLU, glucose; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

To our surprise, long-term outcome data demonstrated that, when the follow-up period was extended to 1 year, the clinical outcome of rescuing with balloon angioplasty alone was still not inferior to that of rescue stenting after the failure of MT in patients of acute BAO, corroborating the clinical use of balloon angioplasty alone as the rescue therapy for acute BAO after

TABLE 4 Comparison of clinical outcomes among patients rescued with different rescue measures.

	Balloon	Apollo	Solitaire	Other self-expanding stent	*P*-value[a]	*P*-value[b]	*P*-value[c]	*P*-value[d]
mRS 0–3 at 90 days	19/59 (32.2)	20/68 (29.4)	12/51 (23.5)	12/34 (35.3)	0.717	0.036	0.692	0.020
Mortality at 90 days	24/59 (40.7)	26/68 (38.2)	24/51 (47.1)	11/34 (32.4)	0.867	0.236	0.024	0.016
Reocclusion within 24h	2/34 (5.9)	2/36 (5.6)	3/28 (10.7)	2/20 (10.0)	0.937	0.252	0.795	0.230
SICH within 48h	3/57 (5.3)	4/67 (6.0)	0	2/32 (6.3)	0.690	0.227	0.107	0.255
mRS 0–3 at 1 year	22/55 (40.0)	30/62 (48.4)	9/45 (20.0)	14/34 (41.2)	0.719	1.000	1.000	1.000
Mortality at 1 year	25/55 (45.5)	28/62 (45.2)	32/45 (71.1)	13/34 (38.2)	0.726	0.383	0.125	0.104

Comparisons were made using the chi-square test with Bonferroni correction for multiple comparisons.
mRS, modified Rankin Scale; SICH, symptomatic intracranial hemorrhage.
P-value[a], balloon angioplasty vs. Apollo stents.
P-value[b], balloon angioplasty vs. solitaire stents.
P-value[c], Apollo stents vs. solitaire stents.
P-value[d], other self-expanding stents vs. solitaire stents.

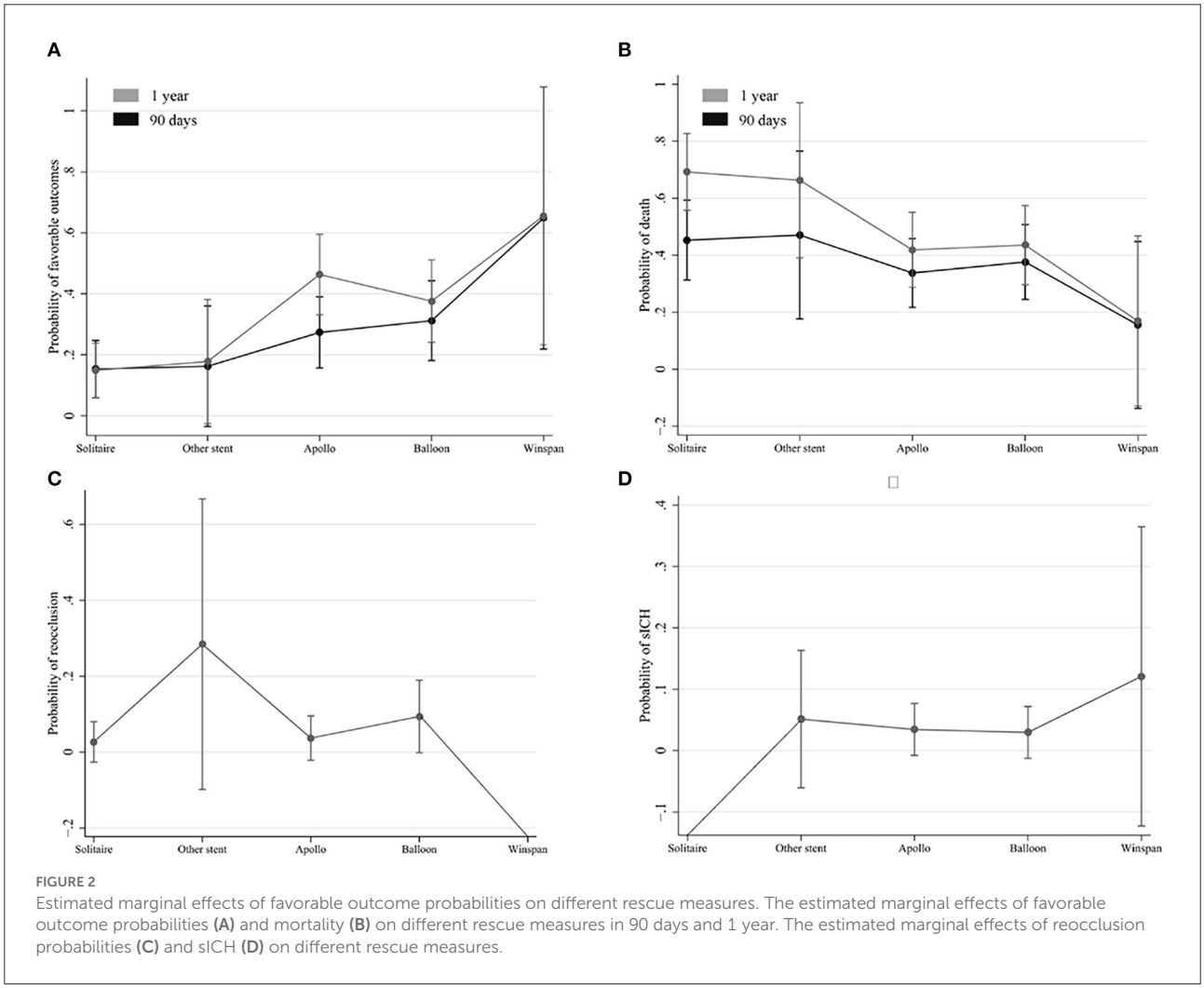

FIGURE 2
Estimated marginal effects of favorable outcome probabilities on different rescue measures. The estimated marginal effects of favorable outcome probabilities **(A)** and mortality **(B)** on different rescue measures in 90 days and 1 year. The estimated marginal effects of reocclusion probabilities **(C)** and sICH **(D)** on different rescue measures.

the failure of MT. As compared with rescue stenting, balloon angioplasty alone has several advantages. Balloon angioplasty alone could prevent vessel damage such as perforator occlusion and in-stent thrombosis and reduce the postoperative use of dual antiplatelet medication, which might help explain the benefits of rescuing with balloon angioplasty alone on short-

and long-term outcomes in the patients with acute BAO. Our results suggest that permanent stenting might not be necessary if sufficient blood flow was successfully achieved and maintained with balloon angioplasty, in line with previous reports (18, 19).

In addition to balloon angioplasty, our subgroup analysis also suggested that Wingspan stenting or Apollo stenting seems to be an effective and safe option for acute BAO after the failure of MT, but the permanent placement of a Solitaire stent is not recommended for acute BAO after the failure of MT. Our results indicated that the short-term clinical outcomes of rescuing with Wingspan stent were superior to those of rescuing with Apollo stent, particularly in patients with occlusion of the middle BA, while the long-term outcomes of these two groups were comparable. It is well known that the BA, especially the middle segment of the BA, may be the most high-risk location for perforator stroke as it may involve a great number of perforators (20). With the Wingspan stent, a self-expanding, laser-cut, nitinol stent designed specifically for intracranial stenosis, it was easier to access and deliver to the target vessel with reduced barotrauma due to its flexibility and appropriate radial force, which theoretically decreases the risk of perforator occlusion and parent vessel dissection or rupture compared with balloon-expandable stents (21), which may help explain the main advantage of using Wingspan stents as rescue therapy in this trial. The Apollo stent is a balloon-expandable stent designed specifically for intracranial stenosis. The use of the balloon-expandable stent with a higher radial force than the Wingspan stent would more likely result in perioperative complications such as vasospasm, arterial dissection, or perforator occlusion (22), which can explain the worse outcome of rescuing with Apollo stents at 90 days. However, as these patients with perioperative complications recovered, it can be considered that using the rescue therapy with Apollo stents achieved comparable long-term clinical outcomes as compared with Wingspan stents in this trial, which was consistent with previous research (23). To save cost, the Solitaire stent was often selected as the rescue implant stent in clinical practices, particularly in developing countries. Nevertheless, according to our results, using the rescue therapy with permanent Solitaire stent implantation led to worse clinical outcomes than using the rescue therapy with balloon angioplasty, Wingspan stent, or Apollo stent. This result can be supported by several pieces of evidence. First, the Solitaire stent—designed as a stent-assisted coiling of aneurysms with a lower radial force and lacking adhesive force against the vessel wall when compared with Wingspan stents—was more likely to cause in-stent acute thrombosis. Second, as reported in a previous study (24), the Solitaire stent was rarely expanded fully. Therefore, after the detachment of the rescue Solitaire stent, on the one hand, the thrombus was easy to form near to or outside the strut, and, on the other hand, it was difficult to achieve sufficient blood flow, which are the reasons contributing to poor outcomes in patients. Due to these reasons, our study suggested that the Solitaire stent cannot be recommended as a rescue implant stent for acute BAO after MT failure.

There are several limitations to this study. First, the device selection was not randomized, and the rescue stent was selected based on the neurointerventionists' preference in each center. Second, the number of patients who underwent rescue therapy with Wingspan stents was small; therefore, further evaluation of a larger cohort will be necessary.

Conclusion

The present study demonstrated that it is reasonable and necessary to administer rescue therapy after the failure of MT for acute BAO. This study also suggested that rescue angioplasty, Wingspan stenting, and Apollo stenting are effective and safe rescue options, but not the Solitaire stent, for acute BAO after MT failure. Future randomized clinical trials with a larger sample size of patients with BAO undergoing EVT are required to illuminate the effect of different rescue measures on the clinical prognosis of the patients.

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 Xinqiao Hospital, Army Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JL, DW, and ZL interpreted the data and drafted the manuscript. JFL and DK contributed to the conception and design of the study. DX, JH, and JS performed the statistical analyses. WL and SL contributed to the acquisition, analysis, and interpretation of data. FL, WZ, and QH provided technical or material support and critically revised 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.2022.992396/full#supplementary-material

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Biomarker of early neurological deterioration in minor stroke and proximal large vessel occlusion: A pilot study

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Background: Plasma neurofilament light chain (pNFL) represents one of the scaffolding proteins of central nervous system axonal injury. The aim of this study was to evaluate pNFL as a predictive biomarker for early neurological deterioration (END) in medically managed patients with large vessel occlusion (LVO) and mild presentation (NIHSS < 6).

Methods: This retrospective study was developed from a prospectively collected stroke database, which was conducted at a large academic comprehensive stroke center in western China. Patients who first presented with acute ischemic stroke (AIS) within 24 h of symptom onset were continuously included. Stroke severity was analyzed at admission using the NIHSS score. The pNFL drawn on admission was analyzed with a novel ultrasensitive single-molecule array.

Results: Thirty-nine consecutive patients were included in the analysis, and 19 (48.72%) patients experienced END. Patients who experienced END had significantly higher pNFL levels (mean, 65.20 vs. 48.28 pg/mL; $P < 0.001$) and larger infarct volume (mean, 15.46 vs. 9.56 mL; $P < 0.001$). pNFL was valuable for the prediction of END (OR, 1.170; 95% CI, 1.049–1.306; $P = 0.005$), even after adjusted for age and sex (OR, 1.178; 95% CI, 1.038–1.323; $P = 0.006$), blood sampling time, baseline NIHSS, TOAST classification, and infarct volume (OR, 1.168; 95% CI, 1.034–1.320; $P = 0.012$). The area under the ROC curve was 85.0% (95% CI, 0.731–0.970; $P < 0.001$). The sensitivity was 73.7%, and the specificity was 80%.

Conclusion: END in minor stroke with LVO was distinguishable from those without END following the determination of pNFL in the blood samples within 24 h of onset. The pNFL is a promising biomarker of END in minor stroke with LVO.

Clinical trial registration: ChiCTR1800020330.

KEYWORDS

biomarkers, early neurological deterioration, large vessel occlusion, minor stroke, neurofilament light

Introduction

Minor stroke is the most common and may represent up to 50% of cases of acute ischemic stroke (1). Proximal large vessel occlusion (LVO) is present in up to 30% of minor strokes (2). Mechanical thrombectomy is the standard of care for patients with LVO presenting with severe symptoms; however, little is known about the best treatment for patients with LVO and mild symptoms. On the one hand, most patients with LVO strokes and mild symptoms have good clinical outcomes; on the other hand, among patients with early neurological deterioration (END), 77% were dead or dependent at 3 months (3). The safety and effectiveness of endovascular therapy have been confirmed by a large number of literature (4–8). Therefore, endovascular therapy should not be given to all patients for LVO, nor should it be stopped because of mild stroke. The higher risk individuals with acute neurological deterioration are the people who need endovascular therapy. It is not difficult to see that the problem now is how to accurately predict END in this population (9).

Neurofilament light chain (NFL), as a protein exclusively expressed in neurons (10), might be a suitable candidate for this purpose because of its potential application prospects in patient monitoring, observation, and intervention research (11). The higher NFL level was found in a TIA patient who developed an ischemic stroke 1 day after blood sampling (12). It might suggest that NFL releasing in ischemic brain injury may have already started before symptoms became clinically apparent.

In light of this, this study aims to investigate the correlation between plasma NFL and END in minor stroke with LVO. We hypothesized that NFL measured within 24 h predicts END in minor stroke with LVO.

Patients and methods

Participants

Data are available on request from the corresponding author upon reasonable request. The study was conducted according to the principles expressed in the Declaration of Helsinki. The ethics committee of General Hospital of Western Theater Command approved sample collection and analysis (No. 2018ky06). All patients or their welfare guardians provided written informed consent for the collection of data, blood samples, and subsequent analyses. This was a single-center retrospective analysis of consecutive patients presenting with mild stroke (National Institute of Health Stroke Scale [NIHSS] <6) and anterior circulation LVO [internal carotid artery (ICA), M1/M2 segment of the middle cerebral artery (MCA), and A1/A2 segment of the anterior cerebral artery (ACA)] from a prospectively collected stroke database (ChiCTR1800020330) (13). From 1 July 2017 to 31 December 2019, acute ischemic stroke (AIS) patients over 18 years old who first presented with stroke symptoms and were confirmed by magnetic resonance imaging (MRI) or computed tomography (CT) were collected into the database. Patients were excluded if they were treated by immediate endovascular therapy or intravenous thrombolysis before END but including those who eventually received rescue thrombectomy because of END. Therefore, rescue thrombectomy refers to the thrombectomy taken when patients have END (NIHSS increased by $\geq$ four points) and disabling clinical symptoms. LVO was determined by reviewing each initial computerized tomography angiography (CTA), magnetic resonance angiography (MRA), or digital subtraction angiography (DSA) report. Early neurological deterioration (END) was defined as four or more points' deterioration on NIHSS score within the first 24 h without parenchymal hemorrhage on follow-up imaging or another identified cause.

To evaluate disease severity, patients were scored by the National Institutes of Health Stroke Scale (NIHSS) score and infarct volume (calculated by MRI-DWI), referring to the previous study protocol (14).

Blood sampling and biomarker measurement

Approximately 8 mL venous blood was collected in glass tubes containing sodium ethylenediaminetetraacetic acid (EDTA) from each subject on admission, and the time from stroke onset to blood collected was recorded. The blood samples were centrifuged at 2,000 × g at 4°C for 10 min within ~40 min of collection. Plasma supernatant was collected, divided into aliquots, and frozen at −80°C until further use. We measured pNFL by the SIMOA platform (Quanterix, Lexington, MA, USA) as described (13, 15). An in-house pool was used as an internal control and included in each assay for evaluating the assay performance. More detailed information on experimental methods can refer to our previous research (13).

Statistical analysis

Data are presented as mean (±SD), median (interquartile range [IQR]), or numbers with percentages. For univariate analysis, the Mann–Whitney U-test, Student's *t*-test, or the chi-square test were used, as appropriate. The association of pNfL levels with END was analyzed by multiple logistic regressions and adjusted for established predictors. Criteria for the entry of variables in the regression analyses were set at $P < 0.05$, together with other clinically significant variables. To assess the diagnostic accuracy of pNfL for discriminating END and Non-END, we calculated the area under the receiver operating characteristic (ROC) curve. The optimal cutoff level for dichotomizing values was selected as the situation maximizing the Youden index. All analyses were performed

using SPSS 26 (IBM, Chicago, IL). Two-tailed $P < 0.05$ was considered significant.

Results

A total of 39 patients with acute LVO presenting with mild symptoms were included in this study. Among them, 19 (48.72%) patients experienced END. The mean age was 64.23(±10.73) years; 48.72% were men; the mean pNFL was 56.53(±14.22); and median clinical severity was three points on the NIHSS (IQR, 2–4). In all cases, the mechanism of END was progressive stroke in the same vascular territory. Baseline demographics and clinical characteristics are shown in Table 1. We found no statistical difference in the baseline characteristics between the groups with or without END, except for infarct volume and pNFL. Patients who experienced END had significantly higher pNFL levels (mean, 65.20 vs. 48.28 pg/mL; $P < 0.001$, Table 1 and Figure 1) and larger infarct volume (mean, 15.46 vs. 9.56 mL; $P < 0.001$).

After multivariate analysis, pNFL levels (OR, 1.170; 95% CI, 1.049–1.306; $P = 0.005$) were significant for prediction of END, even after adjusted for age and sex (OR, 1.178; 95% CI, 1.038–1.323; $P = 0.006$), blood sampling time, baseline NIHSS, TOAST classification, and infarct volume (OR, 1.168; 95% CI, 1.034–1.320; $P = 0.012$; for details, see Table 2).

The highest sensitivity and specificity value required to make a distinction between END and Non-END was obtained using a pNFL cutoff point 55.03 pg/mL. The area under the ROC curve was 85.0% (95% CI, 0.731–0.970; $P < 0.001$). The sensitivity was 73.7%, and the specificity was 80% (Figure 2).

Discussion

Little is known about the best treatment for minor stroke with LVO. Endovascular therapy should not be given to all patients for LVO, nor should it be stopped because of minor stroke. The higher risk individuals with END are the people who need endovascular therapy. In the current study, pNFL levels were analyzed and quantified using a novel ultrasensitive technique in a cohort of END and Non-END patients with different etiologies. This study shows that END frequently occurs (39.4%) in patients with minor stroke and LVO. pNFL levels were shown to be elevated in patients with END compared to those with Non-END within 24 h of onset, and pNFL independently predicted END. The levels of pNFL showed significant diagnostic accuracy in discriminating patients with END from those without END. This is the first study that has investigated the pNfL levels in mild stroke with LVO.

It is unclear which factors can predict END in patients with LVO and mild symptoms. Although rescue endovascular therapy was associated with improved clinical outcomes in patients with neurological deterioration (16), primary endovascular therapy was better than secondary endovascular therapy in the case of neurological deterioration (17, 18). Accurately predicting END in this population may be helpful to select candidates for immediate transfer for additional thrombectomy. No clinical or radiological predictors of acute neurological deterioration ≥4 NIHSS points were observed on multivariable analysis, which is consistent with previous studies (16). Previous studies have indicated that admission glucose level (19), D-dimer level (20), and imaging variables (Volumes of Tmax delay) could identify patients at high risk of END following a minor stroke due to LVO (21, 22). However, these markers cannot reflect the mechanism of END. The direct pathological cause of END is neuronal damage, so looking for markers related to neuronal damage would be useful for predicting and reflecting END. Therefore, we retrospectively analyzed the stroke database of our center (13) and screened the pNFL expression of patients with mild stroke and LVO.

NFL releasing in ischemic brain injury may have already started before symptoms became clinically apparent. Previous studies have shown that pNFL expression was associated with clinical characteristics, stroke severity, and clinical outcome in stroke (11, 23–25). NFL levels also predict functional improvement in the late phase after stroke (26). Recent research results show that higher pNFL in AIS patients after endovascular therapy indicates poor outcome (27), especially the combination of pNFL and NIHSS has higher predictive value (28). These results indicate that pNFL is a specific marker of nerve injury, which can be highly expressed in various nervous system diseases (29). Interestingly, a higher pNFL level was found in a transient ischemic attack (TIA) patient who developed an ischemic stroke 1 day after blood sampling (12). In this study, there is no significant correlation between pNFL and cerebral infarction volume (assessed by DWI), which further indicates that the expression of NFL may be independent of imaging findings (the results are not shown). In addition, NFL is related to the clinical severity (30) and can distinguish different nervous system diseases (31), which indicates that the degree of neuronal damage is related to the expression of NFL and further indicates the feasibility of NFL in differentiating END. It cannot be denied that the expression of NFL also has some influencing factors. First, as mentioned above, NFL may increase based on other nervous system diseases. Therefore, the impact of other nervous system diseases must be excluded in the diagnostic process of using NFL. This study excluded patients with other possible neurological diseases and previous cerebral infarction. Second, the expression of NFL changed dynamically with time after stroke (32), so the effect of blood collection time on NFL cannot be ignored. This study showed that there was no significant difference in blood collection time between the two groups.

Given the association between END and pNFL in patients with minor stroke and acute LVO, restoration of perfusion deficits might be considered a potential treatment strategy for

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TABLE 1 Demographic and clinical characteristics of the patients.

Factors	Total (%)	END (%)	Non-END (%)	*P*
Overall rate, *n* (%)	39 (100)	19 (48.72)	20 (51.28)	
Sex (male), *n* (%)	19 (48.72)	9 (47.37)	10 (50.00)	0.869
Age (y), mean (±SD)	64.23 (±10.73)	66.74 (±7.86)	61.85 (±12.65)	0.158
Vascular risk factors, *n* (%)				
Hypertension	23 (58.97)	14 (73.68)	9 (45.00)	0.069
Diabetes mellitus	12 (30.80)	5 (26.32)	7 (35.00)	0.557
Hyperlipidemia	8 (20.51)	2 (10.53)	6 (30.00)	0.132
Atrial fibrillation	8 (20.51)	2 (10.53)	6 (30.00)	0.132
Smoking	14 (35.90)	7 (36.84)	7 (35.00)	0.905
Drinking	11 (28.21)	6 (31.59)	5 (25.00)	0.648
NIHSS, median (IQR)	3 (2–4)	3 (3–4)	3 (2–4)	0.117
Infarct volume (ml), mean (±SD)	16.64 (±8.76)	15.46 (10.43–23.32)	9.56 (2.29–18.93)	**<0.001**
TOAST classification, *n* (%)				0.208
Large-artery atherosclerosis	18 (46.15)	11 (57.89)	7 (35.00)	
Cardioembolism	10 (25.64)	5 (26.32)	5 (25.00)	
Others	11 (28.21)	3 (15.79)	8 (40.00)	
Event to blood sampling (h), median (IQR)	13.24 (±7.25)	14.37 (±7.59)	12.18 (±6.95)	0.942
Ln HbA1c (%), mean (±SD)	1.84 (±0.23)	1.83 (±0.23)	1.86 (±0.23)	0.484
Ln HsCRP (mg/L), mean (±SD)	1.25 (±0.71)	1.41 (±0.84)	1.10 (±0.54)	1.375
Plasma NfL (pg/mL), mean (±SD)	56.53 (±14.22)	65.20 (±14.29)	48.28 (±8.00)	**<0.001**
90-day mRS (IQR)	2 (1-4)	3 (1-4)	1 (1-2)	**<0.001**

IQR, interquartile range; NIHSS, NIH stroke scale; mRS modified Rankin Scale; pNFL, plasma neurofilament light chain concentration; TOAST, Trial of ORG 10172 in Acute Stroke Treatment.
Bold text indicates a statistically significant difference with a p-value <0.05.

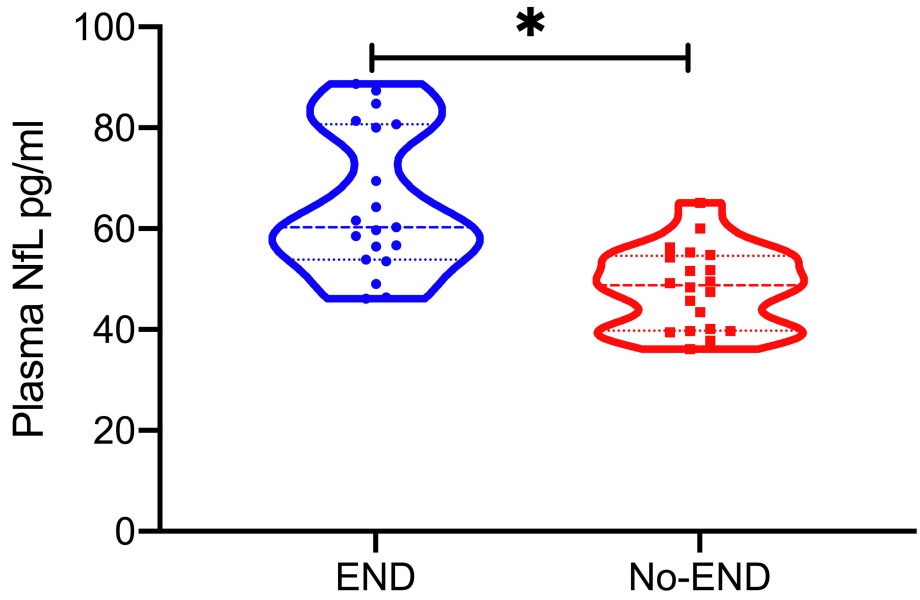

FIGURE 1
Plasma neurofilament light chain (pNFL) concentration in the diagnostic groups is shown as scatterplots. The pNFL concentration was higher in the END group compared with the Non-END group ($^{*}P < 0.001$).

TABLE 2 Odds ratios for pNfL by END compared to Non-END.

Variables	OR	95%CI	*P*
Unadjust pNfL	1.17	1.049–1.306	**0.005**
Model 1 pNfL	1.178	1.038–1.323	**0.006**
Model 2 pNfL	1.168	1.034–1.320	**0.012**

pNFL, plasma neurofilament light chain. Model 1 adjusted for age and sex. Model 2 adjusted for Model 1 and NIHSS, TOAST classification, event to blood sampling (h), and infarct volume. Bold text indicates a statistically <0.05.

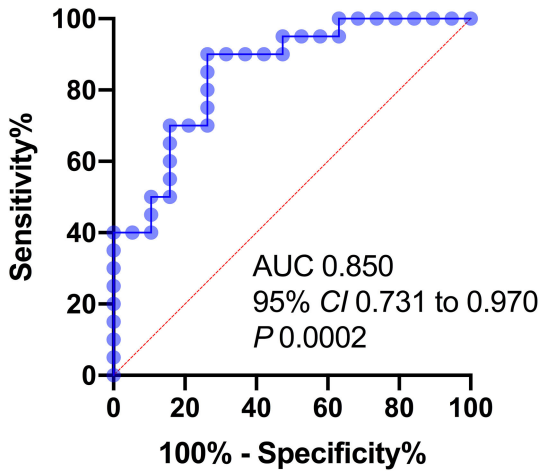

FIGURE 2
Receiver operator characteristic (ROC) curves of variables predicting END 24 h after stroke onset. AUC, area under the curve; NIHSS, NIH stroke scale; pNFL, plasma neurofilament light chain concentration.

patients at high END risk. Since delaying endovascular therapy until neurological worsening appears to reduce its beneficial effect, immediate endovascular therapy might be considered in cases of minor stroke and LVO with a high pNfl. Further well-designed clinical trials should be conducted to prove the benefit of immediate endovascular therapy in minor stroke patients with a high risk of END.

Several limitations to this study should be noted. First, retrospective studies are prone to selection biases. In any case, prospective studies are needed to determine the value of pNFL in making triage decisions to select candidates for primary endovascular therapy. Second, the small sample size due to strict inclusion criteria raises the risk of chance findings. Third, we were limited to a cross-sectional analysis as longitudinal pNFL measurements were not available.

Conclusion

The high expression of NFL in patients with minor stroke and proximal anterior LVO means that they are more prone to END, and these patients may benefit more from early MT treatment. This study provides objective indicators for the formulation of treatment plans for patients with minor stroke due to large vessel occlusion. As a result, further randomized controlled trials are needed to verify this association.

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 General Hospital of Western Theater Command. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JW: conceptualization, methodology, supervision, and software. ZW and SW: data curation, writing the original draft, reviewing, and editing. YL, RW, and LJ: data curation and investigation. BZ and YZ: data curation. QW: conceptualization and investigation. All authors 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

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Diffusion-weighted imaging-based radiomics for predicting 1-year ischemic stroke recurrence

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Purpose: To investigate radiomics based on DWI (diffusion-weighted imaging) for predicting 1-year ischemic stroke recurrence.

Methods: A total of 1,580 ischemic stroke patients were enrolled in this retrospective study conducted from January 2018 to April 2021. Demographic and clinical characteristics were compared between recurrence and non-recurrence groups. On DWI, lesions were segmented using a 2D U-Net automatic segmentation network. Further, radiomics feature extraction was done using the segmented mask matrix on DWI and the corresponding ADC map. Additionally, radiomics features were extracted. The study participants were divided into a training cohort ($n = 157$, 57 recurrence patients, and 100 non-recurrence patients) and a test cohort ($n = 846$, 28 recurrence patients, 818 non-recurrence patients). A sparse representation feature selection model was performed to select features. Further classification was accomplished using a recurrent neural network (RNN). The area under the receiver operating characteristic curve values was obtained for model performance.

Results: A total of 1,003 ischemic stroke patients (682 men and 321 women; mean age: 65.90 ± 12.44 years) were included in the final analysis. About 85 patients (8.5%) recurred in 1 year, and patients in the recurrence group were older than the non-recurrence group ($P = 0.003$). The stroke subtype was significantly different between recurrence and non-recurrence groups, and cardioembolic stroke (11.3%) and large artery atherosclerosis patients (10.3%) showed a higher recurrence percentage ($P = 0.005$). Secondary prevention after discharge (statins, antiplatelets, and anticoagulants) was found significantly different between the two groups ($P = 0.004$). The area under the curve (AUC) of clinical-based model and radiomics-based model were 0.675 (95% CI: 0.643–0.707) and 0.779 (95% CI: 0.750–0.807), respectively. With an AUC of 0.847 (95% CI: 0.821–0.870), the model that combined clinical and radiomic characteristics performed better.

Conclusion: DWI-based radiomics could help to predict 1-year ischemic stroke recurrence.

KEYWORDS

stroke, recurrence, radiology, magnetic resonance imaging, diffusion

Introduction

Stroke remains a leading cause of high disability rate and mortality in the world, accounting for 9–20% of around 10 million strokes that occur each year (1, 2). About 80% of all stroke cases are caused by ischemic stroke (3). According to previous studies, recurrent stroke rates in the western country ranged from 10 to 17% (4). Higher stroke recurrence was reported in Asian populations, ranging from 11.2 to 18.9% in China and Japan (4, 5). One-year recurrence rate was reported in a systematic review and meta-analysis ranged from 5.7 to 17.7% (6).

Studies suggested that a higher risk of disability, dementia, and mortality are associated with recurrent stroke comparing first-time stroke, which might indicate the failure of medical therapy (7–9). Trends and risks of stroke recurrence (10), secondary stroke prevention strategies (4, 11), and demographic characteristics (2) that were evaluated in stroke patients have all been examined in previous studies. Some studies reported laboratory tests, such as peripheral immune cells including neutrophil and lymphocyte counts, infection (12), genetic variants (13), and low-density lipoprotein cholesterol levels (14), were associated with stroke recurrence. In addition, to predict recurrence, artery stenosis, atherosclerotic plaque features, and artery wall change were studied (5, 15–18). Magnetic resonance imaging (MRI) plays an important role in the diagnosis and prognosis of ischemic stroke. However, few studies focused on ischemic stroke lesions based on imaging in predicting stroke recurrence (19, 20). Only a few low-order features of diffusion-weighted imaging (DWI), such as lesion number and presence of lesions, were assessed.

Radiomics is an emerging non-invasive method of extracting high-throughput quantitative features for predicting important clinical outcomes. A previous study demonstrated that radiomics features based on DWI showed good performance in predicting clinical functional outcomes in ischemic stroke patients (3). A recent study that enrolled 522 acute ischemic stroke (AIS) patients showed that the clinical-radiomics model outperformed individual clinical or radiomics models in predicting AIS outcomes (21). To the best of our knowledge, no studies explored the stroke recurrence risk using radiomics features based on DWI. Therefore, we sought to explore DWI-based radiomics for predicting stroke recurrence in 1 year.

Abbreviations: ADC, apparent diffusion coefficient; AIS, acute ischemic stroke; AUC, area under the curve; DWI, diffusion-weighted imaging; LAA, large artery atherosclerosis patients; MRI, magnetic resonance imaging; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; RNN, recurrent neural network; SAO, small artery occlusion; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

Methods

Study population

From January 2018 to April 2021, patients with first-ever ischemic stroke were enrolled in this retrospective study. This study was conducted at our stroke center. The retrospective study had approval from the Institutional Ethics Committee of our hospital (approval number: 2022-013-01 K). Moreover, patients provided written informed consent before MRI examinations. The study was performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

The exclusion criteria were as follows:

Patients with cerebral hemorrhage, traumatic brain injury, previous neurological or psychiatric disorder, cerebral tumor, a history of substance abuse, severe MRI artifacts, a contradiction to MR examination, inability to extract effective features due to the too mall lesions size (<50 voxels), or lost to follow-up were excluded from the study.

The demographic and clinical characteristics were recorded, including age, sex, smoking, drinking, hypertension, hyperlipidemia, diabetes mellitus, atrial fibrillation, the National Institutes of Health Stroke Scale (NIHSS) score on admission, Trial of Org 10172 in Acute Stroke Treatment (TOAST) stroke subtype classification (22), secondary prevention after discharge (antiplatelets, anticoagulants, or statins), a territory of circulation (anterior, posterior, both anterior and posterior), and modified Rankin Scale (mRS) at 90 days. The study flow chart is shown in Figure 1.

Outcome assessments

A recurrent ischemic stroke was defined as a new sudden focal neurological deficit occurring at any time after discharge and confirmed on DWI. In all cases with event recurrence, the date of ischemic event recurrence was ascertained by face-to-face interviews or telephone interviews and confirmed by a review of hospital records. A consensus choice regarding stroke progression or recurrence was made after a thorough examination of these outcome events by one stroke neurologist and one neuroradiologist.

MR acquisition

On scanner 1, an MRI was obtained (EXCITE HD 1.5 T MRI; GE Healthcare, Milwaukee, WI, USA) comprising a 16-channel head/neck coil; and scanner 2 (uMR780 3.0 T MRI; United Imaging Healthcare, Shanghai, China) was equipped with a 24-channel head/neck coil. The detailed scan parameter includes axial fluid-attenuated inversion

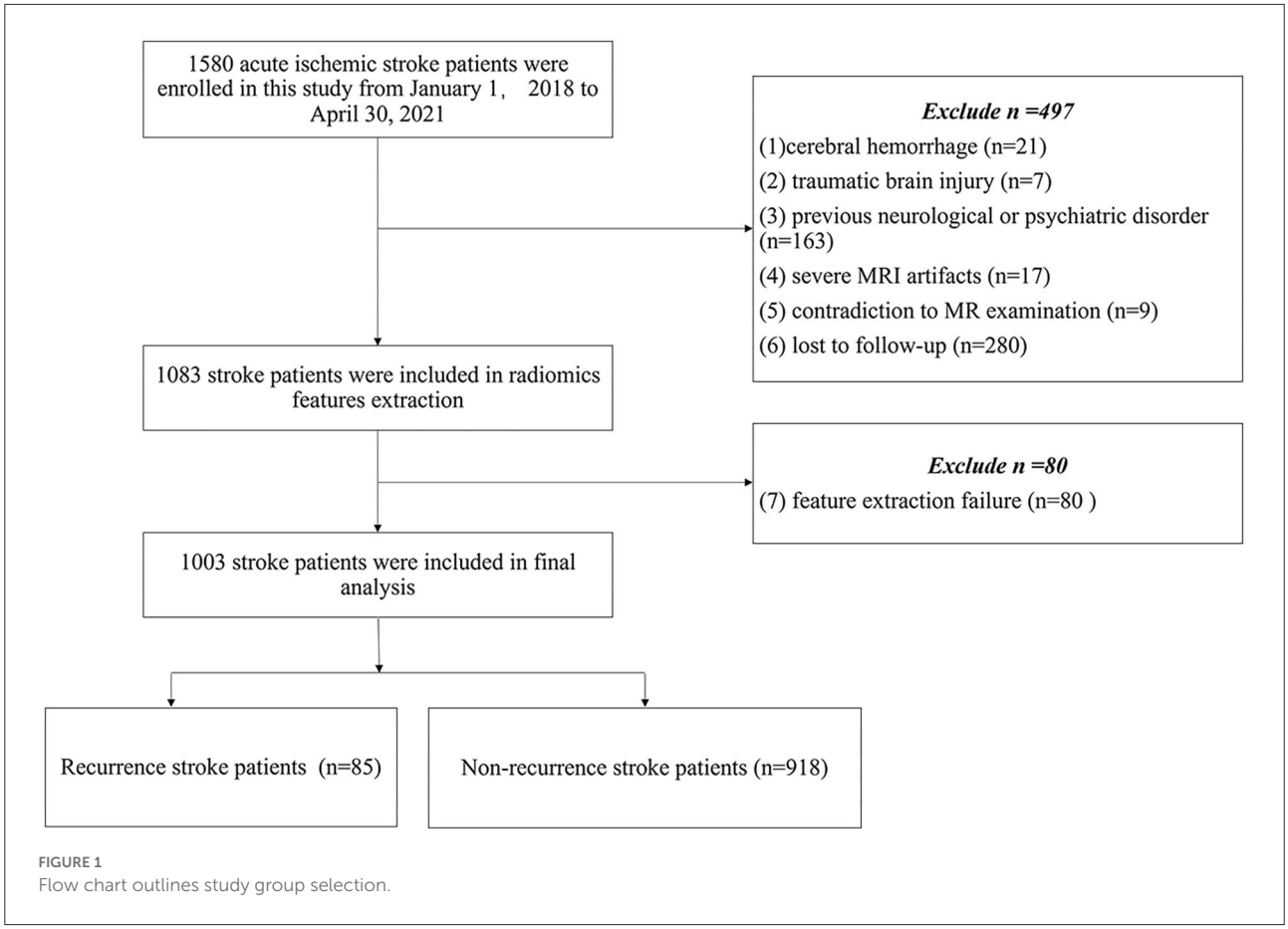

FIGURE 1
Flow chart outlines study group selection.

recovery, DWI including apparent diffusion coefficient (ADC) maps, and T2-weighted and T1-weighted sequences (Supplementary Table e-1). All patients underwent MRI scans within 72 h of symptom onset.

Segmentation of infarction lesions and image preprocessing

First, we randomly selected 100 patients. With ITK-SNAP (http://www.itk-snap.org), infarction lesions were manually segmented by two senior radiologists. Slice-by-slice stacking of DWI images by one neuroradiologist defined the 3D volume of interest of each infarct lesion. Then, we trained 2D U-Net automatic segmentation network on the manually segmented 100 volumes (23, 24). We further segmented all cases based on the trained segmentation network model. Radiomics features were extracted from the ADC map using the segmented mask matrix on corresponding DWI (Figure 2). GitHub (https://github.com/wuguoqingfudan/stroke-segmentation.git) received the automatic segmentation code. In addition, we randomly selected 100 participants segmented by an experienced neuroradiologist and tested the performance of automatic segmentation using the DICE coefficient.

We randomly selected 100 cases from the testing set and calculated the DICE coefficient between the radiologist's annotation results and the network's automatic segmentation results. Before the process of training and testing the network-based image segmentation, image intensity parameters were normalized to 0–1 using window width and window level. Before radiomics feature extraction, we deleted the partial segmentation area (pixel < 20), which is hard to reflect texture features of infarct lesions.

Radiomics feature extraction and selection

First, intensity- and texture-related features were extracted from the ADC map in the image domain. Second, these features were extracted using wavelet domain images generated by performing an 8-wavelet transform on the origin images. Feature extraction was performed on the MATLAB software (MathWorks) (25) and referenced literature (26). The

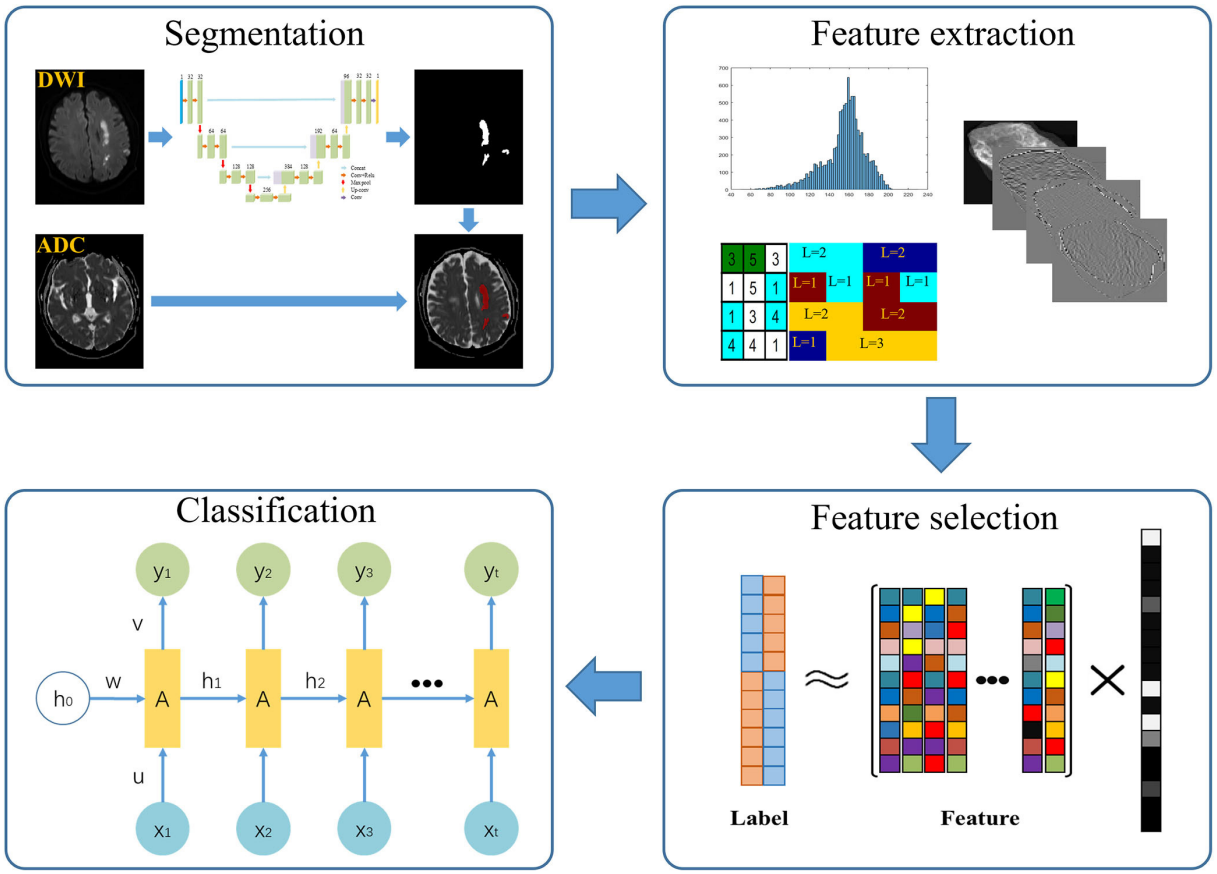

FIGURE 2
Pipeline of radiomics analysis of ischemic stroke on diffusion-weighted imaging.

Supplementary Table e-2 provided more information about the extracted features.

A sparse representation feature selection model was used to select a small number of high discriminative features to reduce the redundancy of extracted features.

The equation feature selection model was as follows:

$$\hat{w} - \arg_w \min \left\| l - Fw \right\|_2^2 + \eta \|w\|_0 (27).$$

Where, $l \in R^m$ represents the label of the training sample,m represents the number of the training sample, $F = [f_1, f_2, ..f_m]^T \in R^{m \times 2K}$ represents the set of features of the training sample, η represents the control parameter of sparse representation, and the absolute values of the coefficient w corresponding to the importance of features. When w is obtained, the key features can be selected by simple threshold comparison.

Data distribution and classification

The study participants were divided into two cohorts, that is, the training cohort and the testing cohort. Further classification was accomplished using a recurrent neural network (RNN) (28). Due to the significant difference in the proportion of positive and negative samples in our study [85 recurrences vs 918 non-recurrences, the proportion was consistent with some previous studies (10, 20)], we used a weighted cross-entropy loss function to optimize the network. Furthermore, we constructed the training cohort with an under-sampling approach in terms of model training data. The training cohort included 57 recurrence patients and 100 non-recurrence patients. The remaining 846 patients were included in the test cohort to confirm the classification's accuracy. The Adam optimizer was used for in-network training, with the learning rate set to 0.0001 and the batch size set to 10. Figure 3 depicts the data distribution. PyTorch was used to create the suggested model. The entire training was carried out on a computer with an Intel Xeon Gold 6128 CPU with 64 GB RAM and an Nvidia TITAN Xp (12 GB).

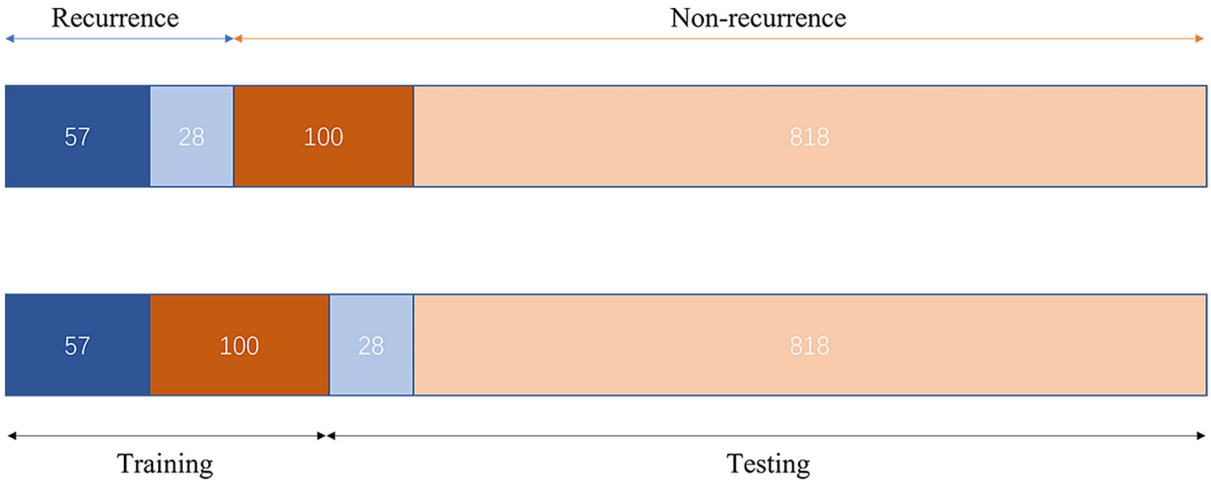

FIGURE 3
Training and testing cohort data distribution.

Statistical analysis

All the statistical analyses were performed with SPSS (version 23.0, SPSS Inc., Chicago, IL, USA). Continuous variables with normal distribution were represented by mean ± standard deviation, non-normally distributed variables by median (interquartile range), and classification variables by frequency (%). We used the Student *t*-test or the Mann–Whitney *U* test for continuous and the χ^2 test for categorical dependent variables (or Fisher's exact test where appropriate) between recurrence and non-recurrence groups.

The receiver-operative curve (ROC) was performed to compare the performance of clinical characteristics and the selected radiomics features in the evaluation of stroke recurrence. The area under the ROC curve (AUC) was constructed by plotting the true-positive rate against the false-positive rate for different binary classification thresholds of the predictors. All P-values were calculated using two-tailed tests, and $P < 0.05$ was considered statistically significant. In addition, we compared the performance of each model using the Delong test. The decision curve analysis (DCV) was implemented to assess the clinical value of the predictive models.

Results

Demographic and clinical characteristics

The final study enrolled 1,003 patients (682 men and 321 women; mean age: 65.90 ± 12.44 years) with acute ischemic stroke. The following factors led to the exclusion of 577 patients: cerebral hemorrhage ($n = 21$); (2) traumatic brain injury ($n = 7$); (3) previous neurological or psychiatric disorder ($n = 163$); (4) severe MRI artifacts ($n = 17$); (5) contradiction to MR examination ($n = 9$); (6) lost to follow-up ($n = 280$); and (7) inability to extract effective features ($n = 80$). The final analysis included 1003 patients (mean age: 65.90 ± 12.44). The clinical and demographic characteristics are summarized in Table 1. Of the 1,003 patients, 85 patients (8.5%) recurred in 1 year, and recurrence stroke patients were older than non-recurrence stroke patients ($P = 0.003$). The stroke subtype was a significant difference between recurrence and non-recurrence groups, and cardioembolic stroke showed a higher recurrence percentage in our study ($P = 0.005$). Secondary prevention after discharge was found significantly different between the two groups. Statins and anticoagulants showed higher recurrence in secondary prevention after discharge in our study ($P = 0.004$ and $P = 0.02$, respectively). There were no significant differences in gender, smoking, drinking, hypertension, hyperlipidemia, diabetes mellitus, atrial fibrillation, circulation territory, and NIHSS at admission and mRS at 90 days between non-recurrence and recurrence stroke patients (all $P > 0.05$).

Difference of stroke subtype in stroke recurrence

Table 2 provides a summary of the demographic and clinical traits of the three primary non-recurrence and recurrence stroke subtypes. The mean interval time between the first stroke and stroke recurrence was 167.11 ± 100.08 days.

TABLE 1 Demographic and clinical characteristics in acute ischemic stroke patients.

Variables		Non-recurrence ($n = 918$, 91.5%)	Recurrence ($n = 85$, 8.5%)	*P*
Age	/	65.55 ± 12.35	69.74 ± 12.79	0.003
Gender	Male	626 (91.8%)	56 (8.2%)	0.662
	Female	292 (91.0%)	29 (9.0%)	
Smoking		578 (92.5%)	47 (7.5%)	0.163
	Yes	340 (89.9%)	38 (10.1%)	
Drinking		791 (91.0%)	78 9.0%)	0.147
	Yes	127 (94.8%)	7 (5.2%)	
Hypertension		296 (90.5%)	31 (9.5%)	0.426
	Yes	622 (92.0%)	54 (8.0%)	
Hyperlipidemia		662 (91.2%)	64 (8.8%)	0.530
	Yes	256 (92.4%)	21 (7.6%)	
Diabetes mellitus		596 (91.3%)	57 (8.7%)	0.693
	Yes	322 (92.0%)	28 (8.0%)	
Atrial fibrillation		817 (91.8%)	73 (8.2%)	0.385
	Yes	101 (89.4%)	12 (10.6%)	
Stroke subtype (TOAST)	Large artery atherosclerosis	488 (89.7%)	56 (10.3%)	0.024
	Cardioembolic stroke	71 (88.8%)	9 (11.3%)	
	Small artery occlusion	280 (94.0%)	18 (6.0%)	
	Other cause	13 (100.0%)	0 (0.0%)	
	Undetermined cause	66 (97.1%)	2 (2.9%)	
Circulation territory	Anterior	629 (91.4%)	59 (8.6%)	0.602
	Posterior	263 (92.3%)	22 (7.7%)	
	Both	26 (86.7%)	4 (13.3%)	
Statins after discharge		350 (94.9%)	19 (5.1%)	0.004
	Yes	568 (89.6%)	66 (10.4%)	
Antiplatelets after discharge		92 (86.8%)	14 (13.2%)	0.064
	Yes	826 (92.1%)	71 (7.9%)	
Anticoagulants after discharge		881 (92.0%)	77 (8.0%)	0.047
	Yes	37 (82.2%)	8 (17.8%)	
NIHSS at admission	/	3 (1,4)	3 (1,4)	0.792
mRS at 90 days	/	1 (0,2)	1 (0,1)	0.908
Symptom onset to DWI scan time (hours)	/	33.56 ± 65.09	39.89 ± 60.25	0.721

The bold values indicate the value of *p* less than 0.05.

Large artery atherosclerosis

Of the 544 large artery atherosclerosis (LAA) patients (362 men and 181 women; mean age: 65.99 ± 12.26 years), 56 (10.3%) patients were found to have a recurrence in 1 year, and recurrence stroke patients were also older than non-recurrence stroke patients ($P = 0.016$). After discharge, stroke patients who did not use antiplatelets had a higher rate of recurrence than those who did ($P = 0.045$). On the contrary, stroke recurrence was found more in stroke patients using statins and anticoagulants after discharge than those without using statins ($P = 0.007$) and anticoagulants ($P < 0.001$). Atrial fibrillation showed more recurrence of stroke in LAA stroke patients ($P = 0.001$). Gender, smoking, drinking, hypertension, hyperlipidemia, diabetes mellitus, NIHSS at admission, and mRS at 90 days were not found significant differences between the non-recurrence and recurrence stroke patients (all $P > 0.05$).

TABLE 2 Demographic and clinical characteristics of different ischemic stroke subtypes (TOAST).

Variables		Large artery atherosclerosis			Small artery occlusion			Cardioembolic stroke		
		Non-recurrence ($n = 488$, 89.7%)	**Recurrence ($n = 56$, 10.3%)**	***P***	**Non-recurrence ($n = 280$, 94.0%)**	**Recurrence ($n = 18$, 6.0%)**	***P***	**Non-recurrence ($n = 71$, 88.7%)**	**Recurrence ($n = 9$, 11.3%)**	***P***
Age	/	65.57 ± 12.09	69.73 ± 13.16	0.016	63.80 ± 12.25	73.39 ± 8.60	< 0.001	73.21 ± 11.048	64.44 ± 15.88	0.036
Gender	Male	330 (90.9%)	33 (9.1%)	0.191	202 (93.5%)	14 (6.5%)	0.787	42 (85.7%)	7 (14.3%)	0.470
	Female	158 (87.3%)	23 (12.7%)		78 (95.1)	4 (4.9%)		29 (93.5)	2 (6.5%)	
Smoking	No	319 (90.9%)	32 (9.1%)	0.223	234 (93.6%)	16 (95.8%)	0.747	51 (94.4%)	3 (5.6%)	0.052
	Yes	169 (87.6%)	24 (12.4%)		46 (6.4%)	2 (4.2%)		20 (76.9%)	6 (23.1)	
Drinking	No	419 (89.1%)	51 (10.9%)	0.281	162 (93.1%)	12 (95.2%)	0.462	66 (88.0%)	9 (12.0)	>0.999
	Yes	69 (93.2%)	5 (6.8%)		118 (6.9%)	6 (4.8%)		5 (100.0%)	0 (0.0%)	
Hypertension	No	158 (88.8%)	20 (11.2%)	0.614	92 (93.9%)	6 (6.1%)	0.967	18 (78.3%)	5 (23.7%)	0.111
	Yes	330 (90.2%)	36 (9.8%)		188 (94.0%)	12 (6.0%)		53 (93.0%)	4 (7.0%)	
Hyperlipidemia	No	342 (88.8%)	43 (11.2%)	0.296	212 94.2%)	13 (5.8%)	0.778	56 (88.9%)	7 (11.1%)	>0.999
	Yes	146 (91.8%)	13 (8.2%)		68 (93.2%)	5 (6.8%)		15 (88.2%)	2 (11.8%)	
Diabetes mellitus	No	328 (88.9%)	41 (11.1%)	0.363	174 (94.1%)	11 (5.9%)	0.930	54 (93.1%)	4 (6.9%)	0.105
	Yes	160 (91.4%)	15 (8.6%)		106 (93.8%)	7 (6.2%)		17 (77.3%)	5 (22.7%)	
Atrial fibrillation	No	453 (91.1%)	44 (8.9%)	0.001	274 (93.8%)	18 (6.2%)	>0.999	21 (70.0%)	9 (30.0%)	< 0.001
	Yes	35 (74.5%)	12 (25.5%)		6 (100.0%)	0 (0.0%)		50 (100.0%)	0 (0.0%)	
Statins after discharge	No	185 (94.4%)	11 (5.6%)	0.007	109 (96.5%)	4 (92.4%)	0.157	22 (84.6%)	4 (15.4%)	0.462
	Yes	303 (87.1%)	45 (12.9%)		171 (3.5%)	14 (7.6%)		49 (90.7%)	5 (9.3%)	
Antiplatelets after discharge	No	38 (80.9%)	9 (19.1%)	0.045	14 (82.4%)	3 (17.6%)	0.074	24 (96.0%)	1 (4.0%)	0.260
	Yes	450 (90.5%)	47 (9.5%)		266 (94.7%)	15 (5.3%)		47 (85.5%)	8 (14.5%)	
Anticoagulants after discharge	No	476 (90.8%)	48 (9.2%)	<0.001	278 (93.9%)	18 (6.1%)	>0.999	52 (85.2%)	9 (14.8%)	0.160
	Yes	12 (60.0%)	8 (40.0%)		2 (100.0%)	0 (0.0%)		19 (100.0%)	0 (0.0%)	
NIHSS	/	3 (2, 5)	3 (1, 4)	0.298	2 (1, 3)	2 (1, 4)	0.192	4 (2, 7)	2 (2, 5)	0.433
mRS at 90 days	/	1 (0, 2)	1 (0, 2)	0.687	0 (0, 1)	0 (0, 1)	0.921	0 (0, 2)	0 (0, 0)	0.252

TOAST, Trial of Org 10172 in Acute Stroke Treatment; NIHSS, National Institutes of Health Stroke Scale. The bold values indicate the value of *p* less than 0.05.

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Small artery occlusion

Our research found 298 patients (216 men and 82 women; mean age: 64.38 ± 12.27 years) with small artery occlusion (SAO). Of the 298 SAO patients, 18 (6.0%) patients were found to have a recurrence in 1 year, recurrence stroke patients were also older than non-recurrence stroke patients ($P < 0.001$). Gender, smoking, drinking, hypertension, hyperlipidemia, diabetes mellitus, atrial fibrillation, NIHSS at admission, and mRS at 90 days were not found significant differences between the non-recurrence and recurrence stroke patients (all $P > 0.05$).

Cardioembolic stroke

Our study found a total of 80 cardioembolic stroke patients (49 men and 31 women; mean age: 72.22 ± 11.89 years). Nine patients (11.3%) out of the 80 patients had a recurrence within a year. Out of these, recurrence stroke patients were younger than non-recurrence stroke patients ($P = 0.036$). Atrial fibrillation showed more non-recurrence stroke in cardioembolic stroke patients ($P < 0.001$). Gender, smoking, drinking, hypertension, hyperlipidemia, diabetes mellitus, NIHSS at admission, and mRS at 90 days were not found significant differences between the non-recurrence and recurrence stroke patients (all $P > 0.05$).

The performance of segmentation of infarction

The DICE coefficient between the radiologist's annotation results and the network's automatic segmentation results is 0.886.

Radiomics feature extraction and selection

A total of 513 radiomics features were extracted, including intensity-related features (18), texture features (39), and wavelet features [8× (18 + 39)]. Finally, a total of 100 features were selected using a sparse representation feature selection model, that is, clinical characteristics ($n = 6$), image domain features ($n = 8$), and wavelet domain features ($n = 86$) (more feature selection was shown in the Supplementary Table e-3).

Performance of prediction model

Figure 4 and Table 3 show the performance of the clinical and radiomics-based prediction model. The area under the curve (AUC) of the clinical-based model and radiomics-based models were 0.675 (0.643–0.707) and 0.779 (0.750–0.807), respectively. With an AUC of 0.847 (0.821–0.870), the model that incorporated both clinical and radiomic characteristics outperformed the model that only used clinical characteristics. The combined model presented showed better than clinical-based model ($P = 0.001$) and radiomics-based model ($P = 0.2$), and radiomics-based model was better than clinics-based model ($P = 0.01$). The combination model had the highest net benefit compared with both the other models (Figure 5).

Discussion

Our study shows that a radiomics prediction model based on stroke infarction lesions derived from the ADC map can accurately predict ischemic stroke recurrence. In this study, we used a 2D U-Net automatic segmentation network based on manually segmented volumes to implement infarction automatic segmentation. In addition, a sparse representation feature selection model was performed to select a small number of high-resolution features to construct the robust prediction model.

A recent study showed that DWI-positive infarction lesion was associated with higher recurrent risk in patients with minor ischemic stroke (MIS) and transient ischemic attack (TIA) (20). Previous studies indicated that large artery atherosclerosis and infarction number were independent predictors of 1-year stroke recurrence with TIA or MIS. Multiple acute infarctions are usually related to embolic pathogenesis (29, 30), which could indicate a relatively unstable cause associated with higher recurrence (31, 32). The majority of patients with a single acute lacunar infarction have pathological changes like fibrinoid necrosis, lipohyalinosis, or other unknown changes. We hypothesized that different stroke subtypes with different pathologic mechanisms could contribute to different recurrent risks. Radiomics can convert medical images into high-throughput quantitative features, mainly comprising histogram and texture features, which have been widely applied in predicting clinical prognosis, pathological grading, and response to treatment (3). The ischemic cascade, which included the production of reactive oxygen species, the release of glutamate, the buildup of intracellular calcium, and the induction of inflammatory processes, is the name given to the pathophysiological mechanisms underlying ischemic stroke. A previous study showed that the texture features based on DWI were closely related to edema after cerebral infarction (33).

In terms of clinical and demographic characteristics, it is not surprising that recurrence stroke patients were older than non-recurrence patients. Different stroke subtypes presented significantly different stroke recurrence incidences. LAA and cardioembolic stroke showed higher recurrence compared to the other subtypes in this study, which was in accordance with the previous study (34). We further analyzed the risk factors of different stroke subtypes in predicting stroke recurrence. After discharge secondary prevention such as statins, anticoagulants, and antiplatelets showed significant

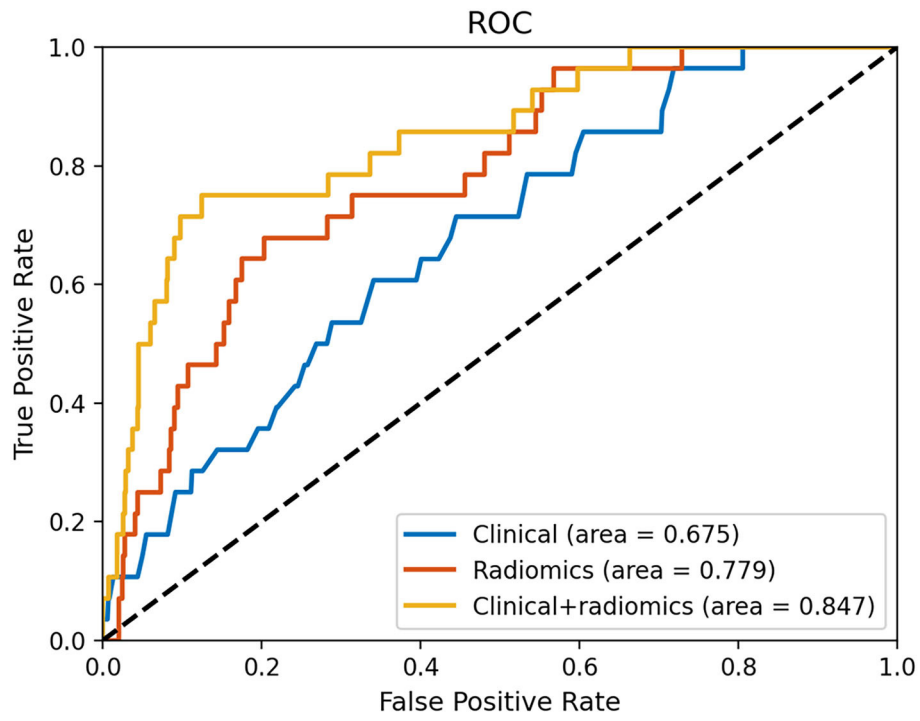

FIGURE 4
Receiver operating characteristic curves based on the clinical characteristics and radiomics.

TABLE 3 Performance of two different prediction models.

	AUC (95% CI)	Accuracy	Sensitivity	Specificity
Clinical	0.675 (0.643-0.707)	0.670	0.536	0.675
Radiomics	0.779 (0.750-0.807)	0.792	0.679	0.796
Clinical+ radiomics	0.847 (0.821-0.870)	0.833	0.750	0.836

differences between recurrent and non-recurrent stroke patients in our study. Antiplatelets could effectively reduce the risk of stroke recurrence in LAA stroke patients. A previous study also demonstrated that less antiplatelet at discharge was associated with a higher recurrence rate (20). Another study showed that there was no evidence of heterogeneity in the effects of antiplatelet therapy by the presence of DWI-positive infarctions vs. absence on the risk of any recurrent stroke (19). Patients with non-recurrence and recurrent strokes did not have significantly different antiplatelet levels, which may be related to the disease's heterogeneity (14). However, the multivariate regression analysis clinical prediction model had a poor performance in our study, with an AUC of 0.675.

In a previous study, imaging parameters (LAA and positive neuroimaging findings) were associated with a higher risk of recurrent stroke rather than clinical characteristics (20). In their study, DWI-positive lesion was associated with higher recurrent risk in patients with transient ischemic attacks. Radiomics based on medical imaging has been extensively used to predict prognosis, pathological type, response to treatment, and so on (35–37). Our previous study investigated the clinical radiomics nomogram for predicting ischemic stroke prognosis with good performance (3). Radiomics extracts high throughput quantitative features by combining radiology and machine learning, which can reflect the heterogeneity of lesions. Few studies explored radiomics based on DWI to predict stroke recurrence. Our study demonstrated that the DWI-based radiomics model could predict stroke recurrence with good performance. Stroke may present with or without underlying arterial pathologies because of the heterogeneity of stroke (14). Recent research demonstrated that lesions on DWI were indicators of microvascular occlusive events that were predisposing to microvessel rupture (19).

This study randomly selected 100 volumes by manual annotation and then used 2D U-net architecture to implement

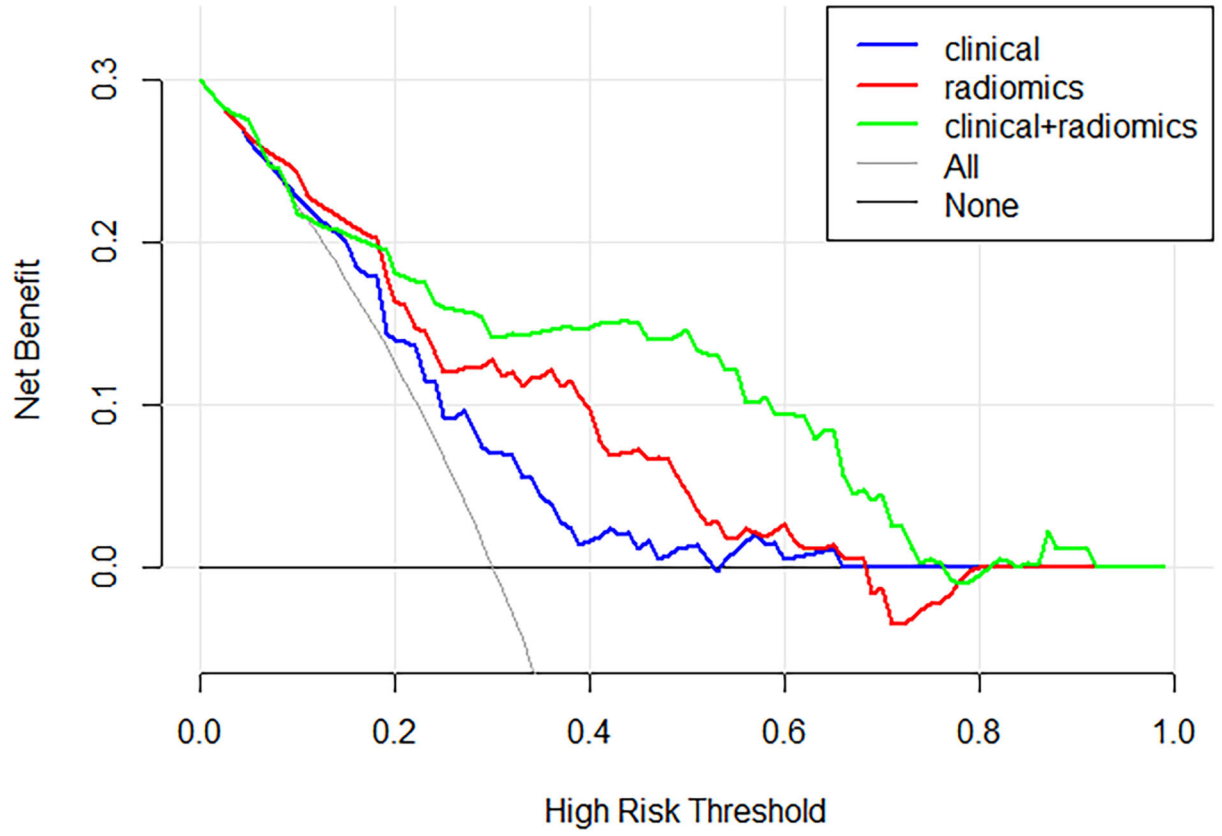

FIGURE 5
Decision curve analysis for each model. The combination model had the highest net benefit compared with both the other models.

infarction lesions automatic segmentation, which was a recommended and efficient segmentation technique (24). 2D U-net convolutional neural networks for automated segmentation, an "end to end" segmentation method with robust accuracy performance, apply what is known as long-skip connections to directly connect opposing layers from contracting to the expanding paths (23, 38). A total of 513 radiomics features were extracted, including texture, intensity-related features, and wavelet features. However, a large amount of redundancy existed in the extracted features. A sparse representation feature selection model was used in our study to reduce redundancy and the risk of overfitting in subsequent classification, which was reported to apply in a previous study (27). In our study, the classification process involved RNN. RNN based on recursive connection is powerful in modeling temporal dynamics and learning appropriate feature representations (28). RNN model has demonstrated application with good performance in various medical tasks including genomic analysis, medical diagnosis, and recognizing patterns in sequential data (39). RNN can further improve the classification performance of the network by making the neurons in the hidden layer communicate with each other by storing the output results (24, 40).

Limitations

There were several limitations in this research. First, due to a loss of follow-up cases in our retrospective study, potential selection bias was unavoidable. Second, we did not keep track of medication details after discharge; instead, we only kept track of whether the patients had taken their medications. Finally, our radiomic-based prediction model performed well in predicting stroke recurrence. However, the corresponding clinical implications of each feature were unclear, and further exploration is needed. Nevertheless, these encouraging results provide a noninvasive method for predicting ischemic stroke recurrence.

Conclusion

In conclusion, we found that the radiomics-based DWI prediction model performed well in terms of predicting stroke recurrence. Our robust prediction performance was aided by machine learning and deep learning algorithms such as U-net architecture automatic segmentation, sparse representation feature selection, and RNN classification.

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 local institutional committee on human research approved the retrospective study and waived the requirement for written informed consent due to its retrospective nature.

Author contributions

YZ and BS designed this study. YS and JZ conducted the study and collected important data. HW drafted 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.

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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.2022.1012896/full#supplementary-material

SUPPLEMENTARY TABLE E-1
Magnetic resonance sequences parameters.

SUPPLEMENTARY TABLE E-2
The category and name of extracted radiomics features.

SUPPLEMENTARY TABLE E-3
The category and name of all selected radiomics features.

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Impact of time between thrombolysis and endovascular thrombectomy on outcomes in patients with acute ischaemic stroke

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Background: Benefits of endovascular thrombectomy (ET) after intravenous thrombolysis (IVT) for patients with acute ischaemic stroke (AIS) have been demonstrated, but analyses of the relationship between IVT-ET time delay and functional outcomes among patients receiving both treatments are lacking.

Methods: We used data from the "Berlin—Specific Acute Treatment in Ischaemic and haemorrhAgic stroke with Long-term outcome" (B–SPATIAL) registry. Between January 1st, 2016 and December 31st, 2019, we included patients who received both IVT and ET. The primary outcome was the 3-month ordinal modified Rankin scale (mRS) score. The IVT-ET time delay was analyzed in categories and continuously. We used adjusted ordinal logistic regression to estimate common odds ratios (cOR) and 95% confidence intervals (CI). Secondary analyses involved flexible modeling of IVT-ET delay and dichotomous outcomes.

Results: Of 11,049 patients, 714 who received IVT followed by ET were included. Compared with having an IVT-ET window >120 min (reference), for an IVT-ET window < 30 min, we obtained adjusted cORs for mRS of 0.41 (95% CI: 0.22 to 0.78); and 0.52 (95% CI: 0.33 to 0.82) for 30 to 120 min. Secondary analyses also found protective effects of shorter time delays against "poor" functional outcomes at 3 months.

Conclusions: In patients with AIS, shorter IVT-ET intervals were associated with better 3-month functional outcomes. While the time-to-IVT and time-to-ET

include the time until medical attention is received, the IVT-ET time delays fall entirely within the domain of medical management and thus might be easier to optimize.

KEYWORDS

ischaemic stroke, time-to-treatment, thrombolysis, thrombectomy, functional outcome, modified Rankin Scale, registry

Introduction

Acute ischaemic stroke (AIS) is one of the most common causes of morbidity and disability worldwide (1). There are two main acute treatment options for AIS, i.e., intravenous thrombolysis (IVT) and endovascular thrombectomy (ET) (2). In 2015, results from five randomized trials provided evidence for the superiority of ET, mostly in combination with IVT ("bridging thrombolysis"), compared to IVT alone (3–7). The benefits of IVT and ET combination therapy may be attributable to the ability of IVT to degrade remaining clot fragments, reduce ET procedure duration, and expedite recanalisation (8). Benefits of both recanalizing treatments, however, are known to diminish with increasing delay from symptom onset (or time last seen well) (9), hence, an earlier start of ET after IVT might result in more favorable outcomes for AIS patients.

Although "time–to–treatment" is a generally well-researched topic in stroke (10, 11), typically measured as the time of symptom onset to treatment initiation, the potential impact of the specific time delay between IVT and ET has not been well studied.

We aimed to estimate the effect of the time delay between IVT and ET on functional outcome as measured by the modified Rankin Scale (mRS) score 90 days after stroke among AIS patients who received both IVT and ET using prospectively-collected data from a large stroke registry in Berlin, Germany.

Materials and methods

Study design and setting

The Berlin—SPecific Acute Treatment in Ischemic or haemorrhAgic stroke with Long term follow–up (B–SPATIAL) registry (Clinicaltrials.gov identifier: NCT03027453) is a prospective, multicentre, observational registry of adult acute stroke and TIA patients presenting at one of 15 hospitals with stroke units in Berlin since January 1st, 2016. Patients aged 18 years or older of any sex with ICD-10 diagnoses of ischaemic stroke (I63), haemorrhagic stroke (I61), or Transient Ischaemic Attack (TIA) (G45.0–G45.3 and G45.5–G45.9) were eligible for inclusion in the registry. The registry includes data from patients transported to hospital by one of three Berlin mobile stroke units (MSUs) (12). Patients or their legal representatives were informed about the purpose and the procedures of the registry and had the opportunity to "opt–out" at multiple time points. Scientific evaluation of the B-SPATIAL registry was approved by the local ethics committee of the Charité–University Medicine Berlin (EA1/208/21).

The present study uses data collected through December 31st, 2019 by dedicated study nurses according to a standardized protocol, including hospital records and data from patient interviews or questionnaires. In cases of no response, information about patients' vital status was obtained via the city registry office 4 months after the index event (12).

In the present study, we restricted our sample to include only ischaemic stroke patients with symptom onset or time last seen well within 6 h of arrival at a participating hospital. We excluded patients with primary haemorrhagic stroke or TIA, as well as those with symptom remission before ambulance or hospital arrival, as they were not considered candidates for acute treatment (12). We included only patients who initiated both IVT and ET treatments in our analyses. Patients who received IVT while simultaneously undergoing ET (intra-arterial thrombolysis) were excluded.

Patient characteristics

We obtained information about age, sex, blood pressure, blood glucose, and comorbidities, including atrial fibrillation, diabetes mellitus, and hypertension. In addition, we extracted clinical information including National Institutes of Health Stroke Scale (NIHSS) scores and vessel occlusion site (internal carotid artery, anterior cerebral artery, middle cerebral artery, and posterior cerebral artery).

Exposure measures

The main exposure variable of interest, the elapsed time between IVT and ET (IVT-ET time delay), was computed as the difference between time of IVT initiation and time of ET initiation. In the analyses, we used both a primary clinical categorization ("short," "medium," "long") of each time-to-treatment (time-to-IVT: <60 min, 60–120 min, >120 min; time to ET: <120 min, 120–280 min, >280 min; IVT-ET time delay: <30 min, 30–120 min, >120 min), as well as a secondary exposure scale, in which we considered the IVT-ET time delay continuously, in 30-min incremental units.

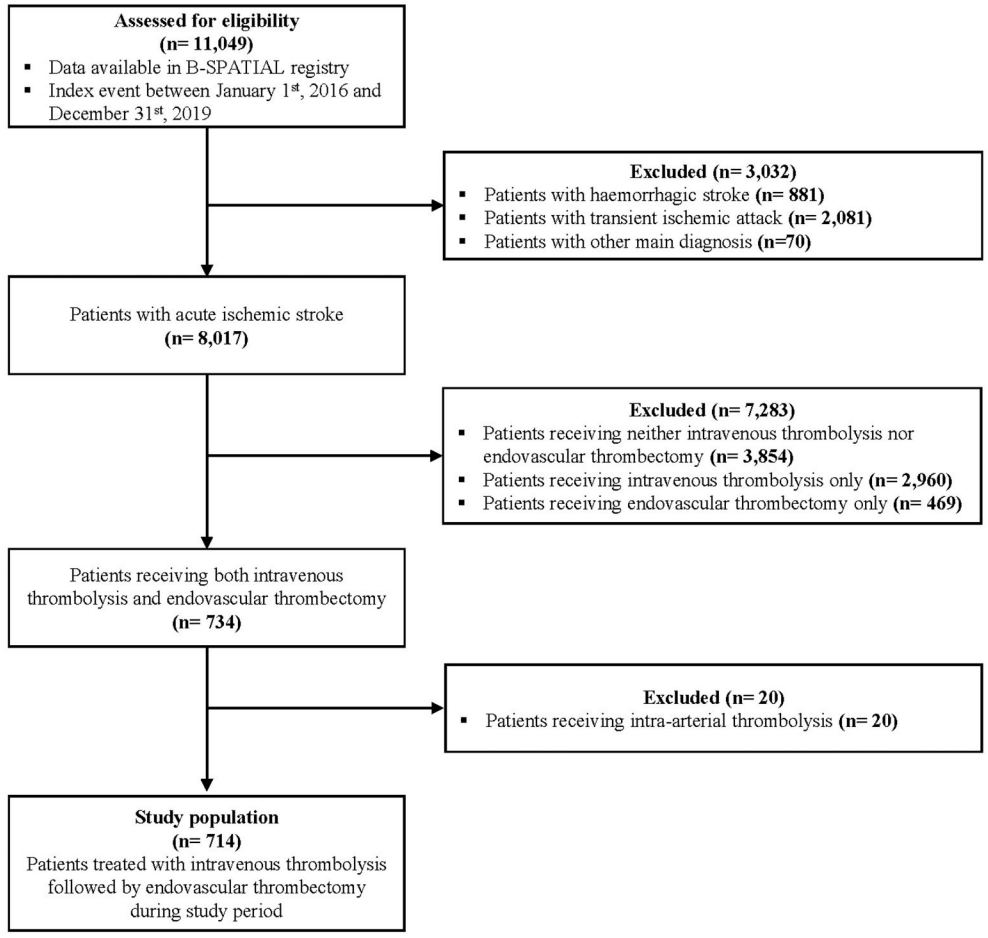

FIGURE 1
Flow diagram of study eligibility. B-SPATIAL, Berlin-Specific Acute Treatment in ischemic and haemorrhagic Stroke with Long-Term outcome.

Outcome measures

Our primary outcome of interest was the functional outcome as defined by the modified Rankin Scale (mRS) score at 90 days after stroke. The mRS is a 7-point ordinal scale ranging from 0 ("no neurological symptoms") to 6 ("death") (13). In line with prior literature (3–7), we also present results using a dichotomous secondary outcome, modeling a "poor" (mRS: 3–6) vs. "favorable" (mRS: 0–2) functional outcome.

Statistical analysis

We present medians and interquartile range limits (IQRL) for all continuous and ordinal variables, means and standard deviations for all normally distributed variables, and frequencies and percentages for categorical variables.

We used ordinal logistic regression (shift analysis) to obtain crude and adjusted common odds ratios (cOR) with corresponding 95% confidence intervals (95% CI) for the primary analysis. We further present results from crude and adjusted ordinal logistic regression models for the exposure variables time-to-IVT and time-to-ET.

The confounding adjustment strategy was determined *a priori* by selecting variables that are thought to be common causes of both the exposure and outcome or risk factors for the outcome. We included the following continuous variables in the adjusted models: age, NIHSS, blood pressure, blood glucose, and time-to-IVT, as well as the following categorical variables: sex, diagnosis of atrial fibrillation, diabetes mellitus, hypertension (or antihypertensive medication use), hospital size, time-to-IVT and vessel occlusion site. We created the variable "hospital size" to capture the relative sizes of the clinics as a proxy for structural factors such as geographic location, experience levels of the hospitals' physicians, treatment processes, and workflow. Of the 15 participating hospitals, five were included in each category: (1) treating <4%, (2) Treating 4–10% or (3) treating >10% of all registry patients.

Missing values were assumed to be missing at random (MAR) and imputed using multiple imputation by chained equations (MICE) with 10 imputed datasets. The primary analyses were performed on the imputed datasets.

In the secondary analysis, we used logistic regression to estimate the effect of the IVT-ET time delay on the dichotomous 3-month functional outcome. To accommodate potential non-linear effects, IVT-ET time delay was modeled using splines (using *mksplines* in Stata) with knots set at every 30 min and using the 60–min knot as a reference. The 60-min reference was chosen based on the so-called "golden hour" of stroke, the time within which the initiation of reperfusion treatments are most effective for eligible acute ischemic stroke patients (14). This secondary analysis was performed in complete cases only (no imputation). We present a graphical representation of the binary odds ratio (OR) estimates for having a "poor" outcome (mRS>2) computed using multivariable logistic regression models adjusted for the aforementioned set of confounders.

Binary OR are commonly misinterpreted as being synonymous with relative risk, which can be especially problematic when the outcome is common (15). Since the prevalence of a "poor" outcome was approximately 50% in our study population, we opted to perform an additional modification to the aforementioned secondary analysis. We again used splines to model the IVT-ET time delay, this time obtaining adjusted relative risk (RR) estimates for the binary outcome using a modified Poisson regression modelling approach with robust standard errors (16). These results for having a "poor" functional outcome were also visualized with the 60-min knot as a reference.

All analyses were performed using STATA/IC 14 software (STATA Corp Ltd.).

Results

Study population

Out of 11,049 patients meeting eligibility criteria for the B-SPATIAL registry between January 1st, 2016 and December 31st, 2019, a total of 714 patients treated with both IVT and ET were ultimately included in this study (Figure 1). Of these, 133 patients were transported by MSUs.

Baseline characteristics

AIS patients receiving IVT and ET consisted primarily of elderly people suffering from moderate to severe strokes (Average age 72 years ±14 and median NIHSS score of 15 (IQRL 10–19). Fifty-one percent of those comprising the study population were female. A full summary of the baseline characteristics including times–to–treatment is displayed in Table 1.

TABLE 1 Baseline clinical and treatment characteristics of patients at hospital admission.

Variable	Acute ischaemic stroke patients receiving intravenous thrombolysis then endovascular thrombectomy ($n = 714$)
Patients transported with MSU, *n* (%)	133 (19)
Age, y, mean (SD)	72 (14)
median (IQRL)	75 (63–81)
Sex†, female, *n (%)*	364 (51)
Hospital size (based on percentage of registry patients treated)	
<4%, *n (%)*	60 (8)
4–10%, *n (%)*	199 (28)
>10%, *n (%)*	455 (64)
Comorbidities	
Atrial fibrillation†, *n (%)*	271 (38)
Diabetes mellitus†, *n (%)*	161 (23)
Hypertension†, *n (%)*	560 (78)
NIHSS†, median (IQRL)	15 (10–19)
Systolic blood pressure ‡, mmHg, mean (SD)	156 (30)
Diastolic blood pressure ‡, mmHg, mean (SD)	85 (17)
Blood glucose ‡, mg/dl, mean (SD)	135 (42)
Vessel occlusion site	
Internal carotid artery, *n (%)*	76 (11)
Anterior cerebral artery, *n (%)*	18 (3)
Middle cerebral artery, *n (%)*	454 (63)
Posterior cerebral artery, *n (%)*	23 (3)
Other or no information available, *n* (%)	143 (20)
Time from symptom onset to IVT, mins, mean (SD),	112 (64)
median (IQRL)	90 (68–135)
Time from symptom onset to ET, mins, mean (SD),	194 (131)
median (IQRL)	169 (130–224)
Time between IVT and ET, mins, mean (SD),	82 (116)
median (IQRL)	66 (44–92)

SD, standard deviation; IQRL, interquartile range limits; NIHSS, National Institutes of Health Stroke Scale; IVT, intravenous tissue–type plasminogen activator; ET, Endovascular Thrombectomy; MSU, Mobile Stroke Unit; †variable had <10% missing values; ‡variable had >10% missing values.

43 (6%) patients had already experienced prior ischemic stroke or TIA according to their medical documentation. 67 (9%) patients developed a symptomatic secondary intracerebral hemorrhage, and 54 (8%) patients died in–hospital. The mRS 90 days after the index event was available for 573 (80%) patients, with a median value of 3 (IQRL 1–5).

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TABLE 2 Ordinal logistic regression results, effect estimates for IVT-ET time delay on mRS score 90 days after index acute ischaemic stroke event.

	mRS at 90 days	
Time delay between intravenous thrombolysis and endovascular thrombectomy	**Unadjusted cOR (95% CI)**	**Adjusted cOR† (95% CI)**
Primary exposure categorization		
<30 mins ($n = 71$)	0.64 (0.35 to 1.17)	0.41 (0.22 to 0.78)
30–120 mins ($n = 551$)	0.71 (0.46 to 1.10)	0.52 (0.33 to 0.82)
>120 mins ($n = 92$)	1 (reference)	1 (reference)
Exposure as a continuous variable		
per 30-min reduction in IVT-ET time delay	0.97 (0.92 to 1.02)	0.94 (0.88 to 1.00)

cOR, common Odds ratio obtained from the ordinal logistic regression models for each exposure category; CI, Confidence interval; IVT, Intravenous Thrombolysis; ET, Endovascular Thrombectomy; mRS, modified Rankin Scale.
†Adjusted for, age, sex, NIHSS, blood pressure, blood glucose, atrial fibrillation, diabetes mellitus, hypertension, hospital size, vessel occlusion site, and time-to-IVT.

We also provide baseline characteristics stratified by IVT-ET time delay groups in Supplementary Table S1.

Impact of IVT-ET time delay on functional outcome

In the primary analysis, after confounding adjustment, we found that having a "short" or "medium" time delay between IVT and ET was associated with a favorable shift in the distribution of mRS scores (shift to lower scores) 90 days after AIS compared with having a "long" time delay (Table 2). Compared to having an IVT-ET time delay of >120 min (reference), for an IVT-ET time delay of fewer than 30 min, we obtained a beneficial adjusted cOR of 0.41 (95% CI 0.22 to 0.78), and for IVT-ET time delays between 30 to 120 min, an adjusted cOR of 0.52 (95% CI 0.33 to 0.82).

Treating the exposure as a continuous variable in the primary analysis, each 30-min reduction in time delay was found to be associated with a favorable shift in the distribution of mRS based on the point estimate, though this result was not statistically significant (adjusted cOR of 0.94, 95% CI 0.88 to 1.00).

The corresponding results for the time-to-IVT and time-to-ET exposures are described and presented in the Supplementary Results and Supplementary Table S2.

Figure 2 shows the unadjusted mRS distribution across the three primary IVT-ET time delay groups. As depicted, the shorter the IVT-ET time delay, the more favorable the shift toward lower 90-day mRS scores. Similarly, Figure 3 shows a higher adjusted binary OR for "poor" 90-day functional outcome with increasing time delay between IVT and ET. Supplementary Figure S1 shows a modified version of Figure 3 using risk ratios instead of ORs.

Discussion

Our findings provide a detailed analysis of the relationship between the elapsed delay in time between IVT and ET and functional outcomes in ischaemic stroke patients who received both treatments. Our results indicate that shorter IVT-ET time delays are associated with better functional outcomes 3 months after ischaemic stroke, corresponding to a favorable shift in the distribution of mRS scores. These effect estimates are similar to the protective effects for reducing time-to-IVT or time-to-ET treatment in both direction and magnitude. Consistent with our findings, prior research has consistently demonstrated detrimental effects of longer time-to-IVT and time-to-ET in different settings (10, 11, 17).

Rapid initiation of ET following IVT appears to have a considerable protective benefit, whereas longer delays between treatments appear to be detrimental in terms of longer-term functional outcomes. Our findings corroborate those of the French Endovascular Treatment in Ischemic Stroke (ETIS) registry (18). Zhu et al. analyzed 1,986 AIS patients in six comprehensive stroke centers, and found that having a longer IVT-ET time delay was associated with a worse functional outcome at 90 days (adjusted OR for the favorable outcome (mRS 0–2) per 30-min increase = 0.91, 95% CI 0.86 to 0.96) (18). Findings from Evans et al. (19) indicated a significant benefit of ET within 90 minutes after IVT (20) in a *post-hoc* subgroup analysis of the IMS III trial.

Recent studies mainly conducted in Europe, North America and Australia suggest that IVT before ET has a beneficial effect, even in patients treated with ET (21). IVT followed by ET was associated with higher reperfusion rates without a significantly higher rate of symptomatic intracranial hemorrhage compared to ET alone. We unfortunately do not have data on the experience level of individual ET operators at the hospitals participating in the B-SPATIAL registry. However, the number of ET treatments performed in the participating hospitals (also counting ETs without prior IVT) ranged from 1 to 238 during the included time period. In two hospitals, the thrombectomy service was first introduced at the end of the inclusion period, explaining the low numbers of ET treatments seen in those two hospitals. Therefore, we assume that most interventionalists were rather experienced.

When interpreting our findings, readers should consider our study's strengths and limitations. At the time of analysis, the B–SPATIAL registry comprised more than 10,000 stroke patients,

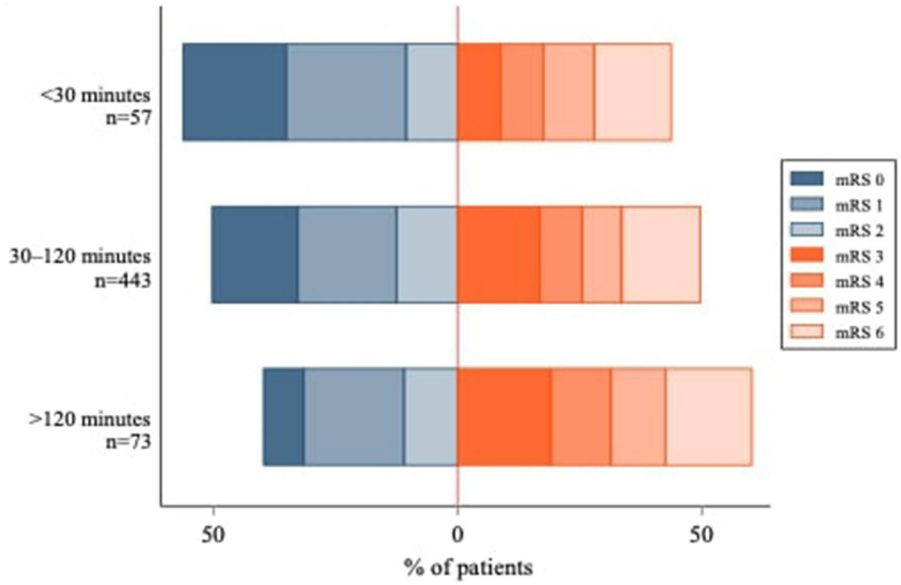

FIGURE 2
Modified Rankin Scale Score Distribution at 90 days by IVT-ET time delay group. The IVT-ET time delay corresponds to the elapsed time between intravenous thrombolysis (IVT) and endovascular thrombectomy (ET). 90-day mRS scores were not available for 141 patients.

yet <10% ultimately received both IVT and ET, meeting our inclusion criteria (714 AIS patients). Despite this being a sufficient sample size for our chosen analyses, we did not have enough power to assess potential effect modification.

International and national efforts such as Safe Implementation of Thrombolysis (SITS), International Stroke Thrombolysis Register, the German Stroke Registry, or The China National Stroke Registry (CNSR) (22–24) may have reached higher numbers than our Berlin-based registry, but differences in data collection between hospitals can affect the quality and quantity of their collected data and follow–up of functional outcome is often incomplete. Our analyses benefitted from the comprehensive and systematic data collection, which was actively promoted in the B-SPATIAL registry through the use of standardized protocols and trained, dedicated study nurses in all hospitals with a stroke unit in Berlin, Germany.

Of note, our study population did not include patients who had recanalisation of their large vessel occlusion through IVT alone. This selection may have led to an overrepresentation of patients with worse outcomes, since rapidly resolving cases did not meet our inclusion criteria.

Finally, we further acknowledge that the routinely collected variables included as covariates may not have fully captured potential causes of IVT-ET time delays that are also causes of functional outcomes. A potential source of residual confounding we considered important is hospital organisation. This includes the hospital's geographic location, angiography facility, diagnostic and therapeutic workflows, and other structural elements. However, since these factors are difficult to quantify, they could not be individually captured in the registry. For this reason, we opted to adjust for hospital size as a proxy variable. Indeed, the exact causes of IVT-ET time delays likely differ per setting. Once identified and implemented, targeted improvement measures are likely to reduce delays and subsequently impact patient outcomes.

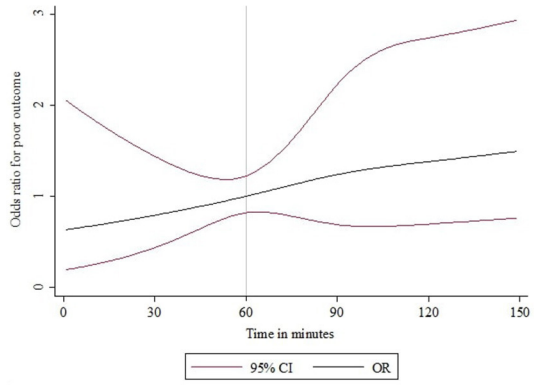

FIGURE 3
IVT-ET time delay and binary OR for "poor" functional outcome (mRS > 2) at 90 days after acute ischaemic stroke. The time delay between IVT and ET was modeled as a continuous exposure variable using splines and the odds ratio estimates are for a "poor" functional outcome (mRS 3–6). An IVT-ET time delay of 60 elapsed minutes was used as the reference.

Our findings indicate a meaningful relationship between the time delay between IVT and ET and the functional outcomes of ischaemic stroke patients three months after stroke. After accounting for confounding including time-to-IVT, our effect

estimates are similar in magnitude to published effect estimates from the literature for time-to-IVT or time-to-ET. While the time-to-IVT and time-to-ET include the time until medical attention is received (e.g., response following emergency call), and are therefore difficult to modify, the elapsed time between IVT and ET falls entirely within the domain of medical management and thus might be easier to optimize.

Data availability statement

The data analyzed in this study was obtained from the Berlin - Specific Acute Treatment in Ischemic or haemorrhAgic stroke with Long term follow-up (B-SPATIAL) registry, the following licenses/restrictions apply: The datasets presented in this article are not readily available because of data protection regulations. Data can be made available in a de-identified manner to researchers upon reasonable request (to the extent allowed by the registry's data protection agreement). Requests to access these datasets should be directed to Jessica L. Rohmann, jessica.rohmann@charite.de.

Ethics statement

The scientific evaluation of B-SPATIAL registry data was approved by the Ethics Committee of Charité-Universitätsmedizin Berlin (EA1/208/21). The B-SPATIAL registry used an opt-out mechanism for patient inclusion. Two months after their index event, patients were informed in writing about the inclusion of their record in the B-SPATIAL registry and had multiple opportunities to opt out.

Author contributions

LW, HA, and BS conceptualized the study. MEb, MEn, CN, PH, and HA were involved in data acquisition. The statistical analyses were planned and conducted by LW and BS, also in consultation with DM and JR. LW, HA, JR, and BS interpreted the results and drafted the manuscript. LW created all figures and tables. HA provided funding for the project and as well as project supervision together with BS and JR. All authors critically revised the final version of the manuscript.

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Conflict of interest

Author JR reports receiving a grant from Novartis Pharma for a self-initiated research project on migraine remission unrelated to this work. Author MEn reports receiving grants from Bayer and fees paid to the Charité-Universitätsmedizin Berlin from AstraZeneca, Bayer, BMS, Pfizer, Daiichi Sankyo, Amgen, GSK, Sanofi, Covidien, and Novartis, outside of this work. Author CN received research grants from German Ministry of Research and Education, German Center for Neurodegenerative Diseases, German Center for Cardiovascular Research, and speaker and/or consultation fees from Boehringer Ingelheim, Bristol-Myers Squibb, Pfizer Pharma, Daiichi Sankyo, Alexion, Abbott, and Bayer. Author HA received research grants from German Ministry of Research and Education and the German Research Foundation for the B-SPATIAL registry and the B_PROUD study and reports personal fees from Bayer Vital, Boehringer Ingelheim, Bristol- Myers Squibb, Novo Nordisk, Pfizer, and from Sanofi outside the submitted work.

The remaining 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

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Therapeutic strategies for intracerebral hemorrhage

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Stroke is the second highest cause of death globally, with an increasing incidence in developing countries. Intracerebral hemorrhage (ICH) accounts for 10–15% of all strokes. ICH is associated with poor neurological outcomes and high mortality due to the combination of primary and secondary injury. Fortunately, experimental therapies are available that may improve functional outcomes in patients with ICH. These therapies targeting secondary brain injury have attracted substantial attention in their translational potential. Here, we summarize recent advances in therapeutic strategies and directions for ICH and discuss the barriers and issues that need to be overcome to improve ICH prognosis.

KEYWORDS

intracerebral hemorrhage, secondary brain injury, therapeutic strategies, neuroinflammation, neuronal death

Introduction

Intracerebral hemorrhage (ICH) is a catastrophic stroke subtype with a high risk of disability and death. It represents 10–25% of all strokes and it afflicts an estimated 2 million people worldwide each year (1, 2). Effective treatment options for ICH are yet to be developed (3). Patients who survive ICH usually suffer from various neurological dysfunctions (4). Previous studies have shown that a variety of etiologies contribute to the development of ICH, among which hypertension is the most common cause (5). Other pathophysiologies such as amyloid angiopathy, brain tumors, aneurysms, arteriovenous malformations, cerebral cavernous malformations, and arteriovenous fistulas also give rise to ICH (6). A series of neuropathologic changes occur in the brain after ICH, including intracerebral hematoma, space-occupying effects due to secondary injury, changes in regional cerebral blood flow, brain edema and neurotoxic injury, and disruption of the blood-brain barrier (BBB) (7–10).

Complications of primary and secondary brain injury develop following ICH (11). Primary injury in the hyperacute phase of ICH is principally due to mechanical damage to the surrounding brain tissues as the hematoma develops (12, 13). The extent of primary injury is determined by the location and volume of the hematoma, and the initial hematoma size (14, 15). A consequence of hematoma formation and expansion is compression of the surrounding brain tissue causing neurological deterioration, intracranial hypertension, brain herniation and even death (16). Secondary brain injury after ICH involves a complex array of processes and consist of BBB damage, brain edema, iron deposition, cytotoxic cascade reaction, neuronal apoptosis, oxidative stress (17), and neuroinflammation (Figure 1) (18, 19).

It is important to explore potential therapeutic strategies that counter the mechanisms of primary and secondary brain injury after ICH (20, 21). In this review, the characteristics of pathophysiology following ICH are presented, as well as the current treatment options, and we emphasize the urgent need for investigating novel treatment strategies.

Preclinical therapeutic strategies targeting ICH

In terms of etiology, intracerebral hemorrhage can be divided into three categories. Hypertension is the leading cause of ICH, a major focus of early treatment is to control blood pressure aggressively (15). A fast-acting agent such as nicardipine or clevidipine is the usual choice. Another common cause of ICH is cerebral amyloid angiopathy (CAA), patients with CAA are at high risk of recurrent hemorrhages and subsequent cognitive deterioration. Prevention of re-hemorrhage emphasizes avoidance of anticoagulation and antiplatelet, if possible, strict blood pressure control, and fall precautions (22). The remaining etiologies are due to secondary

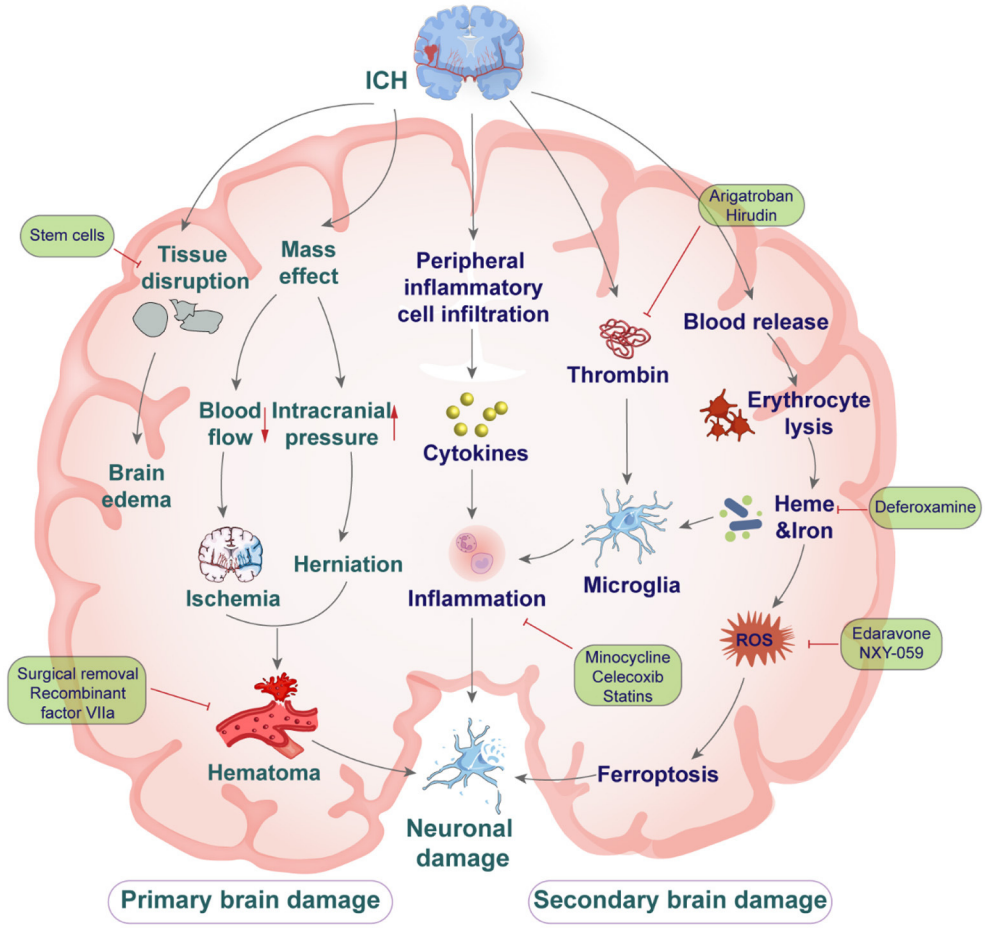

FIGURE 1
The mechanisms of primary and secondary brain injury after intracerebral hemorrhage, and drug intervention.

brain injury. The process of secondary brain injury is triggered by activation of resident cells and infiltration of peripheral immune cells, and inflammatory factors which contribute to the development of brain edema and cell death (23).

Targeting anti-inflammatory responses

Brain injury after ICH occurs through multiple mechanisms including not only space-occupying effects of hematoma and mechanical damage, but also neuroinflammation (18). Neuroinflammation-related mediators include thrombin, matrix metalloproteinases, cytokines, free radicals, and complements. There is growing evidence that inflammation is one of the critical factors in secondary brain injury after ICH (20, 24, 25). The modulation of inflammatory responses in the early and delayed phases of ICH is particularly important, especially in the case of microglia/macrophages, astrocytes, and leukocytes (5, 18). Microglia are the initial and primary active immune defense fundamental to the CNS, which are thought to act as "the first responders" to brain injuries during the early stages of ICH (26). Microglia can undergo morphological and functional changes in response to disease signals. Microglia and monocyte-derived macrophages in the adjacent injured brain tissue of ICH are not readily distinguished from one another, thus we refer to them as microglia/macrophages (10, 27). In clinical studies, it has been shown that up-regulation of CD163 levels can facilitate microglia/macrophages phagocytosis (28); PPAR-γ agonist pioglitazone has been shown to be safe for hematoma regression after ICH (29); minocycline improves outcomes in patients with acute ischemic stroke, but efficacy and safety require further study (30). Preclinical research is oriented to regulate the function of microglia/macrophages. New therapeutic targets such as NLRP3 inflammasome (31), TSPO ectopic protein (32), and transforming growth factor TGF-β1 have been developed (33). Following ICH, astrocytes in the perihematomal area are activated and secrete various cytokines and chemokines, such as aquaporin AQP4 (34), IL-15 (35), IL-33 (36). Neutrophils can infiltrate around the hematoma within 4 h and release various inflammatory factors, which is an important predictor of neurological deterioration in primary ICH (37). In clinical trials, S1PR1 modulators, like fingolimod and RP101075, have shown to improve neurological deficits and promote recovery in patients with primary ICH (38). The cytokine IL-27, which accelerates neutrophil maturation in the bone marrow and reduces the secretion of proinflammatory cytokines, is also worthy of investigation as a potential therapeutic target for ICH (39).

Similarly, Toll-like receptor (TLR) activation and inflammatory signaling pathways regulated by risk-associated molecular patterns are engaged (40). Within 24 h after the onset of ICH, red blood cells are lysed and cytotoxic substances such as hemoglobin, heme and iron are released from the blood clot in the hematoma (24), which further exacerbate brain injury and ultimately lead to tissue damage, BBB dysfunction, and massive brain cell death (41, 42).

Numerous studies in animal models have shown that inhibition of neuroinflammation promotes recovery from ICH. A widely reported preclinical treatment strategy for ICH is the use of minocycline, which by inhibiting microglia and matrix metalloproteinase activation (25, 43–45), reduces brain edema, BBB damage, and brain cell death in collagenase induced ICH model in rat (46, 47). In addition, minocycline is a broad-spectrum tetracycline that may provide neuroprotection through its anti-inflammatory properties, including inhibition of microglia activity and alleviating demyelination in white matter after ICH (46, 48). We showed that minocycline exhibits protective roles in ICH by decreasing EMMPRIN and MMP-9 expression (49), alleviating BBB disruption, ameliorating neuroinflammation, and reducing neuronal degeneration and death (50, 51). Tuftsin fragment 1–3 has been found to attenuate ICH injury and improve neurological function by inhibiting microglia/macrophage activation in rats.

Clinical studies suggest that the anti-inflammatory effects of the cyclooxygenase inhibitors, celecoxib and pioglitazone, may be effective in lowering the neurological sequalae of ICH (52), but further studies are required to confirm this effectiveness. Similarly, statins that inhibit the synthesis of HMG-CoA reductase may not only have anti-inflammatory roles but also have a neuroprotective effect associated with increasing cerebral blood flow after ICH (53). Currently, two statins, rosuvastatin and simvastatin, have shown promising efficacy in clinical trials (54). While statins have encouraging short-term efficacy in the acute phase of ICH, there are no unequivocal clinical data to support the benefit of continued statin use after the acute phase. In addition, the risk of rebleeding that may result from long-term use of such drugs is unclear (55).

Thrombin

Thrombin is a multifunctional serine protease that is rapidly produced after brain hemorrhage and catalyzes the conversion of fibrinogen to fibrin which affects the coagulation cascade. Thrombin may cause brain edema, neuroinflammation, and BBB damage in the early stages of ICH, but it is also essential to prevent initial hemorrhage and hematoma expansion (56). A dual effect of thrombin on blood vessels has been found. Low concentrations of thrombin cause vasodilation and exert neuroprotective effects by upregulating heat shock proteins and iron-related proteins. In contrast, at high concentrations, thrombin causes slow and persistent vasoconstriction, which leads to infiltration of inflammatory cells, disruption of the BBB, formation of brain edema, and neuronal death (24, 57). These findings suggest that inhibition of thrombin may reduce brain edema. Agatroban, a thrombin inhibitor, reduces brain

edema around the hematoma after systemic treatment, while delaying secondary cerebral micro-thrombosis and improving local lateral cerebral blood flow. This observation suggests that thrombin inhibitors may provide a potentially useful neuroprotective strategy. In a similar manner, hirudin, another thrombin inhibitor, may reduce brain edema formation by downregulating aquaporin-4 and 9 in a collagenase-induced ICH model in rat (58–60).

Antiplatelet therapy

More than 25% of patients with ICH have received antiplatelet therapy (APT), which may increase the risk of hematoma enlargement and lead to serious adverse events associated with ICH such as brain edema (42). It was investigated that long-term aspirin use increased in 12 cases of ICH per 10,000 people (61). Emergency treatment with low-dose aspirin (100 mg) within 48 h reduces the risk of recurrent stroke and improves prognosis (62). The risk of high-dose aspirin-induced ICH is increased in the elderly, especially in patients with untreated hypertension, and long-term combined use of aspirin and clopidogrel may elevate the risk of ICH (63).

A multicenter randomized controlled trial (PATCH) showed that patients receiving conventional platelet transfusions had a higher risk of post-ICH functional dependence or even death within 3 months, and therefore a higher chance of serious adverse events during hospitalization compared to the standard treatment group (64, 65). For the use of antiplatelet agents associated with ICH, it seems reasonable to avoid platelet transfusions. In prospective studies in patients with ICH, intravenous vasopressin improved platelet function without significant adverse effects, but to date, the effect of vasopressin on clinical outcomes has not been meaningfully evaluated (66).

Complement inhibition

Current evidence suggests that the complement cascade is activated early after ICH and contributes to brain edema/injury in multiple ways (67). Complement cascade activation produces C3a and C5a, and microglia activation produces inflammatory mediators that subsequently assemble with complements into membrane attack complexes (MAC, C5b-9) on the surface of target cells (68). The formation of MAC may be associated with the release of hemoglobin and iron from erythrocyte lysis which exacerbate brain damage in the vicinity of the hematoma (14). Activation of the complement system is also a powerful driver of the activation of microglia and mast cells, thereby enhancing the inflammatory response of ICH (69).

Inhibition of complement cascade activation by means of antagonists or gene knockout can reduce the extent of brain injury. When ICH mice were treated with C5a-receptor antagonist, a reduction in neutrophil infiltration in the hemorrhagic hemisphere was shown at 24 and 72 h, and some neurological functions were improved. The combination of C5a-receptor antagonist and C3a-receptor antagonist led to synergistic improvements in the neurofunctional outcome while reducing inflammatory cell infiltration and brain edema (70).

However, experimental results on C5a remain controversial, with C5a knockout mice showing more severe brain damage after brain hemorrhage despite the protective effect provided by C5a-receptor antagonist, which may be related to compensatory changes in the organism's responsiveness after C5a loss (71). Therefore, given the role of the complement system in removing apoptotic cells and inducing neurogenesis, therapeutic complement inhibition needs to be delivered in a more targeted manner to maximize its efficacious effects while minimizing potential adverse effects (14).

Improvement in iron chelator management

The process of hemolysis after ICH is featured by the release of erythrocyte contents, one of the main products of which is hemoglobin (Hb) which is then oxidized extracellularly to form oxygen radicals and free heme (72). Hb can also be degraded to ferrous iron, bilirubin and carbon monoxide by the action of heme oxygenase (73). Subsequently, due to the release of excess free iron ions from the hematoma, severe iron deposition in brain tissue may occur, triggering acute brain edema reactions and long-term neurocognitive impairment (74).

Basic studies and preclinical trials have shown that lowering brain iron deposits and reducing iron overload in brain tissue are reasonable strategies for the treatment of ICH. Deferoxamine based iron-lowering therapy significantly reduces brain edema induced by iron overload after ICH, attenuates neuronal death and improves neurological scores (75).

Deferoxamine through iron chelator markedly reduces free iron in brain tissue and is neuroprotective in a variety of animal models of ICH (76). In an autologous blood-induced ICH model in rats (77), deferoxamine attenuated brain edema and brain atrophy and improved neurological function, and these findings were corroborated in piglets (78). In addition, our recent study found that the combination of deferoxamine with minocycline provided better neuroprotection after ICH, with marked reduction in brain injury area, neuronal death and microglia/macrophage activation (79, 80). Preclinical data suggest that deferoxamine may prevent memory dysfunction by restoring iron homeostasis, attenuates neuroinflammation and oxidative stress (Table 1) (94). Meanwhile, clinical studies on deferoxamine are in phase II trials, but its safe dose and the

TABLE 1 Current and past therapeutics for intracerebral hemorrhage.

Intervention	ICH type	Target	Phase	Outcome	Limitation
Minocycline (81)	Primary ICH	Multiple	NCT01805895 Phases 1and 2	400 mg dose of minocycline achieves neuroprotective serum concentrations	Oral administration leads to delayed absorption, not suitable for severe patients
Celecoxib (82)	Primary ICH	COX-2	NCT00526214 Phase 2	Celecoxib limits the expansion of perihematomal edema in the acute phase of ICH	The 90 days functional outcome has not been significantly improved
Desmopressin (66)	Primary ICH	Antiplatelet	Phase II	Intravenous desmopressin was well-tolerated and improved platelet activity after acute intracerebral hemorrhage	Larger studies are needed to determine its potential effects on reducing hematoma growth vs. platelet transfusion or placebo
Fingolimod (38)	Primary supratentorial ICH	Neuroinflammation	Phase II	Administration of oral fingolimod reduced PHE, attenuated neurologic deficits, and promoted recovery	The efficacy of fingolimod in preventing secondary brain injury in patients with ICH warrants further investigation in late-phase trials
Deferoxamine Mesylate (83)	Spontaneous supratentorial ICH	Iron	NCT02175225 Phase 2	DFO can effectively improve nerve function, and the dose of 32 mg/kg/day is safe	Screening of patients with ICH has slight limitations, and the generalizability of the results needs to be considered
Factor VIIa (84)	Acute ICH	Hematoma expansion	NCT00127283 phase 3	Significantly reduced growth of the hematoma	Did not improve survival or functional outcome at 90 days
STICH II (85)	Spontaneous lobar intracerebral hematoma	Surgical evacuation	ISRCTN22153967	Early surgery has clinically relevant survival advantages and does not increase mortality or disability at 6 months	Prospective randomized controlled trials have not been conducted to compare the effect with conservative treatment
PATCH (64)	Supratentorial ICH	Surgical evacuation	NTR1303	Compared to the standard treatment group, patients who routinely receive platelet transfusions have a higher probability of functional dependence at 3 months	1. The sample size was smaller 2. The level of bias through selective inclusion is unknown 3. Adherence to antiplatelet therapy for participants was not measured
MISTIE III (86)	Supratentorial ICH	Surgical evacuation	NCT01827046	Minimally invasive treatment is safe and helps reduce the fatality rate at 365 days	Does not improve nerve function, need to be confirmed in the future
RP101075 (87)	Autologous blood/mouse	S1PR1 agonist	Pre-clinical	RP101075 significantly attenuated neurological deficits and reduced brain edema in ICH mice	Provides insufficient information on the optimal S1PR1 modulation time window
VK-28 (88)	Autologous blood or collagenase/mouse	Iron	Pre-clinical	VK-28 decreased iron-deposition and microglial activation around hematoma, and improved neurologic function	The dose–response and the therapeutic window need to be determined in young and aged animals
Argatroban (89)	Autologous blood or collagenase/rat	Thrombin	Pre-clinical	Systemic administration of argatroban after 6 h can also reduce the formation of ICH-induced edema	Argatroban used in clinical trials may have a higher potential risk of rebleeding
Stem-cell transplantation (90)	Collagenase/rat	Multiple	Pre-clinical	BM-MSCs transplantation reduced hematoma volume and alleviated neurological deficits after ICH	Further research is needed to explore the effect of BM-MSCs in later stages of this condition
Therapeutic hypothermia (91)	Collagenase/rat	Multiple	Pre-clinical	Prolonged mild hypothermia provides persistent histologic and functional protection	Early hypothermia brings complications, such as elevated blood pressure and coagulopathy
TAK-242 (92)	Autologous blood/mouse	TLR4	Pre-clinical	TLR4 antagonist reduced inflammatory injury and neurological deficits, decreased DNA damage and neuronal degeneration	Need to be further verified in clinical trials
PD-1 (93)	Collagenase/mouse	Multiple	Pre-clinical	Significantly attenuated neurological deficits, reduced brain edema, and decreased hemorrhage volume	No direct evidence for the influence of PD-L1 on the apoptosis or proliferation of neurons and astrocytes

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emergence of late complications need to be further investigated (95, 96).

Neuroprotective agents

Neuroprotective agent therapy has been investigated in the acute and chronic stages of ICH. Valproic acid (VPA), an anticonvulsant, may have neuroprotective roles after ICH. VPA was found to enhance the expression of the anti-apoptotic gene Bcl-2, which limits the development of hematoma by inducing the extracellular signal-regulated kinase cAMP response element-binding protein after ICH (97). In addition, VPA not only down-regulates the expression of pro-inflammatory factors such as MMP-9 and FasL, but also acts as an anti-apoptotic neuroprotective agent by inhibiting the activation of Caspase-3. Recent studies have shown that VPA can protect rat cortical neurons from glutamate-induced excitotoxicity and increase the lifespan of cultured cortical neurons (98).

Various endogenous proteins are upregulated to protect the brain from various mechanisms of injury. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates antioxidant defense mechanisms by responding to oxidative stress (99). Nrf2 knockout mice were more susceptible to oxidative stress than wild-type mice. The apoptosis and neurological dysfunction in collagenase-induced ICH model were more severe in Nrf2 knockout mice compare to wildtype mice (100). Erythropoietin (EPO) is a glycoprotein hormone that is an important regulator of cell function, survival, and various molecular cascade responses (101). It is involved in STAT-3 and eNOS pathways and can down-regulate glutamate excitotoxicity, apoptosis and inflammation, thus exerting neuroprotective and anti-apoptotic effects (102). Similarly, programmed cell death 1 (PD-1) represents an important immune receptor that is expressed on a variety of activated immune cells. Related studies have demonstrated that PD-1 inhibits inflammatory cell activation, reduces neurological deficits, hematoma volume and neuronal death in mice, and enhances BBB integrity to a certain degree (93).

Clinical therapeutic strategies targeting ICH

Blood pressure management

Patients with ICH often have elevated blood pressure, possibly due to stress, pain, or high cranial pressure. Hypertension after ICH is often associated with hematoma enlargement and poor prognosis, but the relationship is unclear (12). The treatment of hypertension is currently in a dilemma. On the one hand, the presence of hypertension increases the risk of edema, which may lead to persistent bleeding and rebleeding causing exacerbation. On the other hand, the occurrence of hypertension may be a protective mechanism to ensure cerebral blood flow supply and prevent ischemic stroke (103). Aggressive antihypertensive therapy after ICH can easily lead to a sudden drop in cerebral perfusion pressure and cerebral ischemia, followed by increased intracranial pressure and further neurological impairment.

Guidelines from the American Heart Association and the Stroke Association state that in patients with systolic blood pressure between 150 and 220 mmHg who do not have a contraindication to acute blood pressure lowering, continuous use of antihypertensive agents such as labetalol, esmolol, or nicardipine should be used. It is safe to reduce blood pressure to 130–140 mmHg within a couple of hours, but its effectiveness in improving neurological function remains to be further examined (104). In patients with ICH with systolic blood pressure >220 mmHg, blood pressure can be controlled by continuous intravenous drug infusion with a target systolic blood pressure of 160 mmHg with close monitoring of blood pressure. In the presence of impaired blood flow autoregulation, mean arterial pressure decreases by more than 15–30%, which may exacerbate ischemia in the area surrounding the hematoma and worsen brain injury. Notably, intensive hypotension may lead to adverse renal events (105).

Hematoma expansion

Hematoma expansion has a significant relationship with clinical deterioration and poor prognosis. To date, prevention of hematoma expansion is an effective strategy to reduce the mass occupying effect. Traditionally, hypertension is considered to be one of the main causes of ICH; indeed, persistent elevation of systolic blood pressure is one of the main risk factors for hematoma expansion. Clinical trials have indicated that there is no significant difference in the elevation of systolic blood pressure between the hematoma-dilated and non-hematoma-dilated groups of patients with ICH (73, 106), and that elevation of systolic blood pressure is not an independent factor for hematoma expansion in the acute phase of a ICH when studied with multivariate analysis, but that rapid reduction of blood pressure may contribute to improved functional outcomes (107).

A further approach to prevent hematoma expansion is the use of drugs that alter the coagulation cascade or fibrinolytic process. The results of phase II clinical trial of recombinant factor VIIa show that treatment within 4 h after the onset of ICH limited hematoma expansion and improved clinical regression, but the incidence of thromboembolic events was mildly increased (84). E-aminocaproic acid (EACA) is an antifibrinolytic agent that helps to limit hematoma volume expansion and reduce early morbidity and mortality, but the long-term benefit is uncertain (108). Therefore, the focus of

future studies should be to determine which subgroup of patients would benefit from activated recombinant factor VIIa therapy (63, 65). Clinical trials have demonstrated the benefit of early platelet transfusion in patients with the ICH who have received antiplatelet therapy, while in thrombolytic drug-related ICH, transfusion of coagulation factors and platelets may be an option (109).

Surgical therapeutic strategies targeting ICH

After vascular rupture, a hematoma begins to form and develops within 60 min, causing mechanical damage to the brain parenchyma. The hematoma first causes deformation and displacement of brain tissue, which in turn increases intracranial pressure and elevates the risk of brain herniation and cerebral ischemia (55). Many clinical trials have explored and tested the effectiveness of clot removal and focused on reducing surgical complications (110).

Craniectomy for evacuation of hematoma

Craniotomy is the initial and main method of neurosurgical treatment of ICH. Early removal of hematoma can reduce hematological toxicity, alleviate edema and ischemia around the hematoma, prevent hematoma expansion, and produce a good clinical treatment effect (111). The surgery allows for direct vision, adequate decompression, and precise hemostasis, which not only reduces intracranial pressure but also improves hemodynamics and brain tissue metabolism. However, at the same time, craniotomy causes greater surgical trauma and a higher incidence of postoperative complications, which may have serious prognostic implications (112, 113).

In a multicenter randomized controlled trial, early surgical manipulation was not significantly beneficial to patients compared to conservative treatment (114). In the subsequent STICH II study, in patients with spontaneous superficial ICH without ventricular hemorrhage, early surgical resection of lobar hemorrhage did not result in a better clinical outcome and produced only a slight survival advantage compared with pharmacological treatment (85). Regarding cerebellar hemorrhage, surgical treatment has a better prognosis for patients whose condition deteriorates rapidly due to brainstem compression or hydrocephalus. In patients with spontaneous ICH without ventricular hemorrhage, early surgery may have a small but clinically meaningful survival advantage (115).

Minimally invasive surgery

The application of minimally invasive surgery (MIS) to obtain maximum results at the cost of minimal trauma has helped to improve the surgical outcome of ICH. The application of various minimally invasive techniques has become a mainstream concept worldwide. The most commonly used techniques include hematoma directed drainage, keyhole surgery, and neuro-endoscopic hematoma evacuation (116). The advantages of minimally invasive surgery for ICH are reduced surgical exposure, shorter operative time, less surgical trauma, and the procedure can be performed under local anesthesia.

Meta-analysis of a clinical trial found better treatment outcomes, improved neurological recovery, and reduced long-term mortality with minimally invasive surgical treatment compared to other treatments (conservative and craniotomy) (114).

Further studies have found that minimally invasive surgery combined with thrombolytic therapy within 72 h after ICH is safe, reduces perifocal edema, promotes hematoma liquefaction and drainage *via* catheter, and has a tendency to improve neurological function (117, 118). It is worth noting that minimally invasive surgery is less effective in decompression and is not superior to conservative medical therapy in terms of CNS infection rate and symptomatic rebleeding. Therefore, patients with ICH of undetermined etiology should undergo vascular-related investigations (CT angiography, MR angiography and digital subtraction angiography) prior to minimally invasive surgery to exclude vascular lesions and avoid and reduce the risk of rebleeding (119).

Extraventricular drainage

Intraventricular hemorrhage (IVH) varies in severity and is a common complication of ICH and an independent assessment factor of ICH prognosis. IVH often leads to acute hydrocephalus, which can be treated by lumbar puncture or external ventricular drainage (EVD) (113). In recent years, several studies have shown that fibrinolytic drugs can be used as an adjunct to extraventricular drainage and that their combination can prevent drainage obstruction. CLEAR III, a large multicenter randomized controlled study that included 500 patients, found that EVD combined with rt-PA cleared ventricular hemorrhage with good safety and helped reduce mortality in patients with severe ventricular hemorrhage, but neurological function was not improved. Improvement in neurological function did not occur, and further studies are needed (111, 120). According to another study, EVD + rt-PA combined with lumbar puncture and drainage helped to clear ventricular hemorrhage more rapidly, and the risk of subsequent ventriculoperitoneal

shunts and rebleeding was significantly reduced; a follow-up randomized clinical trial (RCT) is still needed to verify this (116).

Emerging therapeutic strategies targeting ICH

Stem cell therapy

Stem cell therapy has emerged as a potential approach for the treatment of ICH, mainly to modulate the immune response after ICH. The replacement of damaged cells and restoration of function is accomplished by transplanting cells such as mesenchymal stem cells, neural stem cells, embryonic stem cells, stem cells from bone marrow and umbilical cord blood (12). Mesenchymal stem cells (MSC) are umbilical cord-derived cells with powerful immunomodulatory capabilities, and intracerebroventricular transplantation of MSC can prevent further development of brain injury and hydrocephalus after ICH (90). Bone marrow mesenchymal stem cells (BMSC) secrete neurotrophic factors that promote astrocyte proliferation and myelin formation by intravenous injection and improve neurological and behavioral performance in rats with collagenase-induced ICH (121, 122). Recently, human umbilical cord blood (HUCB) has been considered as an ideal cell source for the treatment of ICH. In a collagenase-induced rat ICH model, human umbilical cord blood and its derived single nucleated cell transplantation reduced hematoma volume and improved functional recovery (90, 123).

Potential MicroRNA therapy

MicroRNAs (miRNA, miR) are small conserved non-coding single-stranded RNA. They can regulate inflammatory response after ICH and are viable molecular targets to alter brain function (124, 125). Over the past few years, there has been a surge of research addressing the role of various miRs in the pathophysiology of ICH (126). For example, several studies have demonstrated that miR-223 (127), miR-7 (128), miR-let-7a (129), miR-23b (130) and miR-194-5p (131), among others, have roles in promoting neuroprotection. Given the potential of miR as a viable therapeutic target, several clinical trials are already underway. One clinical trial assesses the efficacy of exosomes overexpressing miR-124 in improving the outcome from acute ischemic stroke (NCT03384433; clinicaltrials.gov) (132). Further studies are required to elucidate the molecular mechanisms of miR dysregulation after ICH and to test the safety and tolerability of miR-based therapeutic strategies.

Nanotechnology

Nanotechnology has been widely used for disease monitoring, diagnosis, and for the treatment of ICH. Moreover, drug delivery strategies that can bypass the BBB physical barrier have benefited from polymer-based nanoparticles (NPs) due to their desirable biocompatibility and ability to improve the bioavailability and pharmacokinetics of specific drugs.

In one study, curcumin Cur was encapsulated in NPs (Cur-NPs), and these Cur-NPs effectively crossed the BBB to enhance the accumulation of curcumin in the brain (133). More importantly, Cur-NPs have the ability to inhibit ferritin formation and thus can be used as an effective treatment for ICH. A similar study combined the covalently bonded iron chelator deferoxamine (DEF) with a carbon nanomaterial, polyethylene glycol-conjugated hydrophilic carbon cluster (PEG-HCC), which was synthesized as a multifunctional nanoparticle of antioxidant with iron chelator property; in addition, DEF-HCC-PEG protected cells from senescence and iron atrophy, and protected neurons from the toxic effects of blood breakdown products in ICH (134).

Therapeutic hypothermia

Hypothermia is an effective neuroprotective agent in rodent models of ischemic stroke (135). It prevents neuronal loss and improves functional outcomes (136). Studies have shown that hypothermia can also reduce mortality and promote neurological recovery in patients with cardiac arrest (137, 138). In a rat whole-blood ICH model, early hypothermia (1 h after injection) resulted in reduced edema but no significant improvement in neurological outcomes, whereas delayed hypothermia (12 h after injection) showed improvement in neurological outcomes. Experts speculate that this is due to early hypothermia leading to impaired coagulation and hypertension, which exacerbates hematoma expansion (139).

Data from clinical studies have shown that patients with ICH treated with sub-cold temperatures (brain temperature of 30–35°C) for 8–10 days have reduced peri-hematoma edema and neutrophil infiltration, improved DNA damage and neurological deficits, and reduced mortality. More than 90% of mild hypothermia treated patients exhibited pneumonia as a side effect but this responds to intravenous antibiotics (140). The Safety and Feasibility Study of Targeted Temperature Management after ICH (TTM-ICH) is currently underway and is expected to provide additional guidance (91, 137).

Neuro-critical care management

Patients with ICH are typically treated in general intensive care units, while studies have shown that managing ICH patients within a neurocritical care system has a more positive impact on their prognosis and may influence clinical endpoints (141). Patients with ICH can benefit from an early and aggressive treatment plan, with care focused on

neurological monitoring, treatment of elevated intracranial pressure or cerebral ischemia, and prevention and treatment of secondary medical complications, which should be performed by experienced nurses, physicians, and other medical staff. Kurtz et al. worked on the management of patients with brain injury in specialized and general ICUs; they highlight differences in indicators such as hemodynamics, intracranial pressure monitoring, tracheotomy, nutritional support and intravenous sedation doses in the NICU (142). Another study found that patients in general ICUs had a 20–40% higher in-hospital mortality rate compared to neurological ICUs (143). The highest priority in the treatment of patients with brain injury is the avoidance of secondary brain injury, so effective management of disease problems and risks, better adherence to established clinical guidelines, more careful monitoring, and more aggressive interventions can lead to a better prognosis for patients with ICH.

Review and outlook

ICH has attracted worldwide attention as a disease that decreases the quality of life of survivors. It is often life-threatening. In the last 20 years, the number of preclinical and clinical studies on ICH has increased considerably and this has led to a better understanding of the mechanisms of injury and potential therapeutic targets for ICH. However, exploring the appropriate and effective treatment method remains a challenge (20).

Patients with ICH require the following measures to be treated effectively: identification of the underlying cause of the hemorrhage, correcting elevated blood pressure, reversing coagulopathy, and providing supportive care in a neurological ICU. Clinical experience shows that the major prognostic interventions after ICH are ultra-early hemostasis, clot evacuation, and intracranial pressure control. Therefore, we need a better understanding of the dynamic process of hematoma growth and to minimize the inflammation caused by hemorrhage and the neurotoxicity produced by blood degradation products (144). Related treatments include ultra-early hemostatic therapy with activated recombinant factor VIIa, acute treatment of hypertension, tight glucose control, and the management of coagulation disorders. Other innovative techniques, such as thrombolytic treatment for ICH, minimally invasive surgery, the development of new anti-inflammatory medications, and the introduction of neuroprotective agents, need further study (e.g., deferoxamine, statins, celecoxib, pioglitazone, etc.). Clinical trials are also actively advancing, and new therapies may be developed in the near future. Other strategies such as hypothermia, neural stem cell transplantation, and professional surgical care, are also expected to contribute additional advances in the coming years (12, 145).

However, the treatment of ICH still faces major problems and obstacles. First, we do not fully understand the pathological mechanism of secondary injury after ICH and the complex molecular pathways leading to brain injury, which limits the development of therapeutic strategies. Although various studies have addressed gene expression and various molecular pathways, the following questions are not entirely clear, for example, whether there is an association between reduced brain edema volume and improved neurological function, and whether the hypometabolism and hypoperfusion surrounding the hematoma is associated with neuronal death (146, 147).

Second, animal models of ICH used in basic research have their own advantages and disadvantages, but none of them fully replicates human ICH; therefore, it is necessary to select an appropriate animal model according to the purpose and conditions of preclinical studies and to critically evaluate its results. Finally, the vast majority of ongoing investigations are still in the preliminary experimental phase and have not advanced to clinical trials, thus the distinctions between the two are not yet well-defined. Therefore, careful consideration should be given to study design, measurement of outcomes, optimal timing of intervention, and heterogeneity among subjects (6, 148).

Conclusions

In summary, the optimal treatment strategy for ICH is effective prevention and long-term supervision for high-risk groups, internal medicine support and systemic treatment, surgical removal of hematoma with minimal trauma, and early rehabilitation by a variety of rehabilitation therapies (12). Although ICH poses a tremendous burden on the world's public health, much relevant research has been done to address the problem and we remain cautiously optimistic on the prospects of effective ICH treatment (147).

Author contributions

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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

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Analyses on safety and efficacy of non-standard dose of r-tPA in intravenous thrombolysis-treated AIS patients

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Background: Intravenous 0.9 mg/kg recombinant tissue plasminogen activator (r-tPA) is one of the most effective treatments in acute ischemic stroke patients. Practically, the dose of r-tPA is still a topic that is constantly being discussed.

Methods: For this observational study, data were obtained from 537 patients who received r-tPA thrombolysis at Shanghai Sixth People's Hospital stroke center over 5 years (2014–2019). Patients were divided into two groups: a non-standard dose group (0.6 mg/kg $\leq$ dose $<$ 0.9 mg/kg) and a standard dose group (0.9 mg/kg). Different outcomes were observed: efficacy: 3 months mRS 0-1 (3m-mRS0-1); safety: symptomatic intracranial hemorrhage within 24 h (24h-sICH) and 3 months mortality (3m-death). We also observed the effect of r-tPA dose coefficient on outcomes in different age groups and baseline National Institute of Health stroke scale (NIHSS) score subgroups.

Results: There were 265 patients who gave the standard dose treatment and 272 gave the nonstandard dose. There was no significant difference between the non-standard dose group and the standard dose group in 3m-mRS0-1, 3m-death, and 24h-sICH ($p = 0.567$, 0.327, and 0.415, respectively). The dose coefficient presents a significant negative correlation ($p = 0.034$, $B = -4.290$) with 3m-death in NIHSS $<$16 sub-group. Door-to-needle time (DNT) is the most important independent outcome-influential factor (MIOIF) in the NIHSS $\geq$16 sub-group. The diabetes history and baseline NIHSS score were the MIOIF in the age $\geq$80-year sub-group.

Conclusions: The non-standard dose group (0.6 mg/kg $\leq$ dose $<$ 0.9 mg/kg) shows no difference in safety and effectiveness than the standard dose group (0.9 mg/kg) in our study. The standard dose should be considered first according to current evidence and Guidelines, but the non-standard

dose (0.6 mg/kg ≤ dose < 0.9 mg/kg) might be an option in the actual diagnosis and treatment process considering the patient's clinical profile and financial condition.

KEYWORDS

acute ischemic stroke, intravenous thrombolysis, r-tPA, dose, sub-group analyses

Introduction

Intravenous thrombolysis using recombinant tissue plasminogen activator (r-tPA) is widely used in acute ischemic stroke (AIS) patients (1, 2). In recent years in China, with the popularization of stroke knowledge, improvement of out-hospital emergency system, and coverage of r-tPA by medical insurance, the ratio of thrombolysis was raised to 24.2% in the year 2018 (3). The main constraints are still largely the patient's own cost and the fear of symptomatic intracranial hemorrhage (sICH).

In the real world, physicians, especially in many developing countries in Asia such as China, may choose r-tPA in a way that does not fully comply with the standard dose for different reasons, such as a patient's financial condition, advanced age, or the onset of a serious illness. Meanwhile, the dose of r-tPA is still a topic that is constantly being discussed. The Japan Alteplase Clinical Trial first demonstrated that AIS patients receiving r-tPA at a dose of 0.6 mg/kg could obtain comparable efficacy and safety to historical controls given 0.9 mg/kg r-tPA (4–8). While the ENCHANTED study (9) showed that compared with the standard dose, the lower dose group was not inferior in the ordinal analysis of modified Rankin scale scores (mRS), and with no significantly higher mortality at 90 days, while the sICH and fatal events occurred within 7 days are reduced. Beside 0.6 mg/kg, other low doses of IV r-tPA have been studied in specific values. In 2017, Cheung-Ter Ong et Al. further compared the efficacy and safety of different doses (0.6, 0.7, 0.8, and 0.9 mg/kg) of r-tPA (10). The early neurological improvement, early neurological deterioration, and the sICH were not significantly different among the four dosage groups. But the clinical functional outcome at 6 months after stroke onset was poorer than in the standard-dose group ($P = 0.02$). All these trials pre-assigned the dose (mg/kg) in a fixed value, but what is the relationship between the doses (as a continuous variable) and the outcome of AIS patients treated with r-tPA? In fact, in clinical practice, many factors affect the prognosis of AIS patients in the guidelines published by AHA/ASA for the Early Management of Acute Ischemic Stroke (1) and the Chinese guidelines for clinical management of ischemic cerebrovascular disease (11). Besides, the idea that thrombolysis should be initiated as quickly as possible because timely treatment is strongly associated with better outcomes, other factors like age and NIHSS score before thrombolysis are also vital factors that affect the outcome. In detail, when the onset to thrombolysis time (OTT) is <3 h, IV alteplase administration is equally recommended for patients ≤80 and >80 years of age, and also it is recommended for patients with severe stroke and with mild but disabling stroke symptoms (1, 11). When OTT is within 3 and 4.5 h, those patients >80 years of age or with severe stroke symptoms (NIHSS > 25), the benefit of IV alteplase is uncertain or lower dose alteplase (0.6 mg/kg) can be given as an alternative (11). Therefore, there are still uncertain opinions on the dose of r-tPA in patients with different major characteristics (like age and NIHSS onset).

In this study, we observed 537 patients with r-tPA treatment in the year from 2014 to 2019 (data from the real world). There are patients given nonstandard dose r-tPA treatment and standard dose r-tPA treatment. We compared the effect of grouping on the outcome. Age subgroups and NIHSS subgroups were also analyzed to find the relationship between the drug coefficient and the outcome of r-tPA-treated AIS patients.

Abbreviations: r-tPA, Recombinant tissue plasminogen activator; 3m-mRS0-1, 3 months mRS 0-1; 24h-sICH, symptomatic intracranial hemorrhage within 24 h; NIHSS, National Institute of Health stroke scale; DNT, Door to needle time; MIOIF, the most important independent outcome-influential factor; AIS, acute ischemic stroke; sICH, symptomatic intracranial hemorrhage; mRS, modified Rankin scale scores; OTT, onset to thrombolysis time; SBP, systolic blood pressure; DBP, diastolic blood pressure; HTN, history of hypertension; DM, history of diabetes mellitus; CHD, history of coronary atherosclerotic heart disease; Af, history of atrial fibrillation; TIA, transient ischemic attack.

Materials and methods

This retrospective observational study protocol was carried out according to the recommendations of the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital and was registered in the Chinese Clinical Trial Registry (accession number: ChiCTR1900024521). All the subjects gave written informed consent according to the Declaration of Helsinki.

Participants and procedures

We have a database for patients who received r-tPA intravenous thrombolysis in our hospital since 2010, including the general situation of the patient, neurological function scores at various stages, laboratory examination results, imaging results, follow-up results, and so on. Our center is one of the 12 municipal stroke treatment centers, which participated in the quality-improvement project for stroke care throughout Shanghai (population of more than 20 million). There are about 100 cases of intravenous thrombolysis every year, about 30 cases can enter the green channel of stroke intervention every month, and about 33% of patients can receive intravenous thrombolytic therapy. About 50% of patients have a DNT time of <60 min, and 25% have a DNT time of <45 min. The incidence of sICH is about 4%. The proportion of patients with good prognosis (mRS score 0–1) is about 57%.

All patients treated with r-tPA met the standard of the scientific statement of the Chinese Stroke Society on intravenous thrombolysis in acute ischemic stroke (12). In our study, we enrolled 537 patients with complete baseline information and follow-up data (21 patients lost to follow-up within 3 months and 14 patients with incomplete key data) from the year 2014 to 2019. Two hundred and sixty-five patients accepted r-tPA with a dose of 0.9 mg/kg, while 272 patients received a lower dose (≥0.6 mg/kg and <0.9 mg/kg) for kinds of reasons (mentioned above). Our team recorded the features of patients, including gender, age, OTT, weightdose of drug, systolic blood pressure (SBP), diastolic blood pressure (DBP), admission glucose, baseline National Institute of Health stroke scale (NIHSS) score, history of hypertension (HTN), history of diabetes mellitus (DM), history of coronary atherosclerotic heart disease (CHD), history of atrial fibrillation (Af), previous stroke or transient ischemic attack (TIA), and followed up 24h sICH and 3-month mRS. All the recorded variables between the two groups were compared. The correlation between the outcomes and these variables (including grouping) was analyzed. Furthermore, patients were divided into different subgroups according to age (≥80 years or <80 years) and NIHSS score before thrombolysis (≥16 or <16). In each subgroup, we studied the correlations between the outcomes and variables.

Statistical analyses

The chi-square test and independent-sample t-test were used for the comparison of baseline data between the standard dose group and the non-standard dose group. In the dose grouping analyses, we also used the chi-square test for categorical variables and the independent-sample t-test for continuous variables, as single factor analysis. Multivariate logistic regression analysis was sequentially performed for further ascertaining the outcome-influential factors, after adjusting for potential confounders (group, age, history of atrial fibrillation to 3m-mRS0-1; group, age, DBP to 24h-sICH; group, history of coronary heart disease to 3m-death). In the age sub-groups and NIHSS sub-groups analyses, chi-square test was also used for studying the relationship between categorical variables to the outcomes, while Mann–Whitney Rank Sum Test was used for testing the continuous variables (not conformed to normal distribution), respectively. Multivariate logistic regression analysis was sequentially used for further ascertaining the outcome-influential factors, after adjusting for potential confounders. The dose coefficient was ascertained through multiple regression analysis, whether it has a significant effect on the outcome after univariate analysis or not.

Results

Comparison of baseline data between standard and non-standard dose groups

From 2014 to 2019, a total of 537 AIS patients received r-tPA. A total of 265 patients accepted the standard r-tPA dose of 0.9 mg/kg, while 272 patients received the non-standard dose (≥0.6 mg/kg and <0.9 mg/kg). The comparison of baseline data between the two groups is shown in Table 1. Gender, OTT, and body weight are significantly different between the two groups ($p = 0.002$, 0.016 and 0.001, respectively).

After equation stepping, the independent influencing factors of each outcome (3m-mRS0-1, 3m-death, and 24h-sICH) were further confirmed in Table 2. We found that OTT, DNT, SBP, baseline Glucose, and NIHSS score had an independent significant effect on 3m-mRS0-1 ($p = 0.007$, 0.016, 0.004, 0.018, and <0.001, respectively). Baseline Glucose, history of fibrillation, SBP, and NIHSS score were significantly related to 24h-sICH ($p = 0.01$, <0.001, 0.003, and <0.001, respectively). Age, DNT, SBP, history of fibrillation, and NIHSS score have significant effect on 3m-death ($p = 0.023$, 0.003, 0.013, 0.022, and <0.001, respectively). Besides these factors, group discrimination was not found to be an independent influencing factor of any outcome index (3m-mRS0-1, $p = 0.314$; 24h-sICH, $p = 0.109$; and 3m-death, $p = 0.196$, respectively).

Sub-group analyses according to baseline NIHSS score

To study the prognosis of acute ischemic stroke patients with different clinical characteristics using thrombolytic therapy more accurately, and to confirm the correlation between drug dose coefficient and prognosis more directly, we conducted sub-group analyses.

In the NIHSS score ≥16 sub-group, 92 patients' records were analyzed. Only DNT was confirmed to be the independent

TABLE 1 General information on non-standard group and standard group patients.

	All samples ($n = 537$)	Non-standard group ($n = 272$)	Standard group ($n = 265$)	χ^2/t value	P
Gender					
Male	366 (68.16)	202 (74.26)	164 (61.89)	9.48	0.002
Female	171 (31.84)	70 (25.74)	101 (38.11)		
Age (year)	65.39 ± 11.62	65.44 ± 11.93	65.33 ± 11.33	0.11	0.916
OTT (min)	176.33 ± 55.78	182.02 ± 56.85	170.48 ± 54.14	2.41	0.016
DNT (min)	74.94 ± 32.76	76.60 ± 34.12	73.24 ± 31.28	1.19	0.235
Weight (kg)	67.93 ± 11.08	69.53 ± 9.77	66.30 ± 12.09	3.40	0.001
SBP (mmHg)	149.35 ± 20.50	149.53 ± 20.70	149.18 ± 20.32	0.20	0.844
DBP (mmHg)	82.74 ± 12.79	83.17 ± 12.37	82.31 ± 13.21	0.77	0.439
Admission glucose (mmol/L)	7.66 ± 3.31	7.46 ± 3.14	7.87 ± 3.47	−1.44	0.150
History of hypertension (Yes)	341 (63.50)	182 (66.91)	159 (60.00)	2.77	0.096
Diabetes history (Yes)	111 (20.67)	54 (19.85)	57 (21.51)	0.23	0.636
History of coronary heart disease (Yes)	29 (5.40)	17 (6.25)	12 (4.53)	0.78	0.377
History of atrial fibrillation (Yes)	120 (22.35)	60 (22.06)	60 (22.64)	0.03	0.871
History of Stroke/TIA (Yes)	68 (12.66)	37 (13.60)	31 (11.70)	0.44	0.507
NIHSS-before thrombolysis	9.10 ± 6.57	9.03 ± 6.69	9.18 ± 6.47	−0.26	0.795
r-tPA Dose/kg	0.84 ± 0.08	0.79 ± 0.08	0.90 ± 0.01	−24.53	<0.001

OTT, onset to thrombolysis time; DNT, door to needle time; SBP, systolic blood pressure; DBP, diastolic blood pressure; NIHSS, National Institute of Health stroke scale.

influential factor to 3m-mRs0-1 ($p = 0.001$). History of atrial fibrillation and SBP were confirmed to be the independent influential factor to 24h-sICH ($p = 0.001$ and 0.023). The DNT, SBP, and NIHSS scores were confirmed to be the independent influential factor to 3m-death ($p = 0.049$, 0.009, and 0.003, respectively) (Figure 1).

In the NIHSS score <16 group, 445 patients were included. The OTT, SBP, and NIHSS score were confirmed to be the independent influential factors to 3m-mRs0-1 ($p = 0.020$, 0.009, and <0.001, respectively). Only the NIHSS score was confirmed to be the independent influential factor to 24h-sICH ($p = 0.001$). History of Af, age, NIHSS score, and dose coefficient was confirmed to be the independent influential factors to 3m-death ($p = 0.026$, 0.014, 0.034, and 0.005, respectively). The higher dose coefficient was related to the less possibility of 3m-death (Figure 2).

Sub-group analyses according to age

There are 51 patients in the age ≥80 years group. The NIHSS score was confirmed to be the independent influential factor to 3m-mRs0-1 and 3m-death ($p = 0.001$ and 0.023). The diabetes history was confirmed to be related to the 24h-sICH and 3m-death independently ($p = 0.012$ and 0.013) (Figure 3).

In the age<80 years sub-group, 486 patients' data were collected. The age, OTT, DNT, SBP, and NIHSS score were confirmed to be related to 3m-mRs0-1 independently ($p = 0.030$, 0.032, 0.009, 0.012, and <0.001, respectively). The history of AF, DBP, and NIHSS scores were the independent influential factors to 24h-sICH ($p = 0.001$, 0.001, and <0.001, respectively). Age, DNT, and NIHSS scores were significantly related to 3m-death ($p = 0.001$, 0.011, and <0.001, respectively) (Figure 4).

Discussion

The selection of r-tPA dosage of intravenous thrombolysis in AIS is still controversial. To resolve the dispute, many prospective studies (4–9, 13) preset drug dose coefficients, included patients according to the dose coefficient group, and compared the efficacy and safety of thrombolytic. But relative to the question of whether the dose of thrombolytic drugs should be standardized or individualized, maybe the real question the physicians are facing is: for individuals receiving thrombolytic therapy, should the given dose be the maximum safe dose that an individual can accept, or using dose titration to maximize the patients' benefits? Many physicians, when they treated AIS patients with thrombolytic therapy, still follow the "drug efficacy and safety" principle. Therefore, our study is retrospective and based on real clinical treatment activities. Physicians face many factors when deciding on a dose, based on things such as a patient's reaction or the cost of the treatment. Thus, the relationship between the dosage of r-tPA and the prognosis of patients in real AIS emergency aid is important.

TABLE 2 Independent influencing factors of each outcome in total patients.

Independent variables	β	S.E.	Wald χ^2	*p*-value	OR (95%CI)
3m-mRS0-1					
OTT (min)	−0.005	0.002	7.181	0.007	0.995 (0.991, 0.999)
DNT (min)	−0.008	0.003	5.814	0.016	0.992 (0.985, 0.998)
SBP (mmHg)	−0.015	0.005	8.278	0.004	0.985 (0.975, 0.995)
Admission glucose (mmol/L)	−0.074	0.031	5.580	0.018	0.928 (0.873, 0.987)
NIHSS-before thrombolysis	−0.198	0.020	100.174	<0.001	0.820 (0.789, 0.852)
24h-sICH					
History of atrial fibrillation (Yes)	1.692	0.449	14.225	<0.001	5.429 (2.254, 13.077)
SBP (mmHg)	0.038	0.013	8.771	0.003	1.038 (1.013, 1.064)
Admission glucose (mmol/L)	0.124	0.048	6.550	0.010	1.132 (1.029, 1.244)
NIHSS-before thrombolysis	0.131	0.032	16.431	<0.001	1.140 (1.070, 1.215)
3m-death					
History of atrial fibrillation (yes)	0.759	0.330	5.273	0.022	2.136 (1.118, 4.081)
Age (year)	0.036	0.016	5.160	0.023	1.036 (1.005, 1.069)
DNT (min)	0.013	0.004	8.615	0.003	1.013 (1.004, 1.022)
SBP (mmHg)	0.020	0.008	6.110	0.013	1.020 (1.004, 1.037)
Admission glucose (mmol/L)	0.078	0.040	3.752	0.053	1.081 (0.999, 1.169)
NIHSS-before thrombolysis	0.148	0.023	41.934	<0.001	1.160 (1.109, 1.213)

OTT, onset to thrombolysis time; DNT, door to needle time; SBP, systolic blood pressure; DBP, diastolic blood pressure; mRS, modified Rankin scale; sICH, symptomatic intracranial hemorrhage; NIHSS, National Institute of Health stroke scale.

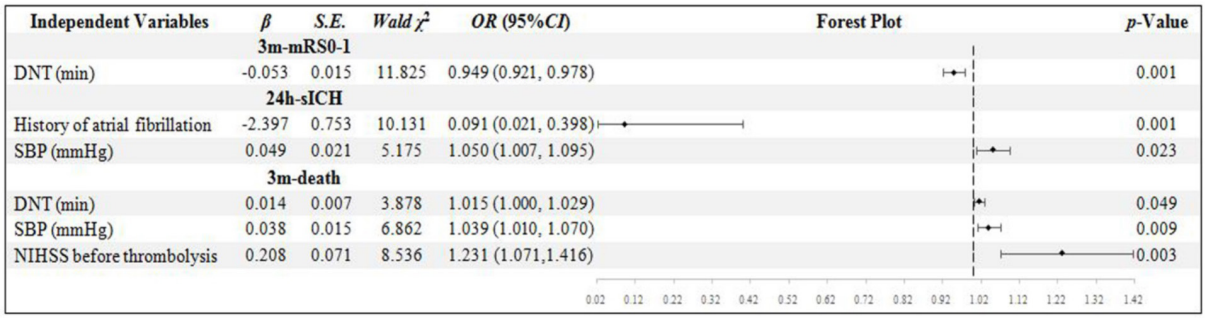

FIGURE 1
Binary logistic regression analysis in NIHSS score ≥ 16 group. DNT, door to needle time; SBP, systolic blood pressure; NIHSS, National Institute of Health Stroke Scale; mRS, modified Rankin scale; sICH, symptomatic intracranial hemorrhage.

In our study, we found that dose grouping was not an independently influential factor in the outcome of patients. The characteristics of the patients, especially the NIHSS score, admission glucose, and SBP still remained the most common independently influential factors to the prognosis, irrespective of the indicators, 3m-mRs0-1 or 24h-sICH and 3m-death. Other common influencing factors included the history of Af, DNT, OTT, diabetes history, and so on. The dose grouping had no significant effect on prognosis in our study.

Indeed, many trials have told us that the standard dose of thrombolytic drugs does not necessarily mean better efficacy, sometimes it does and sometimes it does not. However, in the same clinical trial, there are often significant differences in the comparison of efficacy and safety based on drug dose (9, 10, 14), and it is not consistent about the clinical efficacy and safety corresponding to the drug dose among trials. In our study, the dose coefficient had no independent and significant effect on outcomes, which required us to further refine the analysis. However, if we directly used a large set of data to analyze the correlation between drug dose coefficient and AIS outcome, we might lose the accurate description of the data. After all, no matter the guidance opinions or the clinical practice experience of physicians, the existence of such factors as old age (>80 years) or severe stroke itself would question whether thrombolysis

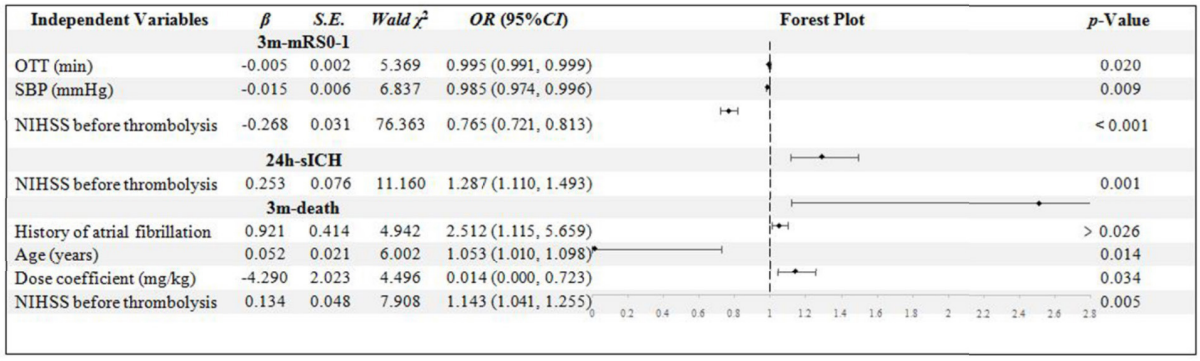

FIGURE 2
Binary logistic regression analysis in NIHSS score < 16 group. OTT, onset to thrombolysis time; SBP, systolic blood pressure; NIHSS, National Institute of Health stroke scale; mRS, modified Rankin scale; sICH, symptomatic intracranial hemorrhage.

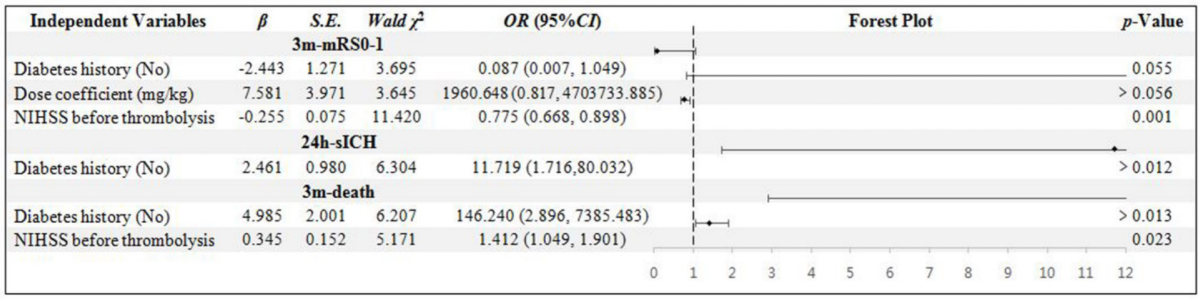

FIGURE 3
Binary logistic regression analysis in age ≥ 80 years group. NIHSS, National Institute of Health stroke scale; mRS, modified Rankin scale; sICH, symptomatic intracranial hemorrhage.

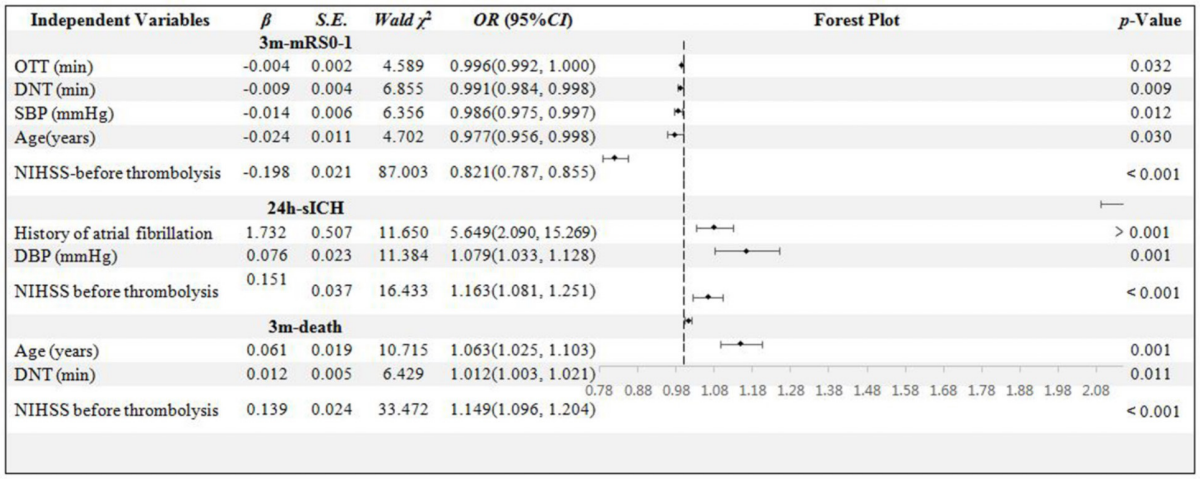

FIGURE 4
Binary logistic regression analysis in age < 80 years group. OTT, onset to thrombolysis time; DNT, door to needle time; SBP, systolic blood pressure; DBP, diastolic blood pressure; mRS, modified Rankin scale; sICH, symptomatic intracranial hemorrhage; NIHSS, National Institute of Health stroke scale.

should even be used (when DNT > 3 h), let alone the choice of drug dosage. This suggested that for AIS patients who are older than 80 years or younger, and whose NIHSS score is greater than or less than a certain critical value, we may need to consider differently about the dosage of r-tPA to reduce the risk of 24h-sICH or 3m-death.

The four subgroups in our study represented patients with four different characteristics. In NIHSS score ≥16 (severe patients) subgroup, MIOIF was DNT, which was correlated to 3m-mRS 0-1 and 3m-death. While in the subgroup of NIHSS score <16, NIHSS was the MIOIF, which was correlated to 3m-mRS 0-1, 24h-sICH, and 3m-death. Multivariate logistic regression analyses showed that the drug dose coefficient was negatively correlated with 3m-death. Combined with the analysis of these two subgroups, we found that in AIS patients with a relatively mild neurological deficit, the higher dose coefficient would reduce 3-month mortality. While, when the nerve function was seriously damaged to a certain extent, the primary factor determining the outcome is DNT. In other words, we should give a standard dose of r-tPA to mild disability AIS patients. But in severe AIS patients, we need to minimize the DNT and give r-tPA to patients as soon as possible.

We used to consider that patients over 80 years of age might have a higher risk of bleeding after thrombolysis, and a lower dose might reduce such risk (1, 11). In the age ≥80 years subgroup, we were surprised to find that the main factor affecting the outcome of thrombolysis is not DNT or dosage, but diabetes history and NIHSS score on set. The results of this subgroup analysis seemed to highlight the risk of thrombolytic therapy for elderly AIS patients with diabetes, both with the severity at the stroke onset. However, such risk factors do not seem to be highlighted in thrombolytic guidelines (1, 11). This may be related to the relatively small sample size (51 cases) in our study. In future, based on the increasing number of patients, more analysis is be needed. In the subgroup of age <80 years, the main factors influencing the outcome of thrombolytic therapy were DNT, age, NIHSS score, and also on.

Mainstream factors

It should be noticed that the dose coefficient tended to affect the 3-month mortality, which was also negatively correlated to criticality.

Anyway, the limited sample size from the single-center database is the limitation of our study. Furthermore, there is a possibility of bias due to the different number of cases between groups when grouping. We are looking forward to enlarging the sample size to further clarify whether the dose coefficient is related to outcomes in r-tPA-treated AIS patients. The issue of r-tPA dosage and side effects will continue to be debated in future clinical work, especially in the use of special populations. Our study might provide a research direction for the dose selection of intravenous thrombolytic therapy for elderly and severe AIS patients.

Conclusion

The non-standard dose group (0.6 mg/kg ≤ dose < 0.9 mg/kg) shows no difference in safety and effectiveness with the standard dose group (0.9 mg/kg) in our study, even in older (≥80 years) or sicker (NIHSS score ≥ 16) ones. While in patients with mild to moderate stroke, the dose reduction was significantly associated with 3-month death from our subgroup analysis. The standard dose should be considered first according to current evidence and AIS guidelines, but the non-standard dose (0.6 mg/kg ≤ dose < 0.9 mg/kg) might be an option in the actual diagnosis and treatment process considering the patient's clinical profile and financial condition.

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 Ethics Committee of Shanghai Sixth People's Hospital. 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

JY and FW were involved in the design of the study and wrote the manuscript. JX and JD collected all the data. FZ checked the database. XZ and KL evaluated the results. RW was involved in the statistical analysis. FC and YZ evaluated the results and revised the manuscript. All authors have approved the final version of 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.

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Predictive value of neutrophil to lymphocyte ratio for ischemic stroke in patients with atrial fibrillation: A meta-analysis

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Objective: Atrial fibrillation (AF) is an important risk factor for stroke, but the currently used CHA2DS2-VASc score has significant limitations in predicting the risk of stroke. It is important to find new biomarkers to predict stroke risk in patients with AF or as a complement to the CHA2DS2-VASc score. Neutrophil-to-lymphocyte ratio (NLR) may be of potential value. This systematic review and meta-analysis evaluated the association between NLR and stroke risk.

Methods: We searched in electronic databases such as PubMed and EMBASE. The final included studies were analyzed by Stata 12.0 software. Subgroup analyses were used to explore sources of heterogeneity. Publication bias was assessed by Egger's test and Begg's test. Sensitivity analyses assessed the stability of outcomes.

Results: A total of 11 studies with a total of 35,221 patients were included. NLR levels are associated with stroke risk in patients with atrial fibrillation (WMD = 0.72, 95%CI = 0.43–1.01). There was a correlation between the occurrence of stroke and NLR level in AF patients (WMD = 1.96, 95%CI = 1.38–2.53). The incidence of stroke was significantly higher in patients with atrial fibrillation with NLR ≥3 than in those with NLR<3 (RR = 1.4, 95%CI = 1.24–1.58).

Conclusion: This study shows that high NLR values are associated with a higher risk of stroke in AF patients. The incidence of stroke in AF patients with NLR ≥3 was 1.4 times higher than that with NLR <3 ($p < 0.001$). NLR may be considered as a complementary risk assessment for CHA2DS2-VASc score, especially for AF patients with CHA2DS2-VASc score <2. NLR may be a potential biomarker for predicting stroke risk in patients with AF.

KEYWORDS

neutrophil to lymphocyte ratio, ischemic stroke, atrial fibrillation, meta-analysis, review

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia and is associated with an increased risk of ischemic stroke. In fact, the actual incidence of AF may be higher than reported because a relatively high proportion of AF is subclinical and undiagnosed (1). Even though anticoagulants are currently effective interventions, 22–36% of patients with AF experience ischemic stroke (2, 3). Clinically, the CHA2DS2-VASc score is commonly used to assess stroke risk in patients with AF. The study showed that the sensitivity of CHA2DS2-VASc $\geq$1 and $\geq$2 are both 100%, but the specificity is only 7 and 14%, respectively (4). In addition, a meta-analysis of patients with atrial fibrillation with a mean follow-up of 2.2 years found that ischemic stroke occurred in 2.3% of patients with direct oral anticoagulants (5). Of these patients, one-third had a CHA2DS2-VASc score of 0–1. This suggests a limitation of CHA2DS2-VASc for low-score patients. Therefore, there is a clinical need for new markers that are easy to measure and reliable to improve the assessment of stroke risk after AF or as a complement to the CHA2DS2-VASc score.

The ratio of neutrophils to lymphocytes (NLR) has been a research hotspot in recent years. Because inflammation is an important pathological process in stroke, which is directly related to the prognosis of patients, and NLR is one of the important reference indicators of inflammation (6). NLR is also an important predictor of AF prognosis (7). It has been reported that NLR can be used to assess stroke risk and prognosis in AF patients (8–10), but there are also studies that suggest that NLR is not an independent biomarker for predicting stroke (11). Therefore, the ability of NLR to independently assess or predict stroke risk in AF patients remains unclear. The aim of this systematic review and meta-analysis was to elucidate the association of NLR with stroke risk in patients with AF and its value as a complementary tool to the CHA2DS2-VASc score in assessing stroke risk after AF.

Methods

Study selection

Studies were included if they met the following criteria: (1) Study subjects were patients with AF; (2) Articles include randomized controlled trials (RCTs), cohort, case-control studies and cross-sectional studies; (3) involving human subjects; (4) The data tested included NLR; (5) There was a history of ischemic stroke or stroke risk assessment; (6) Written in English or translated into English, as well as Chinese.

Exclusion was non-peer-reviewed manuscripts, conference abstracts, and gray literature.

Search strategy

The retrieval period is from the date of establishment of the database to June 6, 2022. Databases searched include: PubMed, Cochrane, EMBASE and major Chinese databases (CAJD, CBMdisc, CDFD, CMFD). Searches include "neutrophil to lymphocyte ratio," "neutrophil to lymphocyte ratio," "neutrophil/lymphocyte ratio," "NLR," "lymphocytes," "atrial fibrillation," "AF," "cerebral infarction" or "stroke," the search strategy is a combination of the above keywords. In addition, omissions were prevented by reviewing the references of the included studies and the included literature of the relevant meta-analyses.

Data extraction

Two investigators (ZYY, LR) independently screened articles and resolved disagreements with the intervention of a third investigator (HBN). Two authors (HBN, LM) independently extracted data on general study information (author, year of publication, country), baseline demographics, and clinical characteristics (age, sex, NLR, stroke history, stroke risk score). A third author (HXM) verified all extracted data. Any disagreements were resolved by the three authors through face-to-face discussions.

Quality assessment

AS the inclusions were case-control studies, three researchers (LM, ZYY, and LR) independently assessed study quality through the Newcastle-Ottawa scale. Overall research quality scores range from low to high on a scale of 0 to 9.

Statistical analysis

Statistical analysis was performed using Stata 11.0 software. The pooled effect for continuous data is the weighted mean difference (WMD), and the pooled effect for dichotomous data is the hazard ratio with a 95% confidence interval. Depending on heterogeneity, a random-effects or fixed-effects model was chosen. In random-effects models, $I^2 \geq 50\%$ or $p \leq 0.10$ indicated significant heterogeneity, and we performed subgroup analyses to identify possible sources. The possibility of publication bias was assessed using the Begg and Egger test.

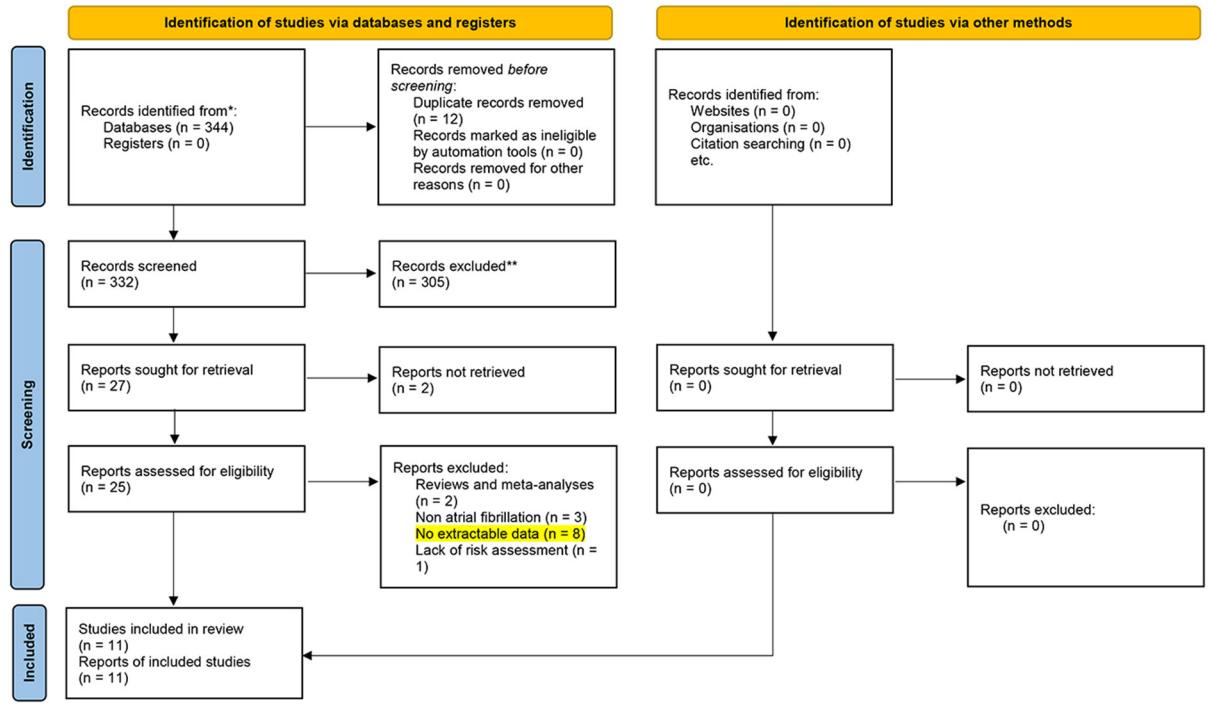

FIGURE 1
The study screening flowchart. *Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers). **If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

Results

Characteristics of included studies

Three hundred and forty four articles were identified from databases and manual searches. Twelve duplicate studies were initially removed, followed by title and abstract screening to exclude 305 studies, followed by full-text evaluation of the screened 27 articles. Ultimately, 11 studies were included for analysis. Figure 1 details the study screening flowchart and the reasons for excluding articles. The 11 articles included 10 case-control studies and one cohort study (7, 12–21). They originated from the three countries: Turkey ($n = 3$), China ($n = 6$), Israel ($n = 2$). In total, there were 35,121 patients with AF, of which 33,853 patients were included in the risk-related analysis because of the stroke risk grouping (941 patients were included to analyze the association of NLR levels with AF risk, and the remaining 32,912 patients were included to analyze the risk of stroke with NLR ≥3 or <3.), and the remaining 1,268 patients were included in the stroke-related analysis because of the stroke outcome grouping.

All observational studies achieved a moderate or high quality score of between 6 and 7 on the Newcastle-Ottawa Quality Assessment Form. Key study characteristics, quality assessments, patient demographics, and clinical variables of the patient population are detailed in Table 1.

NLR levels in different risk groups for AF

Among these 11 papers, six analyzed the correlation between different stroke risks and NLR levels in patients with AF, of which four used CHA2DS2-Vasc score, one used transesophageal echocardiography (TEE), and one used time in therapeutic range (TTR). One of these analyses the association between different stroke risks and different stratified NLR levels in patients with AF. The remaining five articles analyzed the association with NLR levels according to whether or not stroke occurred in AF patients.

There were 941 AF patient, including 438 in the high-risk group for stroke and 503 in the low-risk group for stroke. In pooled analysis, NLR levels were significantly higher in patients with AF at higher stroke risk than in patients with AF at low stroke risk, with a WMD of 0.72 using a random-effects model (95% confidence interval: 0.43 to 1.01; $p < 0.001$, Figure 2), with a significant heterogeneity ($I^2 = 69.7\%$; $p = 0.006$). One of these studies included the NLR of normal people without AF for comparison, and we aggregated the results of this part

TABLE 1 Study details, baseline demographic, and clinical characteristics.

Author	Year	Country	Study design	No. of patients	Male gender (%)	Risk or outcome indicators	NOS*
Zhao Yue et al. (12)	2018	China	Case-control studies	194	83 (43%)	CHA2DS2-VASc score	7
Murat Yalcin et al. (14)	2013	Turkey	Case-control studies	309	145 (47%)	TEE#	6
Sun yulei et al. (15)	2020	China	Case-control studies	100	53 (53%)	CHA2DS2-VASc score	6
Olga Perelshtein Brezinov et al. (13)	2021	Israel	Case-control studies	67	32(48%)	CHA2DS2-VASc score	6
Kahraman Cosansu et al. (7)	2017	Turkey	Case-control studies	271	117(43%)	TTR##	7dx
Gökhan Ertaş et al. (17)	2013	Turkey	Case-control studies	126	80 (63%)	Stroke	6
Gu Xiangting et al. (18)	2019	China	Case-control studies	190	90 (47%)	Stroke	6
Hanikzi Maimaitiyiming et al. (19)	2021	China	Case-control studies	199	114(57%)	Stroke	6
Yang Lei el al. (20)	2020	China	Case-control studies	447	253(57%)	Stroke	6
Rui el al. (21)	2015	China	Case-control studies	306	160(52%)	Stroke	6
Saliba et al. (16)	2015	Israel	Cohort study	32 912	15,932(48%)	CHA2DS2-Vasc/stroke	7

NOS*, the Newcastle-Ottawa Scale; TEE#, transesophageal echocardiography; TTR##, time in therapeutic range.

of the data to show that the outcome indicators were basically unchanged, with a WMD of 0.64 using a random-effects model (95% confidence interval: 0.36 to 0.92), with a significant heterogeneity ($I^2 = 78.3\%$; $p < 0.001$, Supplementary Figure 1).

Further subgroup analysis showed that the heterogeneity was mainly due to different risk assessment methods. After excluding TEE and TTR, the results of the CHA2DS2-VASc score subgroup showed that the WMD obtained by using the random effect model was 0.64 (95% confidence interval: 0.48 to 0.80), with 0% heterogeneity between studies.

NLR levels in patients with AF with or without stroke

A total of five studies with 1,268 patients included 515 strokes and 753 no strokes. After a combined analysis, the NLR levels in patients with AF who had had a stroke were significantly higher than those who had not experienced a stroke, with a WMD of 1.96 using a random-effects model (95% confidence interval: 1.38 to 2.53; $p < 0.001$, Figure 3), with a significant heterogeneity ($I^2 = 54.7\%$; $p = 0.066$).

Association of NLR levels with stroke incidence in patients with AF

A multi-center large sample study in Israel, the study population was divided into eight groups according to the CHA2DS2-Vasc score from 0 to 7, and each group was divided into two categories according to the NLR level ≥3 or <3. The incidence of stroke in the two kinds of NLR levels was calculated separately for each group. Our pooled analysis of stroke incidence in these eight groups by NLR level showed a 140% increased risk of stroke with an NLR≥ 3 (RR = 1.4, 95% confidence interval: 1.24 to 1.58, with 0% heterogeneity, Figure 4).

Publication bias

There was no obvious publication bias found by Begg's Test and Egger's Test (Table 2), this suggests the absence of publication bias in the association of stroke risk with NLR levels and the association of stroke outcomes with NLR levels.

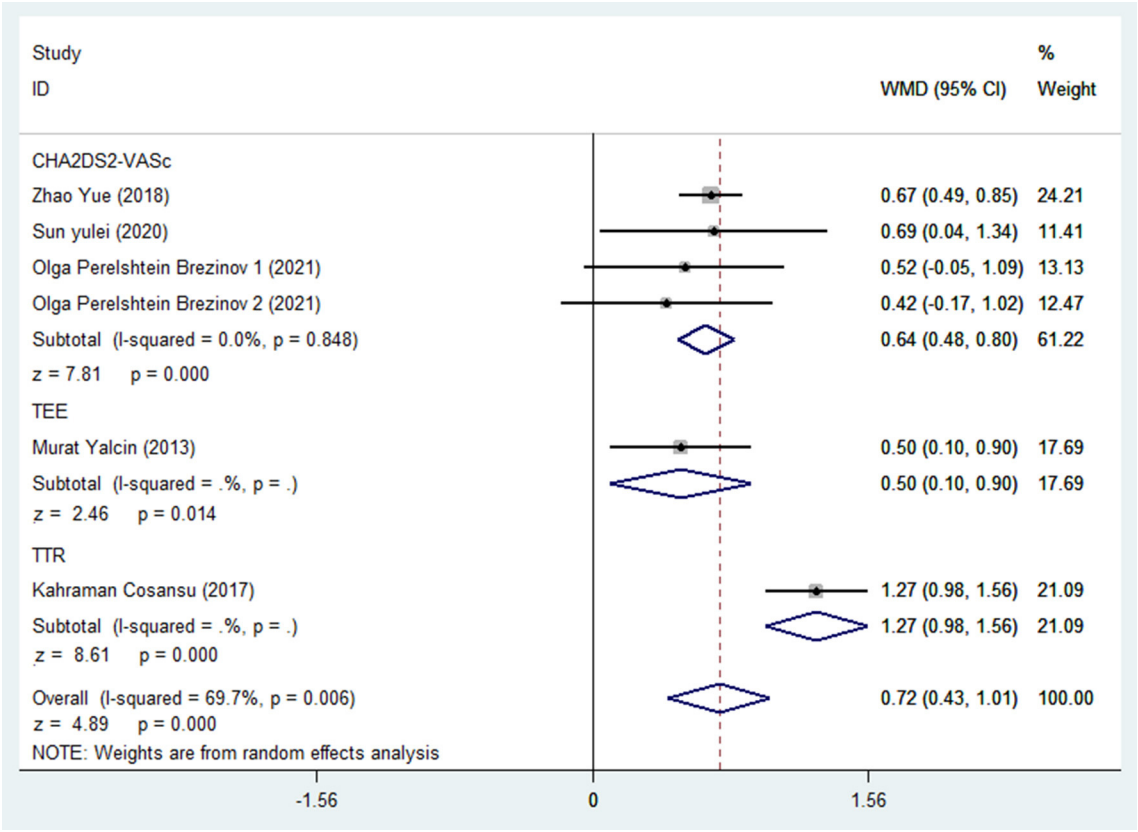

FIGURE 2
Forest plot of WMD for association between different stroke risks and NLR levels in patients with AF.

Sensitivity analysis

On leave-one-out analysis, the association of stroke risk with NLR levels and the results of the association of stroke outcomes with NLR levels did not change substantially. Leave-one-out analysis results are shown in Supplementary Figures 2, 3. There was no material change in magnitude for the results of replacing the effect model of the stroke incidence and NLR level (Table 3, Supplementary Figures 4, 5).

Discussion

Patients with atrial fibrillation are at high risk of stroke and current clinical practice guidelines continue to recommend the use of the CHA2DS2-VASc score for stroke risk stratification in atrial fibrillation to identify anticoagulant candidates (22). However, actual clinical work and relevant clinical evidence have shown the limitations of the predictive value of CHA2DS2-VASc (23–27). The addition of new predictors could help improve the current approach to risk stratification.

Recently, NLR has emerged as a new potential predictor of thrombotic events that can be obtained directly from blood counts. Increased NLR levels have been reported to be associated with atherosclerotic events and as a prognostic predictor of ischemic stroke (28). High NLR levels are associated with stroke severity, adverse functional outcomes, and recurrence of ischemic events in stroke patients (29, 30). Therefore, we sought to investigate the value of NLR levels as a stratified assessment of stroke risk in patients with AF, and as a complement to the CHA2DS2-VASc score.

Through meta-analysis, we found that NLR levels were significantly higher in high-risk AF patients than in low-risk AF patients. Subgroup analysis revealed that heterogeneity stemmed from different approaches to risk assessment. The most significant difference in NLR level was in AF patients with oral vitamin K antagonists, but the INR did not reach the target, followed by B-ultrasonography to confirm the presence of atrial mural thrombus. This may be related to the adaptability of different risk assessment methods, with the CHA2DS2-VASc score being suitable for almost all patients with AF, while "INR" and "transesophageal ultrasonography" are only suitable for some AF patients (Transesophageal ultrasonography

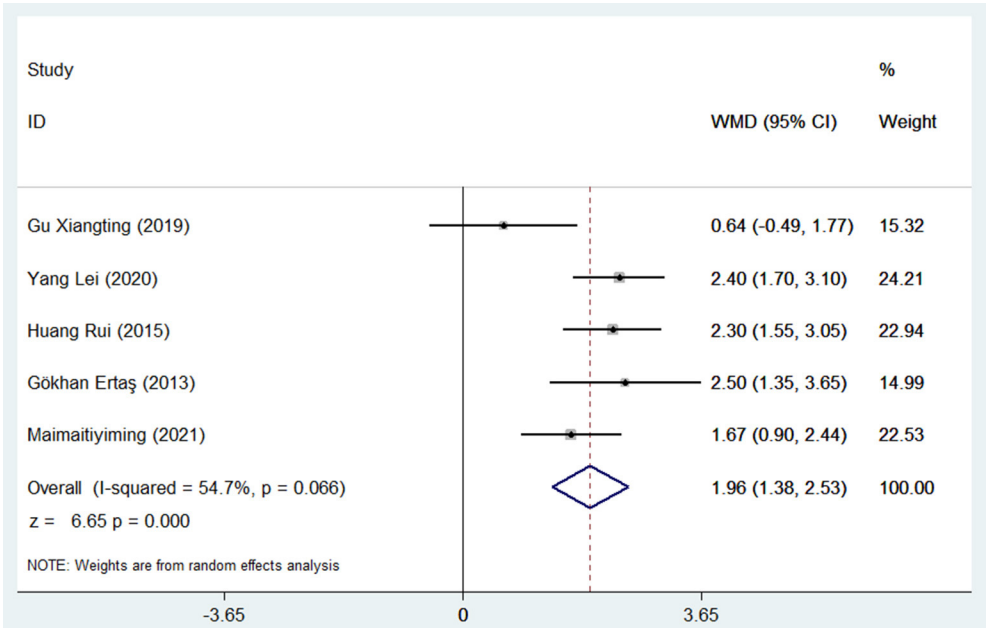

FIGURE 3
Forest plot meta-analysis of associations between different stroke outcomes and NLR levels in patients with AF.

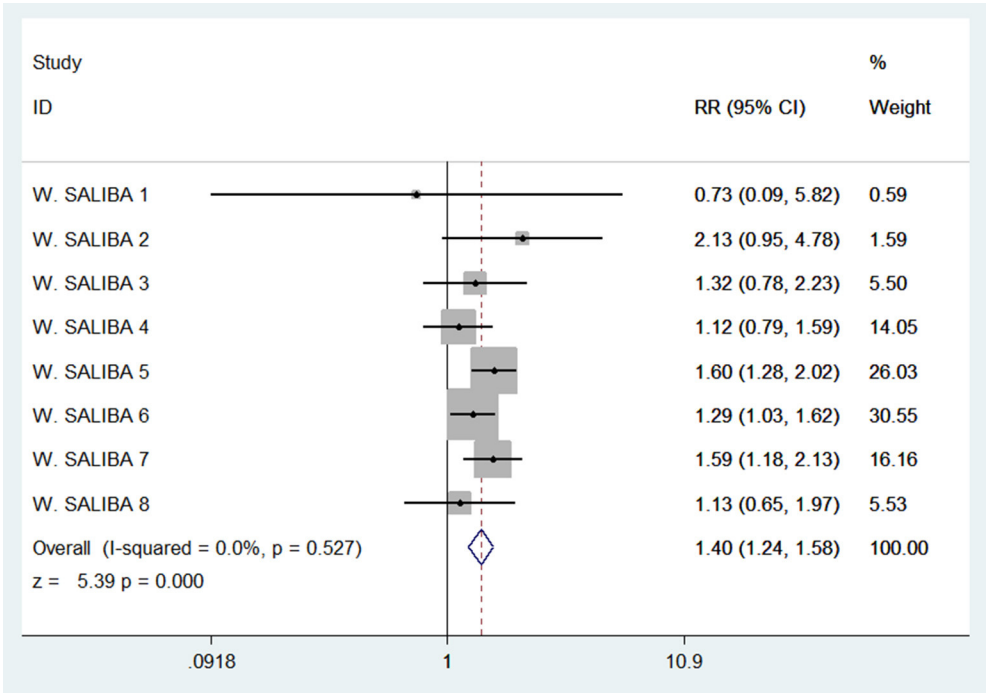

FIGURE 4
Forest plot of associations between stroke incidence and NLR levels in patients with AF.

to determine if there is a thrombus). It has been reported that NLR has become an important prognostic indicator of cardiovascular disease (31). In patients with AF, elevated NLR was independently associated not only with increased risk of new-onset AF after surgery and recurrence of AF after ablation, but also with the presence of LA thrombus and increased risk

TABLE 2 Result of publication bias.

	Begg's test	Egger's test
Stroke risks and NLR* levels	1.0	0.575
Stroke outcome and NLR levels	0.221	0.334

NLR*, neutrophil-to-lymphocyte ratio.

TABLE 3 Sensitivity analysis.

	95% CI	z value	*p*-value
OR	1.42 (1.25–1.62)	5.39	0.000
RD	0.01 (0.00–0.01)	5.12	0.000

of stroke in patients with AF (14, 16, 32). This may be the reason why inflammation is closely related to the pathological mechanism of AF and the mechanism of mural thrombosis. In addition, we attempted to include the normal population in the low stroke risk group for comparison. The NLR level in the high stroke risk group was still significantly higher than that in the low risk group, and the results were stable, with no significant changes in WMD values. Therefore, NLR may serve as a potential predictor of stroke risk in patients with AF.

Current guidelines classify AF into subtypes by pattern, paroxysmal AF (PAF), persistent AF, long- standing persistent AF, and permanent AF (33). Studies suggest that different subtypes of AF have different levels of stroke risk (34, 35). Despite the CHA2DS2-VASc score has high sensitivity for stroke risk, its specificity is low, with a C-statistic of 0.67 for predicting thromboembolic outcome (36). Based on the above, we searched and screened the literature and performed a systematic evaluation to analyze the correlation between different subtypes of AF and NLR levels (Supplementary Figure 6 and Supplementary Table 1). The results revealed that patients with AF had significantly higher NLR levels than those without AF, with a WMD value of 0.57 (95% CI = 0.37–0.78, $p < 0.001$). In the subgroup analysis, the NLR levels of patients with non-paroxysmal AF were not statistically different from those of the AF-free population, with a WMD value of 0.19 (95% CI = −0.16–0.53, $p = 0.295$), unlike the NLR levels of patients with paroxysmal AF, which were significantly higher than those of the AF-free population, with a WMD value of 0.76 (95% CI = 0.42–1.11, $p < 0.001$). We suggest that NLR levels may be more clinically relevant for patients with PAF as a complementary stratification marker for AF stroke risk stratification or CHA2DS2-VASc score.

Further analysis of NLR levels in patients with AF who had a stroke versus those who did not, we found higher levels of NLR in AF patients who had a stroke. This suggests that high NLR levels are strongly associated with stroke in patients with AF. On a case-by-case basis, the heterogeneity was mainly derived from one study, and the possible cause of the heterogeneity could not ultimately be determined because the original data were not available. After the study was excluded, the results remained stable. NLR levels are closely associated with stroke in AF patients.

We performed a meta-analysis of data from a large multicenter Israeli study that divided patients with AF into two groups by NLR levels of ≥3 and <3 and found that the risk of stroke was 1.4 times higher in patients with AF with NLR ≥3 than with NLR <3. In this study, among the AF population with NLR ≥3, 19,726 (83.2%) had a CHA2DS2-VASc score ≥2, and 3,986 (16.8%) had a score <2. Among the AF population with NLR <3, 8502 (92.4%) had a CHA2DS2-VASc score ≥2, and 698 (7.6%) had a score <2. The study conclusively showed that NLR is directly associated with stroke risk in AF, and together with CHA2DS2-VASc score, it can also help to stratify stroke risk in patients with low CHA2DS2-VASc scores, allowing patients to receive timely treatment and reduce stroke risk.

Inflammation has been found to be an important pathological mechanism involved in the occurrence, development and prognosis of stroke. Systemic inflammatory response index (SIRI) can be used as a predictor of futile endovascular reperfusion, and higher SIRI levels are associated with an increased risk of adverse outcomes in stroke patients at 3 months after vascular recanalization (37). In stroke patients receiving intravascular therapy, higher NLR values indicate a higher risk of early neurological deterioration, and can also predict the risk of symptomatic hemorrhagic transformation after vascular recanalization (38, 39). The possible mechanism is that a higher level of NLR is closely related to platelet activation and thrombosis. On the one hand, neutrophils participate in the formation of atherosclerosis by secreting various inflammatory mediators, and aggravate endothelial cell dysfunction (40, 41), which may be related to neutrophils increasing the release of proteolytic enzymes or free oxygen free radicals and other inflammatory mediators (42). On the other hand, higher levels of lymphocytes can upregulate the anti-inflammatory cytokine interleukin (IL)-10 and inhibit inflammatory cytokines including tumor necrosis factor-α and IL-6, thereby exerting anti-inflammatory effects (43). In addition, it has been experimentally demonstrated that regulatory T cells and B cells have regulatory functions to reduce ischemic tissue volume and improve neurological deficits during ischemic stroke (44). NLR is the ratio between cells that mediate two different immune pathways (45). In conclusion, NLR is a potential predictive biomarker of stroke risk in AF patients and may serve as an important complementary risk stratifier to the CHA2DS2-VASc score.

This study also has some limitations. Firstly, NLR reflects the overall inflammatory status of the body. If the patient has mild urinary tract infection or chronic pulmonary interstitial disease without obvious symptoms and signs, the reference value

of NLR may be affected. Therefore, establishing a reference standard for NLR levels of chronic inflammation, excluding the interference of other inflammations, and correcting the NLR value may further improve the value of NLR for stroke risk stratification. Secondly, due to the difficulty in obtaining the original data, this study lacks a separate analysis of patients with CHA2DS2-VASc score <2. Therefore, more relevant research is needed to further update the future research and increase the persuasiveness of NLR as a stroke risk assessment for patients with low CHA2DS2-VASc score. Thirdly, the RR value of this meta-analysis is small, which needs to be treated with caution by clinicians. It is recommended to use NLR on the basis of CHA2DS2-VASc score, and it needs to be evaluated in combination with the patient's own situation. Finally, as described in a previous meta-analysis on NLR, the cutoff values for NLR was not determined (46). The NLR cutoff values of each article in the studies on the relationship between stroke risk and NLR level in patients with AF included in this meta-analysis was different. Different NLR cutoff values may affect the accuracy of the analysis. Therefore, more relevant studies are needed to establish standard NLR cutoff values.

Conclusion

In summary, our study suggests that high NLR values are associated with a high risk of stroke in AF patients. The incidence of stroke in AF patients with NLR ≥3 was 1.4 times higher than that with NLR <3. NLR can be used as a supplemental risk assessment for CHA2DS2-VASc score, especially for AF patients with CHA2DS2-VASc score <2. In the future, more relevant studies are needed to establish the correction formula of NLR and the standard cut-off value of NLR, which can be better applied to clinical prediction of stroke risk in AF.

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.

Author contributions

BH and ML conceived and wrote the manuscript together. RL, YZ, and XH performed literature search, screening, and data collection. All authors contributed to the article and approved the submitted version.

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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.2022.1029010/full#supplementary-material

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Decompressive craniectomy: Comparative analysis between surgical time and better prognosis

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Background: Malignant ischemic stroke is characterized by the involvement of 2/3 of the area of the middle cerebral artery, associated with cerebral edema, intracranial hypertension (ICH) and cerebral herniation, generating high morbidity and mortality. Over the years, several therapies have been studied in an attempt to reverse or reduce the damage caused by this vascular disorder, including decompressive craniectomy (DC), a surgical technique reserved for cases that evolve with refractory ICH.

Methods: This study seeks to perform a comparative analysis on the effectiveness of decompressive craniectomy using four randomized clinical trials and the results found in the retrospective study conducted in a neurosurgical reference center between 2010 and 2018.

Results: The total sample consisted of 263 patients, among which 118 were randomized and 145 were part of the retrospective study. The outcome was analyzed based on the modified Rankin Scale (mRS) for 6 and 12 months. The mean time to perform the DC was 28.4 h in the randomized trials, with the late approach (> 24 h) associated with unfavorable outcomes (mRS between 4 and 6).

Conclusion: Compared to the aforementioned studies, the study by Bem Junior et al. shows that a surgical approach in < 12 h had a better outcome, with 70% of the patients treated early classified as mRS 2 and 3 at the end of 12 months (1). Decompressive craniectomy is currently the most effective measure to control refractory ICH in cases of malignant ischemic stroke, and the most appropriate approach before surgery is essential for a better prognosis for patients.

KEYWORDS

decompressive craniectomy, hemicraniectomy, malignant ischemic infarction, stroke, ischemic stroke

Introduction

The WHO defines ischemic stroke as the "sudden onset of deficient neurological symptoms" attributable to a brain disorder caused by a circulatory disorder lasting longer than 24 h (1). When the ischemic event is associated with brain edema and refractory intracranial hypertension, affecting 2/3 of the middle cerebral artery area, the cerebrovascular accident (CVA) is characterized as malign ischemic stroke and the prognosis is usually poor despite maximal intensive care treatment (2, 3). Several medical therapies have been proposed to reduce development of brain edema and intracranial pressure, such as hyperventilation and osmotic therapy (4–6). Although, several reports suggest that these therapies may be ineffective and lack evidence of efficacy (4, 5).

As the only therapy, surgical decompression has been proposed for patients with space-occupying hemispheric infarction, seeking to relieve the high intracranial pressure (2, 7–9). Therefore, the therapy seeks to create a compensatory space to accommodate the brain and normalize intracranial pressure, reverting brain tissue shifts (2). Randomized double-blind studies describe their results, with an emphasis on the effectiveness of the decompression procedure, but the functional outcome is questionable. In this study, the authors compare the results of retrospective studies carried out in Brazil, and four randomized clinical trials, in search for which is the best surgical time to proceed to DC and this implication in patients outcomes, based on the modified Rankin Scale (mRS).

Methods

This is a comparative analysis of results obtained from a retrospective study of patients undergoing decompressive craniectomy to control intracranial hypertension secondary to malignant ischemic stroke, developed at Hospital da Restauração (HR), between March 2010 and March 2018 by Bem Junior et al. and secondary data was taken from four randomized clinical trials, published on the European continent between 2006 and 2011 (10). The main objective of this research is to compare the results of Bem Junior et al. (10) and four randomized clinical trials since these trials suggest that the best time to perform decompressive craniectomy is between 12 and 24 h, and the results from previous studies indicate that the earlier approach can achieve more favorable outcomes (10).

Retrospective study

Data from the study by Bem Junior et al. was collected from the medical records of the Neurosurgery service of Hospital da Restauração (HR), through a semi-structured collection instrument, which evaluated the following variables: age, sex, comorbidities, time from onset of symptoms to arrival at the hospital, time from symptom onset to neurosurgical procedure, laterality of the ischemic event, preoperative Glasgow Coma Scale (GCS), surgery time, intraoperative complications, postoperative complications, length of hospital stay and clinical/functional status after the procedure, evaluated by the modified Rankin Scale and Glasgow Outcome Scale (10).

The results obtained in the first study were descriptively analyzed using absolute and percentage frequencies, by way of Pearson's chi-square test or Fisher's exact test. The strength of association was analyzed based on the Odds Ratio (OD) and the Confidence Interval (CI). The level of significance in the decision of the statistical tests was 5%, with a confidence interval of 95%. The data was computed using an Excel spreadsheet and the program used to obtain the statistical calculations was IMB SPSS version 25.

Secondary data

The secondary data used in this comparative analysis was taken from four randomized, double-blind clinical trials, and the choice of these trials was guided by the level of the scientific relevance of the articles, as well as by the number of citations of them in the main studies on the subject. The four selected studies were: DESTINY published in 2007, DECIMAL published in 2007, HAMLET published in 2009, DESTINY II published in 2011 (4, 5, 7, 11).

Comparative analysis

The analysis and comparison performed in this article were based on the interpretation of data available in the four clinical trials and the retrospective study developed by the group of Bem Junior et al. (10). The variables analyzed and compared were: the age of patients, time for indication and performance of decompressive craniectomy and clinical outcome, using the modified Ranking Scale (mRS) for the latter.

Results

This comparative analysis was performed with a sample of 263 patients, among which 118 were randomized and 145 were part of the retrospective study.

The clinical characteristics of the 145 patients in the retrospective study are detailed in Table 1. The majority (60%) of the cases were aged between 40 and 64 years, followed by 24.8% who were 65 years of age or older. All cases had an admission National Institutes of Health Stroke Scale (NIHSS) above 15. The time to perform decompressive craniectomy, from the moment of arrival to admission to the operating room, was above 24 h

TABLE 1 Clinical variables of group studied by Bem Junior et al. (10).

Variable	*n* (%)
Total studied	145
Age (years)	
Up to 17	6 (4.1)
18–39	16 (11.0)
40–64	87 (60.0)
65 or more	36 (24.8)
Associated clinical conditions	
Yes	130 (89.7)
No	15 (10.3)
Surgical hours (h)	
<12	26 (17.9)
12 a 24	20 (13.8)
> 24	99 (68.3)

in 68.3%, between 12 and 24 h in 13.8% and < 12 h in 17.9% of the samples.

The outcome was analyzed based on the modified Rankin Scale for 6 and 12 months and is detailed in Tables 2, 3, according to the clinical data considered for this group of patients. During this study, 28 patients died early and weren't included in the outcomes analysis at 6 and 12 months respectively.

The main characteristics of the sample of patients undergoing DC in the randomized studies included in this analysis are shown in Table 4. Only the study by Jüttler et al. (4) exclusively evaluated patients over 61 years of age, with the mean age of cases in the DECIMAL (7), DESTINY (5) and HAMLET (11) studies being 45.5 years.

The severity of the ischemic event in these patients assessed by the NIHSS had a mean number of 21.6. The average time to perform the DC in this sample was 28.4 h. The clinical outcome, analyzed based on mRS for 6 and 12 months is specified in Table 5.

Discussion

Decompressive craniectomy, as a treatment, is an alternative that contributes to positive outcomes in patients who suffer strokes in the 2/3 of the middle cerebral artery territory. The technique used in all cases of the previous study for performing the decompressive craniectomy was a front-temporoparietal hemicraniectomy (12–15 cm) with middle fossa decompression and dural opening.

The assessment of the best surgical time for the procedure is essential for a better outcome. Although it does not reverse the stroke effects and the neuronal loss, the decompressive craniectomy reduces persistent loss in territories beyond the middle cerebral artery or even contralateral by reducing intracranial hypertension and cerebral herniation. The craniectomy approach reported by the four clinical trials took an average of 28.4 h. Analysis using the Rankin Scale or mRS shows that, despite the craniectomy, the relatively late approach (over 24 h) was associated with unfavorable outcomes (mRS between 4 and 6).

The trial by Jüttler et al. had a mean time of 28 h to perform a craniectomy after the onset of symptoms, and demonstrated severe disability (mRS 4–5) and death (mRS 6) in 60 and 33% of cases, respectively, within 6 months (4). In the 12-month follow-up, the prognosis was better, but there were still a high number of patients with severe disabilities (51%) or that died (43%). The study by Hofmeijer et al. (11) in which craniectomy was indicated even later (41 h on average), despite having reported a considerably lower death rate (22%) compared to the study by Jüttler et al. still showed severe patient dysfunction (mRS 4–5) in 53% of cases after 12 months (4, 11). In the study by Jüttler et al. the surgical approach, performed between 24 and 25 h, on average, contributed to a better prognosis, since mortality (mRS 6) remained low and constant (18%), in the periods of 6 and 12 months (5). The trial by Vahedi et al. had, on average, a surgical time of around 20–21 h (7). The approach in a shorter time was essential for a good prognosis, as 75% of the patients survived without severe disability (mRs ≤ 4).

Comparatively, as indicated by data from the retrospective study by Bem Junior (2021) the surgical approach with a time between 12 and 24 h was essential for a more favorable outcome (10). 17% of patients have operated within 12 h of symptom onset and >70% of patients had a relatively favorable outcome (mRS 2 and 3) at 12 months. The performance of a decompressive craniectomy between 12 and 24 h, however, seems to be ideal, according to the data of this study. Of all 20 patients treated in this interval, 95% had a favorable outcome (mRS 2 and 3) 6 months after treatment.

Patients who underwent surgery 24 h after the onset of symptoms had a worse outcome than those who underwent surgery between 12 and 24 h. Of the 75 patients in the first group, 30.7% suffered a moderate to severe disability (mRS 4–5), reinforcing the thesis that performing craniectomy between 12 and 24 h or in < 12 h has a better outcome.

Regarding age, performing decompressive craniectomy in patients over 60 years of age is associated with a worse outcome, as shown by data from the clinical trial by Jüttler et al. (4) who only used older patients in their study. Despite this, data from the four clinical trials show that patients of advanced age may have some benefits from the treatment. In the four studies, the mean age of the participants was 52 years, and mortality ranged between 18 and 25%, except for the DESTINY II study, in which the mean age was 70 years and mortality reached 43% at the end of 12 months (4).

In the previous study, the association between the outcome and the age of the patient shows that the younger ones had a better degree of functionality after 12 months (57.7% for those between 40 and 64 years old) (10). Of the elderly over 65 years who underwent the procedure, only 39.3% had a good outcome.

TABLE 2 Rankin scale evaluation modified at 6 months, shown by the clinical data of Bem Junior et al. (10).

Variable *n* (%)	Rankin Scale (6 months) 4 and 5 *n* (%)	2 and 3 *n* (%)	Total	OR (CI 95%)	*P*-value
Total group	56 (47.9)	61 (52.1)	117 (100.0)		
Associated clinical conditions					$P^{(1)} = 0.060$
Yes	46 (44.7)	57 (55.3)	103 (100.0)	1	
No	10 (71.4)	4 (28.6)	14 (100.0)	3,1 (0,9 a 10,5)	
Surgical hours (h)					
<12	10 (43.5)	13 (56.5)	23 (100.0)	**	$P^{(1)} < 0.001$*
12 a 24	1 (5.3)	18 (94.7)	19 (100.0)	**	
> 24	45 (60.0)	30 (40.0)	75 (100.0)	**	

*Association significant at 5%, **Weren't calculated due to low frequency. (1)Test by Qui-square of Pearson. (2)By Fisher exact test.

TABLE 3 Rankin scale evaluation modified at 12 months, shown by clinical data of Bem Junior et al. (10).

Variable *n* (%)	Rankin Scale (12 months) 4 and 5 *n* (%)	2 and 3 *n* (%)	Total	OR (CI 95%)	*P*-value
Total group	35 (29.9)	82 (70.1)	117 (100.0)		
Associated clinical conditions					$P^{(2)} = 0.756$
Yes	30 (29.1)	73 (70.9)	103 (100.0)	1	
No	5 (35.7)	9 (64.3)	14 (100.0)	1.3 (0.4 a 4.4)	
Surgery hours (h)					$P^{(1)} = 0.916$
<12	6 (26.1)	17 (73.9)	23 (100.0)	1	
12 a 24	6 (31.6)	13 (68.4)	19 (100.0)	1.31 (0.34 a 5.01)	
> 24	23 (30.7)	52 (69.3)	75 (100.0)	1.25 (0.44 a 3.59)	

TABLE 4 Clinical characteristics of the patients included in the random trials.

Study	*n*	Mean age (SD, range)	Sex (% Male)	NIHSS at admission - Mean (range)	Associated pre existing conditions	Surgical time h Mean (SD range)	Mortality
DECIMAL (7)	20	43.5 (±9.7)	45%	22.5 (16–35)	–	20.5 (± 8.3)	25%
DESTINY (5)	17	43.2 (±9.7)	47%	21 (19–26)	–	24.4 (± 6.9)	18%
HAMLET (11)	32	50.0 (± 8·3)	63%	23 (17–34)	–	41 (29–50)	22%
DESTINY II (4)	49	70 (62-82)	25%	20 (15–40)	–	28 (16–50)	43%

TABLE 5 Comparison between outcomes presented by random trials based on mRS at 6 and 12 months.

Study	mRS 6 months			mRS 12 months		
	2–3	4–5	6	2–3	4–5	6
DECIMAL (7)	25%	50%	25%	50%	25%	25%
DESTINY (5)	47%	35%	18%	48%	35%	18%
HAMLET (11)	–	–	–	25%	53%	22%
DESTINY II (4)	7%	60%	33%	6%	51%	43%

Compared to the studies cited, the study Bem Junior et al. (10) shows that the surgical approach in <12 h had a better outcome compared to the surgical time of the other trials, which had an average time of between 20.5 and 41 h. The prognosis of patients in this study, through the Rankin scale, shows that patients operated on within <12 h had a clinically favorable outcome (mRs 2–3) in 55 and 70% of the cases, in the period of 6 and 12 months, respectively (10). While the outcome, although favorable (mRs 2–3), was significantly lower in the other studies, ranging from 7 to 47% in the 6-month period and 6–50% in the 12-month period. Other factors that contribute to a better prognosis of the patients studied by Bem Junior et al. (10), were the length of hospitalization stay, which was fewer in the patients with better clinical outcomes measured by mRS, and the right cerebral hemispheric involvement.

The previous study discussed the importance of the surgical time in decompressive craniectomy, which is rarely discussed in literature, and how the most appropriate approach to the surgical moment is essential for the best prognosis (10). This study not only confirms what the aforementioned trials had already addressed, the choice of decompressive craniectomy as the best treatment for the control of intracranial hypertension secondary to malignant ischemic stroke, but adds that a relatively early approach contributes to a clinically favorable outcome for the patient. At the 6-month follow-up, about 57% of the patients had mRS 2–3 and at the 12-month follow-up, about 74% of the patients also had a grade of 2–3 on the Rankin scale. The individuals in the samples underwent craniectomy at an interval of < 12 h.

Conclusion

The performance of decompressive craniectomy within 24 h after the onset of symptoms seems to be an effective alternative for the reduction of short and long-term neurological damage in patients diagnosed with malignant stroke in the MCA territory. In the study by Bem Junior et al. (10) 52.1 and 70.1% of patients operated on at 6 and 12 months, respectively, and at 24 h, had a relatively favorable prognosis (mRS 2–3) in comparison to other studies, such as Hamlet, with prolonged approach (more than 40 h) and with a more guarded prognosis (25% of patients with mRS 2–3 at 12 months) (10). New studies indicate that performing the procedure within 12 h after the onset of symptoms may be associated with even better results. In addition, the age group that benefits most from the procedure is 65 years or under. Bem Junior et al. (10) corroborate that the early adoption of craniectomy is better since the prognostic factors in the postoperative outcomes are favorable. Further studies on the topic are needed to better assess these variables.

Research limitations

We are aware of the flaws, the study has limitations. We are aware of the selection bias between studies, as the study is a retrospective cohort produced in another country and at different time, and the other studies are prospective. The central proposal of the study is to evaluate and propose what could justify a better outcome, based on early intervention. Furthermore, the anticoagulation use was not analyzed in this research and the cranioplasty was not performed due to service demand.

Author contributions

Conceptualization, methodology, validation, formal analysis, investigation, resources, writing—original draft, project administration, and supervision: LB. Data curation, writing—original draft, investigation, formal analysis, resource, and writing—review and editing: AS, MM, MG, JA, and OF. Conceptualization, validation, formal analysis, resources, project administration, and supervision: NR, NA, MV, and HA. All authors contributed to the article and approved the submitted version.

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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Acidosis in arterial blood gas testing is associated with clinical outcomes after endovascular thrombectomy

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Background: Despite recanalization, some of the patients undergoing endovascular thrombectomy (EVT) still suffer from unfavorable outcomes. Patients with poor prognoses are often accompanied by acidosis in arterial blood gas (ABG) testing. We, therefore, explored the ABG testing results in the early phase of recanalization and analyzed their association with poor prognosis.

Patients and methods: We identified all patients with ischemic stroke and successful endovascular recanalization for anterior circulation vessel occlusion between June 2019 and May 2022. ABG testing was performed in all patients within 0–30 min and 8 h after endovascular therapy. We investigated the relationship between the ABG testing results with symptomatic intracerebral hemorrhage (sICH), hemicraniectomy, and mortality.

Results: A total of 123 patients with stroke after endovascular thrombectomy were analyzed. Of those, eight (6.5%) patients had postinterventional sICH. Acidosis was associated with sICH. Decreased HCO_3^- levels and HCO_3^- levels at 8 h after EVT were independently related to a higher risk of sICH. Twelve (9.8%) patients underwent hemicraniectomy for postischemic malignant edema and similar results were found for hemicraniectomy. Increased lactate at 8 h after EVT and decreased HCO_3^- levels at 8 h after EVT were closely associated with hemicraniectomy. Twenty-two (17.9%) patients died within 3 months. Decreased HCO_3^- levels were independently related to mortality, as were decreased pH levels at 8 h after EVT and decreased HCO_3^- levels at 8 h after EVT.

Conclusion: Acidosis is associated with clinical outcomes after endovascular therapy and may help to select patients with poor prognosis in the acute early phase of recanalization.

KEYWORDS

acidosis, arterial blood gas, hemorrhage, reperfusion, stroke, thrombectomy

Introduction

Endovascular thrombectomy is the optimal treatment option in patients with large vessel occlusion acute ischemic stroke (1). Although 90% of patients achieved successful recanalization and the procedure is generally relatively safe, some of the patients still suffer from unfavorable outcomes at 90 days (2–4). Reperfusion injury and hemorrhagic transformation, which are mainly attributed to impaired cerebral blood flow autoregulation and disrupted blood–brain barrier (BBB), have been identified as important complications in the acute phase of recanalization (5–7).

Early identification of potential candidates who may suffer from neurological deterioration should be performed for a timely experimental treatment to improve the outcome of patients with reperfusion therapies. While timely neurological assessment is unavailable in patients who received general anesthesia or mild sedation during endovascular reperfusion therapies. Transcranial duplex (TCD) sonography or transcranial color-coded sonography (TCCS) may be one of the early hemodynamic predictors of outcome, while there are numerous confounding factors affecting middle cerebral artery (MCA) flow velocity. It may be difficult to reverse the prognosis when the intracranial lesion is detected on cerebral computed tomography (CT) or MRI 24 h routinely after the endovascular procedure or in the event of clinical deterioration.

In clinical practice, we often find that patients with poor prognoses have decreased bicarbonate (HCO_3^-) levels in arterial blood gas (ABG) testing or even have metabolic acidosis or elevated lactate in the early phase of recanalization. Patients with cerebral infarction are less likely to suffer from hemodynamic disorders in the early stages, so changes in the acid–base balance in peripheral blood may be related to cerebral metabolism. There have been few studies exploring the relationship between ABG testing results and poor prognosis in patients with reperfusion therapies. We, therefore, explored the ABG testing results in the early phase of recanalization and analyzed their association with poor prognosis in patients with successful revascularization.

Patients and methods

Patients and study design

Between June 2019 and May 2022, we identified consecutive patients with anterior circulation large vessel occlusion who had undergone endovascular recanalization therapy at Shengli Oilfield Central Hospital, an urban university tertiary hospital and national advanced stroke center. In the treatment protocol for patients with endovascular therapy in our hospital, patients were intubated under general anesthesia by dedicated neuroanesthesiologists in the operating room, followed by endovascular treatment. Then, patients were administrated in the postinterventional period at our neurointensive care unit. Patients would receive mild sedation and close blood pressure monitoring, and they would be evaluated for extubation 12–24 h after endovascular therapy. Patients were included if they underwent endovascular thrombectomy with successful reperfusion. Successful reperfusion was defined as modified thrombolysis in cerebral ischemia (mTICI) $\geq$ 2b. Additional inclusion criteria were admission Alberta Stroke Program Early CT Score (ASPECTS) $\geq$ 6. Patients with hemodynamic instability, hepatic, or renal dysfunction on admission will be excluded from the study. Patients with secondary metabolic abnormalities, such as diabetic ketoacidosis, mitochondrial disease, respiratory acidosis, respiratory alkalosis, or severe aspiration pneumonia were also excluded. Clinical characteristics, including demographic characteristics, past medical history, drug usage, the National Institutes of Health Stroke Scale (NIHSS) scores, intravenous thrombolysis, occlusion location, onset-to-groin puncture time, final mTICI grades, postoperative blood pressure, stroke complication, and outcome were collected. All patients were treated according to current stroke guidelines (1). The Hospital Institutional Review Board and the Ethics Committee of the Shengli Oilfield Central Hospital approved the study. Analysis of patient data was performed in accordance with the Declaration of Helsinki. All patients performed cerebral CT or MRI 24 h after thrombectomy with successful revascularization, and additional CT was also performed in case of clinical deterioration. The primary endpoints were symptomatic intracranial hemorrhage (sICH), all-cause 90-day mortality, and whether or not hemicraniectomy was performed during hospitalization. sICH was defined as any apparent extravascular blood in the brain or within the cranium that was associated with clinical deterioration, as defined by an increase of >2 points in one category or >4 points in total on the NIHSS (6, 8). Functional status was assessed 3 months after stroke onset using the modified Rankin Scale (mRS). The mRS was collected through telephone interviews.

TCD and ABG testing

Transcranial duplex sonography (devices: Delica EMS-9PB, Delicate Manufacturer, Shenzhen, China) and ABG testing (devices: GEM premier 4000, Werfen Co. Barcelona, Spain) were performed in all patients within 0–30 min and 8 h after endovascular therapy as bedside assessment at neurointensive care unit. Absolute values for mean blood flow (MBF) velocities and pulsatility indices (PI) were analyzed in the treated (ipsilateral) MCA. pH, actual bicarbonate (HCO_3^-) levels, and lactate levels in ABG testing from radial artery were recorded and analyzed.

Statistical analysis

The baseline characteristics were described as frequencies, percentages, median, and interquartile ranges. Comparisons between groups were made using the Mann–Whitney *U*-test for continuous variables. Receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC) were conducted. Multivariable logistic regression analyses were performed for each group to determine factors that could be considered independent predictors of clinical outcomes. Clinically relevant variables and those showing $P < 0.05$ in univariate analysis were included in the multivariate model. The model was determined by the method of ENTER (default with the menu system). $P < 0.05$ was considered to be statistically significant. All statistical analyses were carried out using SPSS 19.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 6 (GraphPad, La Jolla, CA).

Results

Characteristics of enrolled subjects

During the investigational period, 167 patients received endovascular recanalization therapy with anterior circulation large vessel occlusion. A total of 44 patients were excluded. Twenty-two patients with incomplete vessel recanalization (TICI 0-2a) and nine patients with hemodynamic instability were excluded. Seven patients with secondary metabolic abnormalities were excluded, including one patient with mitochondrial disease, four patients with severe aspiration pneumonia, and two patients with diabetic ketoacidosis. Two patients were excluded due to the lack of second ABG results and TCD results, and these patients had gone to the operating room for hemicraniectomy within 8 h. Four patients were lost to follow-up. A total of 123 patients were enrolled in the study. The flow chart is illustrated in Figure 1.

Table 1 summarizes the baseline characteristics and outcomes of the study cohort. Fifty-three (43%) patients were women, with a median age of 66 years [interquartile range (IQR), 63–74], median NIHSS score of 17 (IQR, 13–20), and median ASPECT score of 9 (IQR, 8–10). Distribution of initial arterial occlusion location was as follows: Terminal intracranial internal carotid artery 23 (18%), M1-middle cerebral artery 56 (46%), M2-middle cerebral artery 28 (23%), and tandem occlusion 16 (13%). Sixty-six patients (54%) received intravenous thrombolysis before endovascular thrombectomy (EVT). After a median time of 256 min (221–312) from symptoms onset to

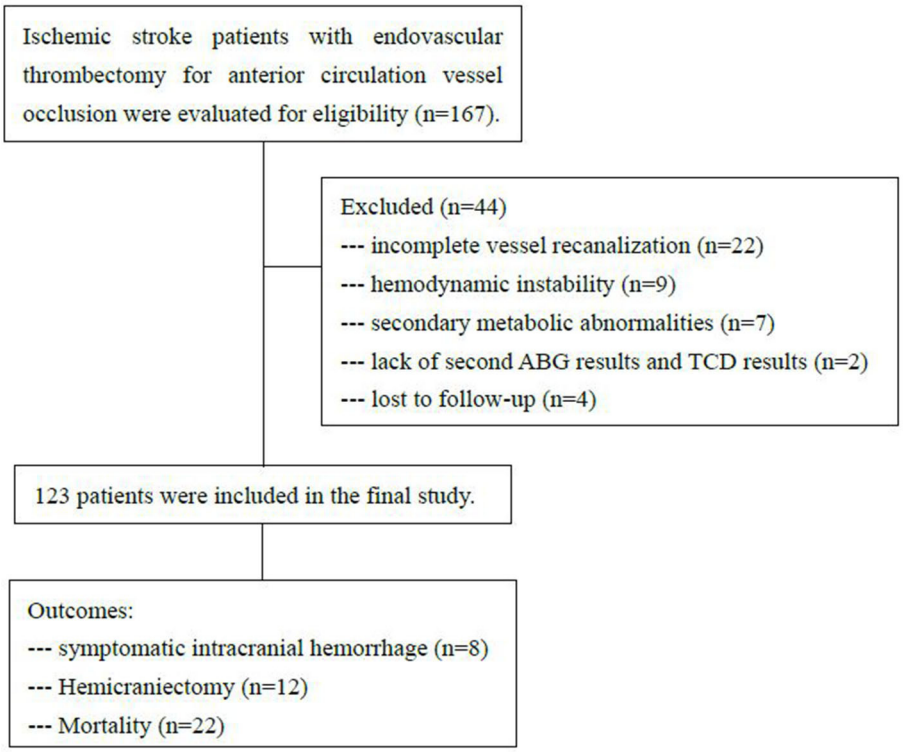

FIGURE 1
Research flow chart. ABG, arterial blood gas (ABG); TCD, transcranial duplex.

TABLE 1 Baseline characteristics and outcomes of the study cohort.

Variable	
Demographics	
Age, year, median (IQR)	66 (63–74)
Female sex, *n* (%)	53 (43%)
Diabetes mellitus, *n* (%)	41 (33%)
Hypertension, *n* (%)	68 (55%)
Hyperlipidemia, *n* (%)	57 (46%)
Atrial fibrillation, *n* (%)	42 (34%)
Smoker on admission, *n* (%)	34 (28%)
Anticoagulation use, *n* (%)	10 (8%)
Antiplatelet use, *n* (%)	39 (32%)
Clinical features	
NIHSS, median (IQR)	17 (13–20)
ASPECTS, median (IQR)	9 (8–10)
MBP, mmHg, median (IQR)	79 (73–86)
Occlusion site, *n* (%)	
TICA	23(18%)
M1-MCA	56 (46%)
M2-MCA	28 (23%)
Tandem occlusion	16 (13%)
Procedural features	
IV tPA, *n* (%)	66 (54%)
Onset-to-Groin, min, median (IQR)	256 (221–312)
Final mTICI 3, *n* (%)	80 (65%)
Final mTICI 2b, *n* (%)	43 (35%)
Outcomes	
mRS score ≤2 at 90 days, *n* (%)	58 (47%)
sICH, *n* (%)	8 (6.5%)
Hemicraniectomy, *n* (%)	12 (9.8%)
Mortality, *n* (%)	22 (17.9%)

ASPECTS, alberta stroke program early CT score; IQR, interquartile range; IV tPA, intravenous tissue-type plasminogen activator; MBP, mean blood pressure; MCA, middle cerebral artery; mRS, modified rankin scale; mTICI, modified thrombolysis in cerebral ischemia score; NIHSS, national institutes of health stroke scale; sICH, symptomatic intracranial hemorrhage; TICA, terminal artery carotid artery.

groin puncture, 80 (65%) patients achieved a final mTICI 3 and 43 (35%) achieved mTICI 2b.

Patients with symptomatic intracranial hemorrhage

A total of eight (6.5%) patients developed sICH after thrombectomy in this study. Patients with sICH had significantly lower HCO_3^- levels ($P = 0.028$). pH levels at 8 h after EVT and HCO_3^- levels at 8 h after EVT were also significantly reduced in patients with sICH ($P = 0.006$ and $P = 0.001$). Although there was a tendency for MBF velocity and PI to increase in patients with sICH, only PI at 8 h after EVT was significantly increased ($P = 0.037$). The detailed results are shown in Table 2. Univariate and multivariate logistic regression were used to identify independent predictors associated with sICH. After the univariate analysis, age, NIHSS score, ASPECTS, and onset-to-groin puncture time were included in the final model. Using the multivariate logistic regression analysis, decreased HCO_3^- levels were independently associated with sICH [odds ratio (OR) = 0.720, 95% confidence interval (CI) 0.541–0.958, $P = 0.024$], as were decreased HCO_3^- levels at 8 h after EVT (OR = 0.696, 95% CI: 0.509–0.952, $P = 0.023$). The detailed results are illustrated in Figure 2 and Table 3. According to the ROC curve, the AUC of the HCO_3^- levels for predicting sICH was 0.730 (95% CI: 0.496–0.964, $P = 0.030$), and HCO_3^- levels at 8 h after EVT was 0.824 (95% CI: 0.674–0.975, $P = 0.002$). The detailed results are demonstrated in Figure 3A.

Patients with hemicraniectomy

A total of 12 (9.8%) patients underwent postischemic malignant edema. Hemicraniectomy was regarded as a surrogate for malignant edema, and we investigated the relationship between relevant variables and hemicraniectomy. Patients with hemicraniectomy had significantly increased MBF velocity at 8 h after EVT ($P = 0.048$). Lactate levels and lactate at 8 h after EVT were obviously higher in patients with hemicraniectomy ($P = 0.027$ and $P = 0.007$). pH levels, HCO_3^- levels, pH levels at 8 h after EVT, and HCO_3^- levels at 8 h after EVT were significantly reduced in the hemicraniectomy group (all $P < 0.05$). The detailed results are shown in Table 4. After the univariate analysis, hyperlipidemia, atrial fibrillation, NIHSS score, ASPECTS, tandem occlusion, and onset-to-groin puncture time were included in the multivariable analysis. Increased lactate at 8 h after EVT was an independent predictor of hemicraniectomy (OR = 2.910, 95% CI: 1.006–8.419, $P = 0.049$), as was decreased HCO_3^- levels at 8 h after EVT (OR = 0.762, 95% CI: 0.599–0.970, $P = 0.027$). The detailed results are shown in Figure 2 and Table 3. The AUC of lactate at 8 h after EVT for predicting hemicraniectomy was 0.731 (95% CI: 0.568–0.894, $P = 0.009$). HCO_3^- levels at 8 h after EVT were 0.818 (95% CI: 0.716–0.920, $P < 0.001$). The detailed results are illustrated in Figure 3B.

Patients with mortality

Twenty-two (17.9%) patients died within 3 months (mRS score = 6) in this study. The mortality group had higher PI at 8 h

TABLE 2 Comparison between favorable and poor prognosis according to the outcome: Symptomatic intracranial hemorrhage.

Variables	No sICH	sICH	*P*-value
MBF velocity after EVT (cm/s)	71 (57–85)	81 (71–100)	0.126
PI after EVT	1.04 (0.84–1.14)	0.94 (0.88–1.29)	0.734
MBF velocity at 8 h after EVT (cm/s)	71 (59–86)	83 (71–99)	0.079
PI at 8 h after EVT	1.05 (0.90–1.18)	1.17 (1.08–1.30)	0.037
pH level	7.36 (7.34–7.40)	7.31 (7.27–7.41)	0.215
Lactate level (mmol/L)	1.1 (0.9–1.9)	1.5 (1.1–2.1)	0.113
HCO_3^- level (mmol/L)	22.6 (20.5–25.0)	17.6 (15.6–23.5)	0.028
pH level at 8 h after EVT	7.39 (7.36–7.41)	7.34 (7.29–7.37)	0.006
Lactate at 8 h after EVT (mmol/L)	1.3 (1.0–1.8)	1.3 (0.9–2.5)	0.471
HCO_3^- level at 8h after EVT (mmol/L)	24.2 (21.0–25.6)	18.8 (16.1–22.4)	0.001

Data are shown as median and interquartile range unless otherwise indicated.
EVT, endovascular thrombectomy; HCO_3^-, bicarbonate; MBF, mean blood flow; PI, pulsatility indices; sICH, symptomatic intracranial hemorrhage.

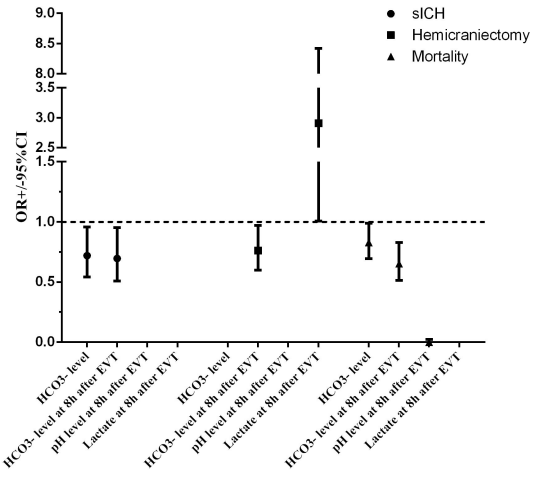

FIGURE 2
Adjusted odds ratio (OR, midpoints) and 95% CIs (error bars) derived from multivariable analysis for clinical outcomes of patients with endovascular thrombectomy. ASPECTS, Alberta stroke program early CT score; CI, confidence interval; EVT, endovascular thrombectomy; HCO^{3-}, bicarbonate; NIHSS, national institutes of health stroke scale; OR, odds ratio; sICH, symptomatic intracranial hemorrhage.

after EVT ($P = 0.010$) and lactate at 8 h after EVT ($P = 0.041$). HCO_3^- levels, pH levels at 8 h after EVT, and HCO_3^- levels at 8 h after EVT were significantly lower in the mortality group (Table 5). Adjusting diabetes mellitus, NIHSS score, ASPECTS, tandem occlusion, onset-to-groin puncture time, and decreased HCO_3^- levels were independent predictors of mortality within 3 months in the multivariable analysis (OR = 0.829, 95% CI: 0.695–0.989, $P = 0.037$), as were decreased pH levels at 8 h after EVT (OR = 0, 95% CI: 0–0.022, $P = 0.015$) and decreased HCO_3^- levels at 8 h after EVT (OR = 0.652, 95% CI: 0.514–0.828, $P < 0.001$). The detailed results are presented in Figure 2 and Table 3. The AUC of HCO_3^- levels for predicting mortality was 0.700 (95% CI: 0.578–0.821, $P = 0.003$). pH levels at 8 h after EVT were 0.747 (95% CI: 0.629–0.866, $P < 0.001$), and HCO_3^- levels at 8 h after EVT were 0.837 (95% CI: 0.751–0.923, $P < 0.001$). The detailed results are shown in Figure 3C.

Discussion

Despite revascularization after thrombectomy, a relevant proportion of patients with large vessel occlusion acute ischemic stroke do not achieve a favorable prognosis. Early identification of patients with possible neurological deterioration may be critical to improving prognosis. Our study demonstrated that ABG testing results were closely related to clinical outcomes after EVT. Our main finding was that acidosis in arterial blood gas testing was strongly associated with poor prognosis. Decreased HCO_3^- levels at 8 h after EVT were independently related to higher odds of sICH, hemicraniectomy, and mortality. Decreased HCO_3^- levels, decreased pH at 8 h after EVT, and elevated lactate levels at 8 h after EVT were also presented in patients with poor clinical outcomes. To the best of our knowledge, this was the first study to investigate the relationship between ABG testing results and clinical outcomes after successful recanalization. Our findings may be useful for screening patients with possible neurological deterioration in the acute early phase of recanalization. ABG could detect exacerbations earlier than routine TCD, CT, or MRI.

After successful recanalization, a series of complex pathophysiological changes occur in the cerebral tissue, which may involve the intricate interplay of mitochondrial dysfunction, overload of calcium, excitotoxicity, free radical-mediated toxicity, endothelial pathology, and edema formation

TABLE 3 Logistic regression analysis of independent factors for prognosis in patients with endovascular reperfusion therapies.

Variable	*B*	SE	Wald	*P*-value	OR	95 % confidence interval for EXP(B)
HCO_3^- level*	−0.329	0.146	5.085	0.024	0.720	0.541–0.958
HCO_3^- level at 8 h after EVT*	−0.362	0.160	5.134	0.023	0.696	0.509–0.952
Lactate at 8 h after EVT[#]	1.068	0.542	3.885	0.049	2.910	1.006–8.419
HCO_3^- level at 8 h after EVT[#]	−0.271	0.123	4.889	0.027	0.762	0.599–0.970
HCO_3^- level[&]	−0.188	0.090	4.353	0.037	0.829	0.695–0.989
pH level at 8 h after EVT[&]	−19.364	7.923	5.973	0.015	0	0–0.022
HCO_3^- level at 8 h after EVT[&]	−0.427	0.122	12.290	<0.001	0.652	0.514–0.828

*Multivariable logistic regression analyses were performed for symptomatic intracranial hemorrhage. [#]Multivariable logistic regression analyses were performed for hemicraniectomy, adjusting for hyperlipidemia. [&]Multivariable logistic regression analyses were performed for mortality, adjusting for diabetes mellitus, NIHSS score, ASPECTS, tandem occlusion, and onset-to-groin puncture time.
ASPECTS, alberta stroke program early CT score; EVT, endovascular thrombectomy; HCO_3^-, bicarbonate; NIHSS, national institutes of health stroke scale.

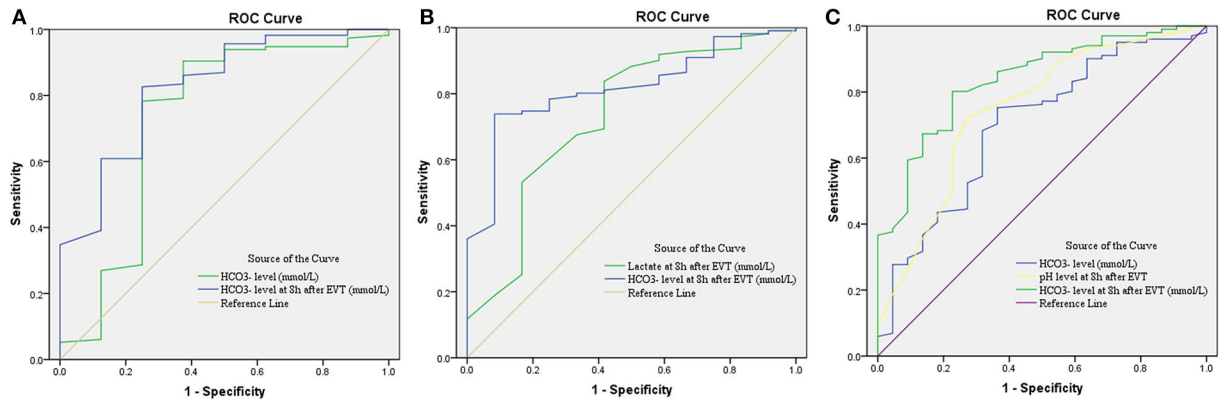

FIGURE 3
Receiver operating characteristic (ROC) curve for predicting sICH **(A)**, hemicraniectomy **(B)**, and mortality **(C)** in patients with endovascular thrombectomy. CI, confidence interval; EVT, endovascular treatment; HCO_3^-, bicarbonate; sICH, symptomatic intracranial hemorrhage.

TABLE 4 Comparison between favorable and poor prognosis according to the outcome: Hemicraniectomy performed during hospitalization.

Variables	No hemicraniectomy	Hemicraniectomy	*P*-value
MBF velocity after EVT (cm/s)	71 (56–85)	77 (72–91)	0.077
PI after EVT	1.04 (0.84–1.14)	1.06 (0.96–1.20)	0.215
MBF velocity at 8 h after EVT (cm/s)	71 (58–87)	80 (77–90)	0.048
PI at 8 h after EVT	1.05 (0.90–1.17)	1.14 (1.03–1.25)	0.079
pH level	7.37 (7.34–7.40)	7.31 (7.29–7.36)	0.014
Lactate level (mmol/L)	1.1 (0.9–1.9)	1.8 (1.2–2.1)	0.027
HCO_3^- level (mmol/L)	22.9 (20.6–25.1)	19.4 (16.1–20.3)	<0.001
pH level at 8 h after EVT	7.39 (7.36–7.42)	7.35 (7.31–7.37)	<0.001
Lactate at 8 h after EVT (mmol/L)	1.2 (0.90–1.7)	2.2 (1.3–2.5)	0.007
HCO_3^- level at 8h after EVT (mmol/L)	24.2 (21.1–25.6)	20.2 (17.2–21.2)	<0.001

Data are shown as median and interquartile range unless otherwise indicated.
EVT, endovascular thrombectomy; HCO_3^-, bicarbonate; MBF, mean blood flow; PI, pulsatility indices.

TABLE 5 Comparison between favorable and poor prognosis according to the outcome: Mortality.

Variables	mRS < 6	mRS = 6	*P*-value
MBF velocity after EVT (cm/s)	71 (55–85)	76 (71–92)	0.055
PI after EVT	1.04 (0.88–1.13)	0.97 (0.81–1.18)	0.815
MBF velocity at 8 h after EVT (cm/s)	71 (58–86)	78 (70–93)	0.151
PI at 8 h after EVT	1.05 (0.90–1.13)	1.20 (1.01–1.28)	0.010
pH level	7.37 (7.34–7.40)	7.34 (7.30–7.39)	0.058
Lactate level (mmol/L)	1.1 (0.9–1.9)	1.2 (1.0–2.0)	0.161
HCO_3^- level (mmol/L)	22.9 (20.7–25.2)	20.5 (16.6–23.6)	0.003
pH level at 8 h after EVT	7.39 (7.36–7.42)	7.35 (7.31–7.39)	<0.001
Lactate at 8 h after EVT (mmol/L)	1.2 (0.9–1.7)	1.4 (1.2–2.1)	0.041
HCO_3^- level at 8 h after EVT (mmol/L)	24.2 (22.2–25.9)	19.6 (17.9–21.5)	<0.001

Data are shown as median and interquartile range unless otherwise indicated.
EVT, endovascular thrombectomy; HCO_3^-, bicarbonate; MBF, mean blood flow; mRS, modified Rankin Scale; PI, pulsatility indices.

(9–13). In another view, recanalization after EVT does not always lead to downstream reperfusion, a state that has been known as the no-reflow phenomenon, which may be due to capillary compression and luminal narrowing in cerebral circulation (14–16). These pathophysiologic derangements may result in cerebral microcirculation disorders. The larger the infarct size, the more severe the microcirculation disorders may be. Impaired microcirculation could lead to excessive acid production, increased toxic substances, and elevated lactic acid. Due to exacerbation of BBB disruption after reperfusion, these acid productions are released into peripheral blood (17). Dysfunctional cerebral blood flow autoregulation after thrombectomy aggravates these processes (18).

The existence of a buffer system in the body will maintain pH in the normal range during the initial phase. However, HCO_3^- as the main component of the biological buffer may change at the onset. Under normal conditions, lactate is continually being produced and metabolized, maintaining dynamic balance. When lactate production predominates, lactate levels rise. Impaired microcirculation can lead not only to elevated lactate levels but also to excessive production of other acids. For example, in the area of cerebral infarction, the Na^+/H^+ exchange isoform 1 (NHE1) function is augmented (19). As a result, Na^+ enters the cells in return for H^+ extrusion, which may aggravate acidosis (20). Thus, bicarbonate, as the main component of biological buffer, is a more comprehensive and earlier indicator of cerebral metabolism, whereas lactate is only a major part of metabolic acidosis. Acidosis (drop in pH) may present when the buffering system fails to compensate. Excluding causes such as systemic hypoperfusion and other causes of secondary metabolic abnormalities, pH, lactate levels, and HCO_3^- levels may be optimal candidates for embodying a cerebral metabolic state, which may indicate the severity of cerebral postischemic reperfusion injury.

Previous studies have explored cerebral metabolism in cerebral ischemia under experimental and clinical conditions by using microdialysis catheters. In animal experiments, the biochemical pattern of cerebral tissue during ischemia is characterized by a marked increase in cerebral lactate (21, 22). Elevated intracerebral lactate and glutamate were also demonstrated in patients with MCA infarcts and malignant brain swelling (23, 24). In addition, the Na^+/H^+ exchange is augmented and tissue pCO_2 rises during ischemia, which may also contribute to cerebral tissue acidosis (19, 25). Acidic amino acids, lactate, and other acidic metabolites were released into the peripheral blood through the damaged BBB, and these products resulted in altering biological buffers, even metabolic acidosis.

The previous study has shown that bedside TCD indicated a significantly higher MBF velocity in the recanalized MCA in patients who experienced ICH following recanalization (26). Although there was a tendency for MBF velocity to increase in patients with sICH in our study, the MBF velocity was not a statistically significant difference between the two groups. The difference between our results and the previous study could be related to the fact that TCD in the present study was performed within 0–30 min and 8 h after EVT, Markus et al. performed a TCD examination within 24 h after EVT. However, the two studies were similar in that MBF velocity was not an independent predictor of sICH following recanalization. Therefore, MBF velocity may not be an optimal biomarker to identify patients with possible neurological deterioration in the acute early phase of recanalization.

Cerebral metabolism is accompanied by complex pathophysiological changes in patients with large vessel occlusion acute ischemic stroke. After successful recanalization, cerebral metabolism may suffer from aggravation due to

postischemic reperfusion injury. There are more published articles using bedside TCD to explore cerebral blood flow in patients with cerebral infarction (27, 28). However, it is surprising that so scant attention has been given to bedside cerebral metabolic markers. Cerebral metabolic indicators may contribute appropriate information on cerebral impairment much earlier. In this context, ABG testing as an efficient bedside screening method is certainly more readily available than other examinations and provides more information about prognosis.

In this study, we observed that decreased HCO_3^- levels at 8 h after EVT were a reliable predictor of clinical outcome in patients with successful revascularization. In the case of conventional treatment after recanalization, decreased HCO_3^- levels at 8 h after EVT indicated that acid production from cerebral metabolism is still increasing, implying deteriorating cerebral function. This study demonstrated that increased lactate at 8 h after EVT was closely associated with hemicraniectomy, which was regarded as a surrogate for malignant edema. Increased lactate levels may result from microcirculatory disorders provoking secondary brain impairment. Due to severe deterioration of cerebral function and excessive acid production release, patients who died may be accompanied by a decrease in pH levels after endovascular therapy.

Some limitations need to be considered in this study. First, all newly admitted patients had their ABG testing routinely collected within 30 min and reviewed 8 h later in our intensive care unit. BBB permeability was increased in the ischemic hemisphere 1 h after reperfusion (29), and whether an earlier review of the ABG testing could provide useful information about the prognosis needs to be further explored. Second, although we included only patients with ASPECTS $\geq$ 6, we could not obtain the final infarct volume. The correlation of final infarct volume with ABG testing results may provide more useful information. Third, the sample sizes were relatively small, this was a single-center study, and more patients need to be included to confirm these findings. Fourth, a lot of patients with malignant cerebral edema may not choose to undergo hemicraniectomy due to age, hemispheric laterality, and other reasons. But the prognosis of these patients may be worsened and eventually be classified in the death group.

Conclusion

In the present study, we have indicated that post-EVT ABG testing might be an effective bedside method for assessing prognosis in patients with large vessel occlusion acute ischemic stroke. Acidosis in arterial blood gas testing is associated with clinical outcomes after endovascular therapy and may help to select patients with poor prognosis in the acute early phase of recanalization.

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 study was approved by the Ethics Committee of the Shengli Oilfield Central Hospital (Approval No. Q/ZXYY-ZY-YWB—LL202251). The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZT and MX conceived and designed the study. RS, LL, JX, PL, GW, HS, RL, YZ, SH, YL, and PZ acquired the data. RS and LL analyzed the data, which was discussed with ZT and MX. RS and ZT drafted and critically revised the manuscript. All authors gave final approval for manuscript publication and agree to be accountable for all aspects of this work. 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

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Admission neutrophil-to-lymphocyte ratio to predict 30-day mortality in severe spontaneous basal ganglia hemorrhage

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Background: Spontaneous intracerebral hemorrhage (ICH) usually occurs in the basal ganglia and is highly lethal and disabling. The aim of this study was to evaluate the predictors of 30-day mortality in patients with severe spontaneous basal ganglia hemorrhage.

Methods: This retrospective study included patients with severe basal ganglia intracerebral hemorrhage treated in the Third Affiliated Hospital of Soochow University from 2012 to 2018. Demographic, clinical, laboratory and neuroradiological data were collected. The short-term prognosis was evaluated and divided into death within 30-days and survival over 30-days. We studied the factors affecting the prognosis of patients with severe intracerebral hemorrhage, analyzed the parameters related to neutrophil-to-lymphocyte (NLR) at admission, and evaluated the predictive effect of NLR on 30-day mortality.

Results: A total of 105 patients was included in this retrospective study. The 30-day death group had a larger hematoma, a higher probability of ventricular hemorrhage, a higher ICH score and a lower Glasgow Coma Scale (GCS) score on admission. Meanwhile, the patients in the death group had higher White blood cells (WBC) counts, neutrophil counts, NLRs and C-reactive protein (CRP) levels. The risk factors for 30-day death were related to the ICH volume, GCS score, ICH score, WBC count, neutrophil count, NLR and CRP. The univariate receiver operating characteristic (ROC) curve of the risk factors showed that the NLR had the best prediction performance. Mathematical predictive models for ICH patients showed that the model with NLR had better prediction accuracy.

Conclusions: The NLR is expected to be a potential biomarker for predicting the prognosis of patients with severe basal ganglia hemorrhage.

KEYWORDS

intracerebral hemorrhage, basal ganglia, mortality, NLR, neutrophil, lymphocyte

Introduction

Stroke is the second leading cause of disability and death in the world (1). As a developing country, the incidence and burden of stroke are also increasing rapidly, and the death related to stroke has become the leading cause of death among Chinese residents (2). In China, the prevalence of stroke increased by 13.2% from 2013 to 2019, with an annual growth rate of 2.2%. The incidence of stroke in adults over 40 years old is 2.58% (about 17.5 million people). The age group with the highest weighted incidence rate of stroke is 70–79 years old, which is 18 folds that of 40–49 years old (3). Spontaneous intracerebral hemorrhage (ICH) is the most serious acute cerebrovascular disease and accounts for 20% of all strokes (4). Only 20% of patients can recover to independent functioning within 6 months after onset, and the mortality rate is close to 60% within 1 year (5). The basal ganglia are located deep in the white matter of the brain and represent an important nerve functional area that is closely related to sensory, motor, visual, behavioral and other functions (6). It also has a high incidence of spontaneous cerebral hemorrhage. Because of the rapid disease development, high mortality and disability rate among patients with severe basal ganglia cerebral hemorrhage (Glasgow Coma Scale, GCS score $\leq$ 8 and hematoma volume $\geq$ 30 cm^3) (7), reliable prediction indicators are needed to help us judge the condition and generate prognoses.

The ratio of the neutrophil count and lymphocyte count in the peripheral blood is known as the NLR, and it can dynamically monitor the body's immune ability and systemic inflammatory state (8). Its ability to predict the clinical outcome has been verified in clinical models of ischemic stroke (9), aneurysm (10), Parkinson's disease (11), and glioma (12). Using ICH models, previous reports found that the NLR of patients was negatively correlated with clinical prognosis (13–15). At present, studies about predicting the prognosis of patients with ICH by NLR mainly focused on patients with mild hematoma volume (16, 17), while few reports had focused on NLR-based prognoses for patients with severe ICH (GCS score $\leq$ 8 and hematoma volume $\geq$ 30 cm^3). Furthermore, the existing literatures roughly included patients with ICH into the study, and did not distinguish the location. It is well known that the location and volume of bleeding are important factors affecting the prognosis of patients.

In the current study, we limited the bleeding location, hematoma volume, and admission status of patients, and only included patients with severe cerebral hemorrhage located in the basal ganglia (GCS score $\leq$ 8 and hematoma volume $\geq$ 30 cm^3), in order to investigate the predictive role of NLR in this highly fatal and disabling stroke.

Methods

Study selection

We retrospectively analyzed 105 patients with severe basal ganglia intracerebral hemorrhage admitted to the Department of Neurosurgery, the Third Affiliated Hospital of Soochow University, from 2012 to 2018 (Figure 1). The inclusion criteria were as follows: (A) head CT images met the diagnostic criteria of cerebral hemorrhage; (B) the hematoma was located in the unilateral basal ganglia; (C) the volume of hematoma, which was calculated according to a previous report (18), was more than 30 ml; (D) admission GCS score was $\leq$8; and (E) age was $\geq$18 years old. The exclusion criteria were as follows: (A) admission occurred more than 24 h after onset; (B) secondary cerebral hemorrhage in the basal ganglia was caused by cerebral aneurysm, vascular malformation, tumor, trauma, and coagulation dysfunction; (C) anticoagulants and immunosuppressants were used; (D) a history of infection in the past 2 weeks; (E) a history of stroke in the past 6 months; and (F) a history of hematological or malignant tumor. This study was approved by the institutional ethics committee (2021–03). All subjects in this retrospective study were anonymous, and the authors were unable to obtain information that could identify individual participants during or after data collection.

Data collection

Demographic information, medical histories, GCS and head CT scan results were retrieved from the EMR system. The ICH score was calculated according to the clinical data and CT results (19). All patients provided blood samples on admission and received medical management according to the guidelines of the American Heart Association/American Stroke Association Stroke Council. For comparison, patients were divided into those who survived more than 30-days (n = 94) or up to 30-days (n = 11).

Statistical analysis

SPSS software version 25.0 was used for the data analysis. Continuous normally distributed variables are presented as the mean ± SD and were analyzed with independent sample t-tests. Categorical variables were expressed as percentages, and the chi-square test was used. To compare the diagnostic efficiency of different parameters, the receiver operating characteristic curve (ROC) and area under the curve (AUC) were analyzed. The diagnostic sensitivity, specificity and accuracy of each variable were calculated, and the optimal cutoff value was determined by the Youden index. Univariate and multivariate logistic

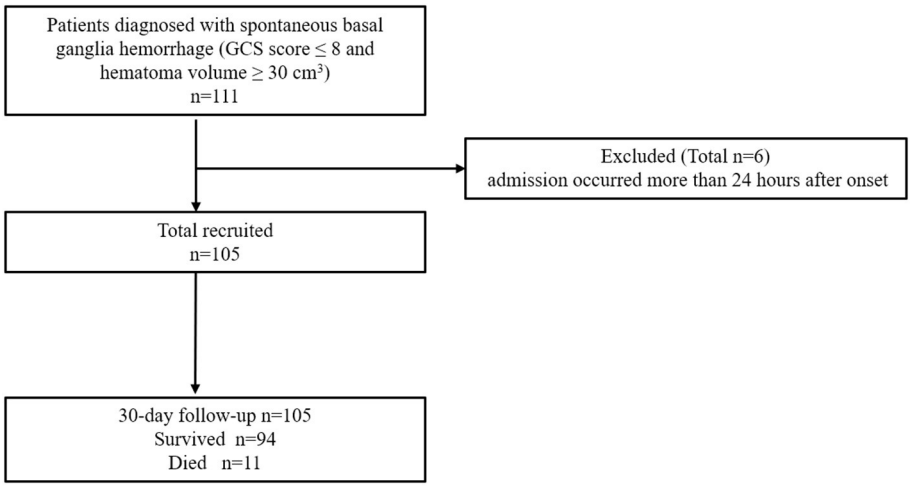

FIGURE 1
Patient selection flow chart. GCS, Glasgow coma scale.

regression analyses were performed to detect the correlation between the two variables, and odds ratios (ORs) were calculated to establish the logistic model. ROC and Youden index analyses were performed to establish the best mathematical model for the prediction of ICH patients. The ROCs of all parameters and models were compared by EmpowerStats software. All statistics were tested by a two-sided test, and $p < 0.05$ was considered statistically significant.

Results

A total of 105 patients were selected from 2012 to 2018, of whom 11 (10.5%) died within 30-days after onset. The patients were classified into different groups according to whether they died within 30-days after onset. The demographic data, clinical characteristics and laboratory parameters of the two groups at admission are shown in Table 1. According to the analysis of the clinical characteristics of the subgroups, the death group had a larger amount of hematoma (median 75, $p = 0.006$), a higher probability of ventricular hemorrhage (72.7%, $p = 0.049$), a higher ICH score on admission (3.0 ± 1.0, $p = 0.019$), and a lower GCS score (median 5, $p = 0.003$) (Table 1). Meanwhile, laboratory examinations found that the patients in the death group had a higher WBC count (17.4 ± 9.0, $p = 0.002$), neutrophil count (14.2 ± 8.2, $p = 0.004$), NLR (median 8.8, $p = 0.002$) and C-reactive protein level (median 49, $p = 0.012$) in their peripheral blood samples, and the differences were significant (Table 1).

To explore the relationship between the risk factors and the outcome prognosis, we performed a correlation analysis. The results showed that the risk factors for 30-day death did not include intraventricular hematoma ($p = 0.062$) but were related to the hematoma volume ($p = 0.012$), GCS score ($p = 0.005$), ICH score ($p = 0.029$), WBC count ($p = 0.010$), neutrophil count ($p = 0.011$), NLR ($p = 0.001$) and CRP level ($p = 0.018$) (Table 2). These risk factors were calibrated for age, gender and GCS score (Table 3).

To compare the diagnostic efficacy of risk indicators, we analyzed the univariate ROC curve of ICH volume, GCS score, ICH score, WBC count, neutrophil count, NLR and CRP level (Table 4). The AUC and 95% confidence interval of the ICH volume, GCS score, ICH score, WBC count, neutrophil count, NLR, and CRP level were (0.7065, 0.5307–0.8823), (0.7660, 0.6128–0.9191), (0.6248, 0.4312–0.8183), (0.6963, 0.5304–0.8623), (0.6939, 0.5415–0.8463), (0.7901, 0.6754–0.9049), and (0.7311, 0.5869–0.8754), respectively. The results show that the AUC of NLR is the largest.

We established a mathematical predictive model of ICH to further conduct multivariate logistic regression analyses. Death within 30-days was set as the dependent variable and ICH volume, GCS score, ICH score, WBC count, neutrophil count, and CRP level as the independent variables. The equation of ICH prediction Model 1 was $-4.90143 + 0.01894 \times$ (ICH volume) $-0.22312 \times$ (WBC) $+ 0.36191 \times$ (neutral count) $+ 0.01277 \times$ (CRP) $+ 0.74191 \times$ (ICH Score) $-0.38027 \times$ (GCS score) (Table 5). Then, the independent variable NLR was also introduced on the basis of Model 1, and a logistic regression analysis was carried out to construct multifactor joint prediction Model 2. The regression equation was $-7.47229 + 0.02976 \times$ (ICH volume) $+ 0.54289 \times$ (WBC) $-0.53591 \times$ (neutral count) $+ 0.40806 \times$ NLR $+ 0.00730 \times$ (CRP) $+ 0.49988 \times$ (ICH Score) $-0.66796 \times$ (GCS score) (Table 5). A ROC curve analysis was applied to compare the diagnostic efficacy

TABLE 1 Baseline characteristics of patients according to 30-day outcome.

	Survived ($n = 94$)	Died ($n = 11$)	p-value
Demographics			
Male, *n* (%)	61 (64.9)	8 (72.7)	0.605
Age (years)	52.4 ± 14.1	54.5 ± 12.4	0.640
Medical history, *n* (%)			
Prior stroke	11 (11.7)	0 (0.0)	0.230
Hypertension	57 (60.6)	9 (81.8)	0.169
Diabetes mellitus	10 (10.6)	1 (9.1)	0.874
Admission assessment			
Duration from onset to hospitalization (h)	3 (2–4)	3 (2–4)	0.484
Right basal ganglia hemorrhage, *n* (%)	37 (39.4)	2 (18.2)	0.169
ICH volume (mL)	52(40–70)	75(53–94)	0.006
IVH, *n* (%)	39 (41.5)	8 (72.7)	0.049
GCS score	7 (5–7)	5 (3–6)	0.003
ICH score	2.5 ± 0.6	3.0 ± 1.0	0.019
Laboratory values			
WBC ($\times 10^9$/L)	12.2 ± 4.6	17.4 ± 9.0	0.002
Neutrophil count ($\times 10^9$/L)	9.2 ± 4.8	14.2 ± 8.2	0.004
lymphocyte count ($\times 10^9$/L)	2.3 ± 1.3	1.6 ± 1.2	0.064
NLR (%)	3.8 (1.9–8.5)	8.8 (6.5–15.6)	0.002
D-dimer	3.0 ± 8.0	3.8 ± 2.6	0.742
CRP (mg/L)	14.0 (5.0–54.7)	49.0 (27.9–135.6)	0.012

GCS, Glasgow coma scale; ICH, Intracerebral hemorrhage; IVH, Intraventricular hemorrhage; WBC, White blood cells; NLR, Neutrophil-to-lymphocyte ratio; CRP, C-reactive protein.

TABLE 2 Risk factors associated with 30-day death.

Independent variable	OR (95% CI)	p-value
ICH volume	1.0 (1.0, 1.1)	0.012
IVH	3.8 (0.9, 15.1)	0.062
GCS score	0.6 (0.4, 0.8)	0.005
ICH score	3.0 (1.1, 7.8)	0.029
WBC	1.2 (1.0, 1.3)	0.010
Neutrophil count	1.2 (1.0, 1.3)	0.011
NLR	1.2 (1.1, 1.4)	0.001
CRP	1.0 (1.0, 1.0)	0.018

GCS, Glasgow coma scale; ICH, Intracerebral hemorrhage; IVH, Intraventricular hemorrhage; WBC, White blood cells; NLR, Neutrophil-to-lymphocyte ratio; CRP, C-reactive protein.

of Model 1 and Model 2 (Table 4), which showed that the prognostic efficacy of Model 2 was better than that of Model 1 (AUC: 0.9304 vs. 0.8383, $p = 0.0270$).

Discussion

Stroke is mainly divided into ICH and ischemic stroke. A study from bigdata observatory platform for stroke of China showed that the ICH group had the highest rate of in-hospital mortality (0.9% for ischemic stroke, 5.1% for ICH). Meanwhile, the 1-year fatality and disability rates of patients with ICH were also higher than those of patients with ischemic stroke (20). Spontaneous intracerebral hemorrhage mostly occurs in the basal ganglia and is related to hypertension, and it presents high disability and mortality rates (21). However, the relevant treatment strategies and clinical prognoses are still controversial (22). Patients with severe basal ganglia hemorrhage have catastrophic outcomes, and the 30-day mortality is over 30% (23). There are two main aspects of brain injury caused by intracerebral hemorrhage: the primary destructive effect of hematoma and secondary injury caused by the space-occupying effect (24). Some studies suggest that the factors related to the prognosis of patients with ICH include the volume of hematoma, the ICH score, and whether the hematoma breaks into the ventricle (14); however, convenient and reliable biological markers for judging the prognosis are lacking.

Inflammatory responses are widely involved in the process of a variety of diseases and can even be used as an independent risk factor for prognosis (25–28). After intravascular recanalization in patients with acute ischemic stroke (AIS), recanalization and reperfusion of previously hypoxic brain regions increase the proinflammatory function of platelets, and the activated thrombus inflammatory reaction may aggravate the ischemia reperfusion injury. Moreover, T cells and platelets will further accelerate the progression of cerebral infarction and expand the infarct area (29). AIS patients who received endovascular therapy and successfully recanalized had higher systemic inflammatory response index (SIRI, SIRI = absolute neutrophil count × absolute monocyte count/ absolute lymphocyte count) at admission, the risk of poor neurological prognosis at 3 months was also increased. SIRI is an independent risk factor for the prognosis of patients with ineffective recanalization (30). Similarly, a retrospective study found that the increase of neutrophil count and NLR before thrombolysis in AIS was independently associated with ICH after thrombolysis and deterioration of prognosis at 3 months (31). Moreover, elevated NLR also indicates an increased risk of symptomatic intracerebral hemorrhage in AIS patients after vascular recanalization therapy (32). In addition, studies on patients with hemorrhagic stroke found that elevated NLR is

TABLE 3 Adjusted risk factors for 30-day mortality in ICH patients.

Variable	Unadjusted		Adjusted[a]	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
ICH volume	1.0 (1.0, 1.1)	0.012	1.0 (1.0, 1.1)	0.014
GCS score	0.6 (0.4, 0.8)	0.005	0.6 (0.4, 0.8)	0.006
ICH score	3.0 (1.1, 7.8)	0.029	2.9 (1.1, 7.8)	0.032
WBC	1.2 (1.0, 1.3)	0.010	1.2 (1.1, 1.4)	0.007
Neutrophil count	1.2 (1.0, 1.3)	0.011	1.2 (1.0, 1.4)	0.008
NLR	1.2 (1.1, 1.4)	0.001	1.2 (1.1, 1.4)	0.002
CRP	1.0 (1.0, 1.0)	0.018	1.0 (1.0, 1.0)	0.027
Variable	Unadjusted		Adjusted[b]	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
ICH volume	1.0 (1.0–1.1)	0.012	1.0 (1.0–1.0)	0.093
ICH score	3.0 (1.1–7.8)	0.029	2.2 (1.0–6.1)	0.152
WBC	1.2 (1.0–1.3)	0.010	1.2 (1.0–1.4)	0.016
Neutrophil count	1.2 (1.0–1.3)	0.011	1.2 (1.0–1.4)	0.015
NLR	1.2 (1.1–1.4)	0.001	1.3 (1.2–1.6)	0.001
CRP	1.0 (1.0–1.0)	0.018	1.0 (1.0–1.0)	0.109

GCS, Glasgow coma scale; ICH, Intracerebral hemorrhage; WBC, White blood cells; NLR, Neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; [a]Adjustment by age and gender; [b]Adjustment by age, gender and GCS score.

TABLE 4 Comparisons of risk factors of ICH patients.

	AUC	95%CI	Cut-off	Specificity	Sensitivity	Accuracy	+LR	−LR
ICH volume	0.7065	0.5307–0.8823	74.0000	0.8298	0.5455	0.8000	3.2045	0.5478
GCS score	0.7660	0.6128–0.9191	5.5000	0.7340	0.7273	0.7333	2.7345	0.3715
ICH score	0.6248	0.4312–0.8183	3.5000	0.9787	0.2727	0.9048	12.8182	0.7431
WBC	0.6963	0.5304–0.8623	13.4050	0.6596	0.7273	0.6667	2.1364	0.4135
Neutrophil count	0.6939	0.5415–0.8463	6.3250	0.3723	1.0000	0.4381	1.5932	0.0000
NLR	0.7901	0.6754–0.9049	5.5250	0.5638	1.0000	0.6095	2.2927	0.0000
CRP	0.7311	0.5869–0.8754	26.0500	0.6064	0.9091	0.6381	2.3096	0.1499
Model 1	0.8385	0.7234–0.9536	−2.6904	0.6277	0.9091	0.6571	2.4416	0.1448
Model 2	0.9304*	0.8450–1.0000	−1.0338	0.9681	0.8182	0.9524	25.6364	0.1878

GCS, Glasgow coma scale; ICH, Intracerebral hemorrhage; WBC, White blood cells; NLR, Neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; AUC, Area under curve; +LR, Positive-likelihood ratio; -LR, Negative- likelihood ratio. Model 1, −4.90143 + 0.01894 * (ICH volume) −0.22312 * (WBC) + 0.36191 * (Neutrophil count) + 0.01277 * (CRP) + 0.74191 * (ICH score) −0.38027 * (GCS score). Model 2, −7.47229 + 0.02976 * (ICH volume) + 0.54289 * (WBC) −0.53591 * (Neutrophil count) + 0.40806 * (NLR) + 0.00730 * (CRP) + 0.49988 * (ICH score) −0.66796 * (GCS score). *Compared with AUC of Model 1, $p < 0.05$.

a good predictor of early neurological deterioration and poor functional status at 3 months (15, 33). Once ICH occurs, the inflammatory reaction is activated, and then inflammatory cells migrate, infiltrate local brain tissue, release cytokines, affect the stability of the blood–brain barrier, aggravate tissue edema, and even lead to hematoma expansion, resulting in a vicious cycle (34, 35). Therefore, evaluating the inflammatory response in patients with ICH will help us to predict the outcomes. The NLR is the ratio of the neutrophil count and lymphocyte count in peripheral blood, which is easy to obtain in the clinic and can be used to dynamically monitor the body's immune ability and systemic inflammatory state. The predictive ability of the NLR for clinical outcome has been verified in a variety of clinical models of brain diseases (9–12). In patients with aneurysmal subarachnoid hemorrhage, NLR is an independent risk factor for poor functional prognosis (10). In addition, NLR is defined as an independent factor for early deterioration of neurological function in patients with AIS after thrombolytic

TABLE 5 Construction of predictive mathematical models for ICH patients.

	OR (95% CI)	*p*-value
Model 1		
ICH volume	1.0191 (0.9892–1.0499)	0.2127
White blood cells	0.8000 (0.4631–1.3821)	0.4238
Neutrophil count	1.4361 (0.8103–2.5451)	0.2151
CRP	1.0128 (0.9979–1.0280)	0.0923
ICH score	2.1000 (0.6034–7.3079)	0.2436
GCS score	0.6837(0.4185–1.1169)	0.1289
Model 2		
ICH volume	1.0302 (0.9895–1.0726)	0.1485
White blood cells	1.7210 (0.7937–3.7316)	0.1692
Neutrophil count	0.5851 (0.2505–1.3668)	0.2157
NLR	1.5039 (1.1166–2.0255)	0.0072
CRP	1.0073 (0.9876–1.0274)	0.4683
ICH score	1.6485 (0.3839–7.0793)	0.5014
GCS score	0.5128 (0.2577–1.0202)	0.0570

GCS, Glasgow coma scale; ICH, Intracerebral hemorrhage; NLR, Neutrophil-to-lymphocyte ratio; CRP, C-reactive protein.

therapy (36). NLR is a promising predictor of clinical outcomes in patients with ischemic and hemorrhagic stroke. Furthermore, increased NLR is associated with a higher risk of ischemic stroke (4). However, the predictive role of the NLR in patients with ICH is still controversial. Several scholars have found that the NLR level was negatively correlated with the short-term prognosis in ICH patients (13–15), and other studies reported that the NLR at admission was not associated with the 30- or 90-day mortality (13, 16). The possible reason for these contradictory results is that the previous studies did not screen and distinguish the bleeding area, hematoma volume and admission status of the selected patients in detail, which seriously affected the prognosis and resulted in biased results. Therefore, if we can improve the homogeneity of enrolled patients, it will improve the accuracy of predictions. In view of this, we established a mathematical model that focuses on patients with severe basal ganglia intracerebral hemorrhage (GCS score $\leq$ 8 and hematoma volume $\geq$ 30 cm^3) and explored the relationship between NLR in peripheral blood routine at admission and 30-day mortality. Briefly, the NLR at admission is positively correlated with the 30-day mortality. To improve our understanding of this dangerous disease and improve the accuracy of disease prediction, we carried out univariate and multivariate logistic regression analyses and established mathematical predictive models. The univariate ROC analysis showed that the ICH volume, GCS, ICH score, WBC, neutrophil count and NLR could be used to predict the 30-day mortality in patients with severe basal ganglia ICH, and NLR was the most reliable. Further construction of the combined predictive model showed that compared with Model 1, predictive Model 2, which was composed of the NLR at admission, was superior to the single NLR and Model 1, and the specificity and accuracy were obviously improved. In conclusion, the NLR is expected to be a potential biomarker for predicting the prognosis of patients with severe basal ganglia hemorrhage.

Some shortcomings were observed in this study. First, this was a retrospective study in which patients were selected from a single center; thus, the screening may be biased. Second, although inflammatory factors may be associated with the enlargement of hematoma and the aggravation of brain edema (34, 37, 38), but we did not detect the expression level of inflammatory factors or their relationship with NLR and prognosis.

Basal ganglia intracerebral hemorrhage is a type of stroke with high mortality and disability rate, and has a trend of youth, which brings heavy burden to society and families. Therefore, how to quickly, accurately and economically identify the risk factors of patients with cerebral hemorrhage is particularly important, which will help to provide reference for follow-up treatment. It is known that the neuroinflammatory reaction triggered by cerebral hemorrhage will lead to a series of reactions and affect the prognosis of patients (39). Neutrophils invade brain tissues at the early stage of lesions, release proinflammatory cytokines and other cytotoxic products, and promote the secondary damage of potential living tissues (40). It can also increase the permeability of blood brain barrier and lead to brain edema after stroke (40). In addition, neutrophils adhere to the blood vessel wall, which can form secondary obstruction in the cerebral microvessels (41). On the contrary, lymphocyte count is considered to have neuroprotective effects and help to improve neural function (42). The peripheral blood NLR is a relatively easy to obtain and widely used clinical marker, which is routinely used to respond to systemic inflammatory reaction. Therefore, the analysis of NLR is helpful to understand the potential pathophysiology of ICH, and can also be used as a factor to predict the prognosis of patients with ICH, providing inspiration for clinical practice and future research of this public health concern.

Data availability statement

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

Ethics statement

This study was approved by the Ethics Committee of The Third Affiliated Hospital of Soochow University. All methods were carried out in accordance with relevant guidelines and

regulations. Informed consent was obtained from the patient for anonymized information to be published in this study.

Author contributions

JS was a major contributor to design the study, analyze the data, and draft the manuscript. YL and LW collected and organized the patients' data. WX was patient management. WG participated in the design of the study and coordination of the whole work. 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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Predictors of 30-day mortality among patients with stroke admitted at a tertiary teaching hospital in Northwestern Tanzania: A prospective cohort study

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Background: Stroke is the second leading cause of death worldwide, with the highest mortality rates in low- to middle-income countries, particularly in sub-Saharan Africa. We aimed to investigate the predictors of 30-day mortality among patients with stroke admitted at a tertiary teaching hospital in Northwestern Tanzania.

Methods: This cohort study recruited patients with the World Health Organization's clinical definition of stroke. Data were collected on baseline characteristics, the degree of neurological impairment at admission (measured using the National Institutes of Health Stroke Scale), imaging and electrocardiogram (ECG) findings, and post-stroke complications. The modified Rankin scale (mRS) was used to assess stroke outcomes. Kaplan–Meier analysis was used to describe survival, and the Cox proportional hazards model was used to examine predictors of mortality.

Results: A total of 135 patients were enrolled, with a mean age of 64.5 years. Hypertension was observed in 76%, and 20% were on regular anti-hypertensive medications. The overall 30-day mortality rate was 37%. Comparing patients with hemorrhagic and ischemic stroke, 25% had died by day 5 [25th percentile survival time (in days): 5 (95% CI: 2–14)] versus day 23 [25th percentile survival time (in days): 23 (95% CI: 11–30) (log-rank $p < 0.001$)], respectively. Aspiration pneumonia was the most common medical complication, occurring in 41.3% of patients. ECG abnormalities were observed in 54.6 and 46.9% of patients with hemorrhagic and ischemic stroke, respectively. The most common patterns were as follows: ST changes 29.6 vs. 30.9%, T-wave inversion 34.1 vs. 38.3%, and U-waves 18.2 vs. 1.2% in hemorrhagic and ischemic stroke, respectively. Independent predictors for case mortality were as follows: mRS score at presentation (4–5) [aHR 5.50 (95% CI: 2.02–15.04)], aspiration pneumonia [aHR 3.69 (95% CI: 1.71–13.69)], ECG abnormalities [aHR 2.28 (95% CI: 1.86–5.86)], and baseline stroke severity [aHR 1.09 (95% CI: 1.02–1.17)].

Conclusion: Stroke is associated with a high 30-day mortality rate in Northwestern Tanzania. Concerted efforts are warranted in managing patients with

stroke, with particular attention to individuals with severe strokes, ECG abnormalities, and swallowing difficulties to reduce early morbidity and mortality.

KEYWORDS

stroke, predictors, morbidity, mortality, Tanzania

Introduction

Stroke is the second leading cause of death and the third leading cause of death and disability-adjusted life years (DALYs) combined worldwide, according to the 2019 Global Burden of Disease report (1). In contrast to high-income countries (HIC), where there was a decrease in age-standardized stroke incidence, DALY, and mortality, low-middle-income countries (LMIC) had the highest age-standardized stroke-related mortality of more than 3.6 times (86%) with 87% DALYs (1). This decline in stroke morbidity and mortality in HIC reflects advancements in stroke management, leading to more favorable outcomes.

Countries in sub-Saharan Africa (SSA) have a high stroke burden and mortality (1): Stroke in Africa occurs at a younger age, which has significant socioeconomic implications (2–5). Previous hospital-based studies in Tanzania have reported varied 30-day case fatality rates, ranging from 30 to 60%, with a limited characterization of predictors of mortality (5, 6). In SSA, stroke severity, infections, elevated glucose levels, and fever are known predictors of 30-day mortality (5–7). There is an urgent need to find solutions to mitigate the rising number of strokes and the associated mortality in Tanzania. We aimed to investigate the predictors of 30-day mortality among patients with stroke admitted at a large tertiary teaching hospital in Tanzania.

Materials and methods

Study design and population

This cohort study was conducted at a tertiary teaching hospital, Bugando Medical Center (BMC), in Northwestern Tanzania. BMC offers specialized care to all in and outpatients from all over the country, particularly along the shores of Lake Victoria. Patients with stroke who met the World Health Organization (WHO) clinical definition were consecutively recruited between February 2022 and May 2022 after obtaining written informed consent from the patient, or next of kin (for those unable to consent due to stroke-related disabilities) (8).

Data collection

Data were collected and managed using an electronic data-capturing database developed and hosted by VervigR. Information captured included baseline data, including gender, age, residency, marital status, level of educational achievement, and at least three available mobile numbers from the patient and next of kin. We also recorded any previous history or prescriptions for hypertension and diabetes mellitus and inquired about smoking and alcohol consumption.

Clinical measurement

Physical examination included assessment of the Glasgow coma scale score, temperature, pulse rate, and rhythm. Blood pressure readings were taken using a standard digital sphygmomanometer (Omron 10, Healthcare); three readings were taken 5-min apart. Hypertension was defined as systolic blood pressure (SBP) of ≥140 mmHg and/or diastolic blood pressure (DBP) of ≥90 mmHg or previous/current use of anti-hypertensive medications (9). Waist and hip circumference were measured using a tape measure and recorded in centimeters. The waist-hip ratio was computed and interpreted according to the WHO guidelines (10).

Laboratory investigations

We aseptically collected 15 ml of venous blood for complete blood count (Sysmex 1000 machine) and random total cholesterol (BIO-SYSTEMS machine). Capillary fingertip blood samples were collected to check for random blood glucose (RBG) levels and HIV rapid testing using a glucometer GLUCOPLUS^{TM} and SD Bioline, respectively. A fasting blood glucose (FBG) sample was collected the following morning for patients with RBG levels of ≥11.1 mmol/l. Diabetes mellitus diagnosis was defined as an RBG reading of ≥11.1 mmol/l or an FBG reading of ≥7 mmol/l. For patients who were HIV reactive to SD Bioline, confirmatory testing was performed using Unigold Biotech.

Medical complications

This included clinical seizures, infections: urinary tract infection and aspiration pneumonia (confirmed aspiration pneumonia or probable) (11), and the presence of bedsores (12, 13). Other complications included new onset fever lasting more than 24 h from an unknown source (13). Presumed venous thromboembolism was clinically diagnosed using Well's score (14). Miscellaneous complications were defined as any documented complication resulting in a specific medical or surgical intervention (e.g., gastrointestinal hemorrhage, constipation, episodes of cardiac failure, cardiac arrhythmias, and arthritis) (13).

Brain imaging

A non-contrasted brain computed tomography scan, acquired on a 128-slice CT Scanner (Siemens Somatom Perspective, Siemens

Abbreviations: mRS, modified Rankin scale; NIHSS, National Institute of Health Stroke Scale; SSA, sub-Saharan Africa; WHO, World Health Organization.

Healthcare GmbH, Germany), was performed on all patients on admission, and images were interpreted by a neuro-radiologist. For ischemic stroke, the Alberta Stroke Program Early CT (ASPECTS) score was dichotomized to <7 and ≥7 and used for analysis (15). Hemorrhagic transformation was defined per European Cooperative Acute Stroke Study (ECASS II) (16). Midline shift was defined as any measurable shift of midline cerebral structures seen on axial view, specifically the septum pellucidum and/or the pineal gland (17). For those with hemorrhagic stroke, the location was documented, and the hematoma volume was measured using the modified ABC/2 method (18). We calculated the intracerebral hemorrhage (ICH) score with scores ranging from 0 to 5 (19).

Cardiovascular assessment

A 12-lead electrocardiography (ECG) (model ECG-3312B) was performed on all patients, and results were interpreted by a cardiologist for the presence of ST-segment depression or elevation, T-wave abnormalities, U-waves, and the presence of atrial fibrillation.

Stroke outcomes

Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS) on admission (20). Stroke outcomes were assessed using a modified Rankin scale (mRS) with scores ranging from 0 (no symptoms) to 6 (death) on admission up to 30 days (20). The date of death was recorded, and the time-to-event was computed using the date difference between the date of last contact (date of death or date of the last follow-up) and the date of symptom onset.

Study variables

The dependent variable was case mortality, and the independent variables included were as follows: demographic data, medical co-morbidities and complications, stroke severity, stroke subtype, and ECG changes.

Data analysis

Data analysis was done using STATA software version 15.0. Continuous variables were summarized and presented as means and standard deviation (SD) or medians and interquartile range [IQR]. Categorical variables were summarized as frequencies and proportions. Kaplan–Meier analysis was used to describe survival, where the 25th percentile survival time with respective 95% confidence intervals was estimated, and significant differences in survival times by stroke subtype were tested using the log-rank test. Crude and adjusted analyses were done using a Cox proportional hazards model. Hazard ratios (HRs), 95% confidence intervals (CIs), and corresponding *p*-values were obtained from the models adjusting for potential confounders. Factors for multivariable analyses were selected based on prior knowledge of the possible associations between stroke and mortality. These included age, sex, and previous history of stroke. Other variables with a *p*-value of <0.20 in the univariable model were included in the multivariable analysis, and a significance level was set as a *p*-value of <0.05.

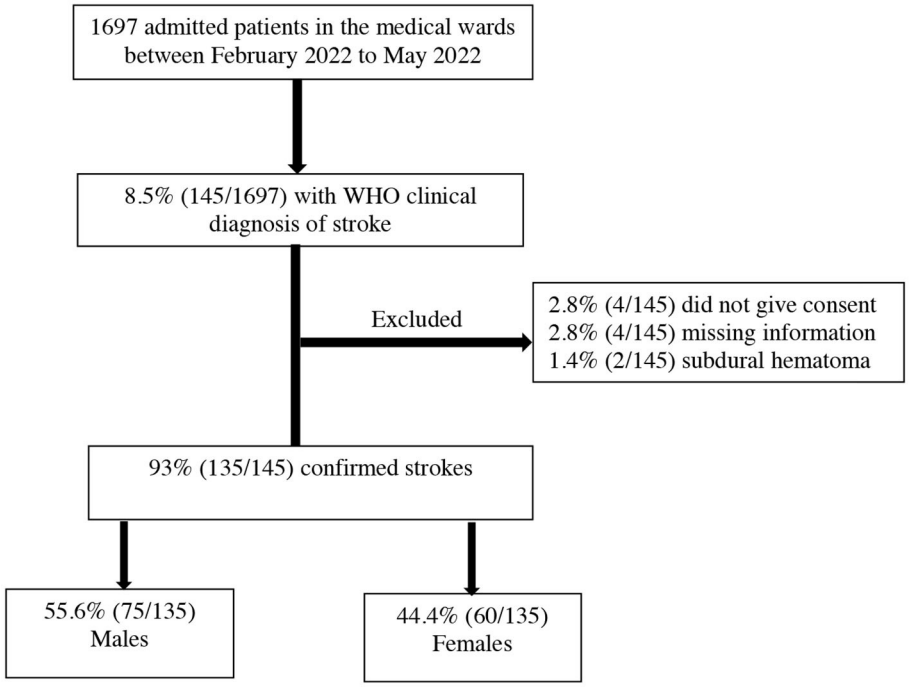

FIGURE 1
Recruitment flowchart.

Results

The proportion of strokes compared to the total medical admissions

During the study period from February 2022 to May 2022, there were 1,697 medical admissions. Out of these admissions, 8.5% (145/1697) met the WHO's clinical definition of stroke. We excluded 6.9% (10/145) patients for the following reasons: 2.8% (4/145) did not give consent to participate in the study, 2.8% (4/145) had missing information, and 1.38% (2/145) had subdural hematoma as summarized in Figure 1. The final proportion of patients with stroke compared to the total medical admissions was 7.9% (135/1697) (95% CI 6.7–9.4%).

Demographic characteristics of the study patients

The mean age was 64.5 ± 14.7 years, and the majority were male patients, 55.6% (75/135). Notably, 57% (77/135) had secondary education or higher and 52.6% (71/135) were uninsured. The proportion of patients with a history of smoking or alcohol consumption was 28.9% (39/135) and 72.6% (98/135), respectively, as summarized in Table 1. The mean time from stroke symptom onset to hospital arrival was 2.67 ± 4.9 days.

A prior history of hypertension and diabetes mellitus was present in 76.3% (103/135) and 16.3% (22/135) of subjects, respectively. One-quarter of hypertensive (27/103) and one-half of patients with diabetes (11/22) were taking relevant regular medication. The mean time from stroke onset to brain imaging was 3.43 ± 3.8 days, and ischemic stroke occurred in two-thirds (81/135) of the patients. The median mRS and NIHSS scores on admission were 4 (IQR 3, 4) and 20.5 (17, 21), respectively. At presentation, 80.7% (109/135) were hypertensive and 3% (4/135) were HIV positive, as summarized in Table 2.

TABLE 1 Demographic characteristics of the study patients.

Variable	Frequency	Percentage
Age in years		
<60	52	38.5
≥60	83	61.5
Mean ± SD	64.5 ± 14.7	
Sex		
Female	60	44.4
Male	75	55.6
Marital status		
Ever married	132	97.7
Never married	3	2.2
Education level		
No education	34	25.2
Primary education	24	17.8
Secondary and above	77	57
Insurance status		
Insured	64	47.4
Not insured	71	52.6
History of smoking		
Never	96	71.1
Ever	39	28.9
History of alcohol drinking		
Never	37	27.4
Ever	98	72.6

TABLE 2 Clinical characteristics of the study patients.

Characteristic	Frequency	Percentage
Prior history of hypertension	103	76.3
Not on regular medications	76	73.8
On regular medications	27	26.2
Prior history of Diabetes mellitus	22	16.3
Not on regular medications	11	50
On regular medications	11	50
Atrial fibrillation		
No	131	97
Yes	4	3
HIV status		
Negative	131	97
Positive	4	3
Waist-hip ratio		
Normal	83	61.5
Increased	52	38.5
High blood pressure on admission (≥140/90 mmHg)		
No	26	19
Yes	109	80.7
Stroke type* (*n* = 125)		
Hemorrhagic	44	35.2
Ischemic	81	64.8
Functional status on admission		
mRS 0–3	60	44.4
mRS 4–5	75	55.6
Median mRS (IQR)	4 (3, 4)	
NIHSS score on admission		
Median (IQR)	20.5 (17, 24)	
Medical complications		
No complications	72	53.3
Presence of complications	63	46.7

mRS, modified Rankin scale; NIHSS, National Institute of Health Stroke Scale.
*A total of 10 patients had a normal CT brain scan.

Post-stroke medical complications occurred in 46.7% (63/135) of patients. Confirmed aspiration pneumonia was the leading medical complication, seen in 41% (26/63), followed by probable aspiration pneumonia in 21% (13/63) and pyrexia of unknown cause in 14% (9/63) (Figure 2).

Neuroimaging and ECG abnormalities among patients with hemorrhagic stroke

Neuroimaging and ECG features of the study patients with hemorrhagic stroke are presented in Table 3. A quarter (11/44) had a hemorrhage in multiple locations (both the cortical and subcortical regions), followed by the thalamus in 22.7% (10/44). In more than half (24/44), the volume of hematoma exceeded 30 ml and 63.6% (28/44) had an intraventricular extension. ECG abnormalities were present in 54.6% (24/44); the most common patterns were T-wave inversion in 34.1% (15/44), followed by ST segment changes in 29.6% (13/44).

Neuroimaging and ECG abnormalities among patients with ischemic stroke

The majority were observed to have multi-territory infarction; 62.9% (51/81) and 53.1% (43/81) had midline shifts. ECG abnormalities were present in 46.9% (38/81); the most common patterns included T-wave inversion in 38.3% (31/81), followed by ST changes in 30.9% (25/81) (Table 4).

Survival experience and mortality by stroke subtype

Approximately one-third (50/135) of the patients with stroke had died by 30-day follow-up. Overall, one-quarter of the patients had died by day 17 [25th percentile survival time (in days): 17 (95% CI: 9–24)] (Figure 3). Comparing patients with hemorrhagic and ischemic stroke, 25% of the patients had died by day 5 [25th percentile survival time (in days): 5 (95% CI: 2–14)] compared with day 23 [25th percentile survival time (in days): 23 (95% CI: 11–30)], respectively (log-rank $p < 0.001$) (Figure 4).

Predictors of 30-day mortality

In the multivariable-adjusted analysis, independent predictors of 30-day mortality were NIHSS score on admission (30-day mortality increased by 9% for every unit increase in NIHSS score) [aHR 1.09 (95% CI: 1.02–1.17), $p = 0.012$], degree of disability on admission (mRS 4–5) [aHR 5.50 (95% CI: 2.02–15.04), $p = 0.001$], aspiration pneumonia [aHR 3.69 (95% CI: 1.51–13.6), $p = 0.005$], and ECG abnormalities [aHR 2.28 (95% CI: 1.86–5.86), $p = 0.044$] (Table 5).

Discussion

In this study, we found that the 30-day post-stroke mortality rate was 37%. This mortality is high compared to mortality rates observed in HIC; for instance, the rate is 12.7% in patients admitted to Canadian stroke services (22). Furthermore, our mortality rate is higher than other comparable studies conducted in LMIC, for example, Nigeria, where the 30-day mortality rate was 23.8% (23). The differences in mortality could be attributed to the nature of the study design: The Nigerian study was a retrospective study of first-ever strokes, whereas this study was prospective and included

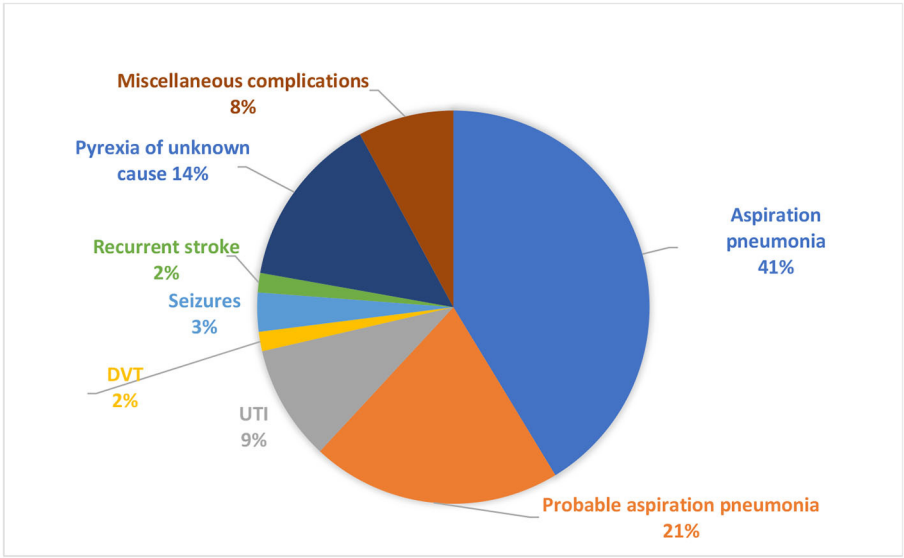

FIGURE 2
Medical complications post-stroke.

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TABLE 3 Neuroimaging and ECG abnormalities among patients with hemorrhagic stroke ($N = 44$).

Characteristic	Frequency	Percentage
Hemorrhage location		
Basal ganglia	9	20.4
Frontal lobe	2	4.6
Parietal lobe	3	6.8
Temporal lobe	6	13.6
Thalamus	10	22.7
Pontine	1	2.3
External capsule	2	4.6
Multiple locations	11	25.0
Hemorrhage size (ml)		
<30	20	45.4
≥30	24	54.6
Presence of intraventricular extension		
No	16	36.4
Yes	28	63.6
Intracerebral hemorrhage score (ICH)		
<3	24	54.6
≥3	20	45.4
ECG abnormalities		
No	20	45.4
Yes	24	54.6
ST changes		
No	31	70.4
Yes	13	29.6
T-wave inversion		
No	29	65.9
Yes	15	34.1
U wave		
No	36	81.8
Yes	8	18.2

TABLE 4 Neuroimaging and ECG abnormalities among patients with ischemic stroke ($N = 81$).

Characteristic	Frequency	Percentage
Infarct location ($n = 81$)		
Occipital lobe	3	3.7
Basal ganglia	4	4.9
Frontal lobe	3	3.7
Parietal lobe	7	8.6
Temporal lobe	4	4.9
Thalamus	4	4.9
Pontine	2	2.5
External capsule	3	3.8
Multi-territory	51	62.9
ASPECT score		
<7	40	55.6
≥7	32	44.4
Midline shift		
No	38	46.9
Yes	43	53.1
Hemorrhagic transformation		
No	69	85.2
Yes	12	15.8
Hemorrhagic transformation subtypes*		
HI1	8	66.7
HI2	3	25
PH1	1	8.3
PH2	0	0
ECG abnormalities		
No	43	53.1
Yes	38	46.9
ST changes		
No	56	69.1
Yes	25	30.9
T-wave inversion		
No	50	61.7
Yes	31	38.3
U wave		
No	80	98.8
Yes	1	1.2

HI, hemorrhagic transformation; PH, parenchymal hematoma, *N = 12.

all strokes (first and recurrent). Our findings are quite alarming and highlight the barriers associated with the management of acute stroke in a resource-limited setting. It has been well demonstrated that the presence of specialized stroke units, specialists, the use of intravenous thrombolytic agents, and endovascular procedures are highly effective at reducing morbidity and mortality from stroke (24). However, these services and resources are neither readily available nor affordable in SSA. In the current study, half of the patients had no health insurance coverage, which is a major barrier to accessing affordable, good-quality healthcare services for patients with stroke in Tanzania. A recent systematic review on health financing for universal health coverage in SSA reported that 27 out of 48 countries are affected by direct out-of-pocket payments for healthcare services of >30%. Therefore, the cost is likely to be a significant barrier to accessing healthcare, thus contributing to the high burden of preventable deaths (21).

In the present study, patients with hemorrhagic stroke had higher mortality than those with ischemic stroke, with one-quarter of patients dying by day 5 vs. 23. This is similar to

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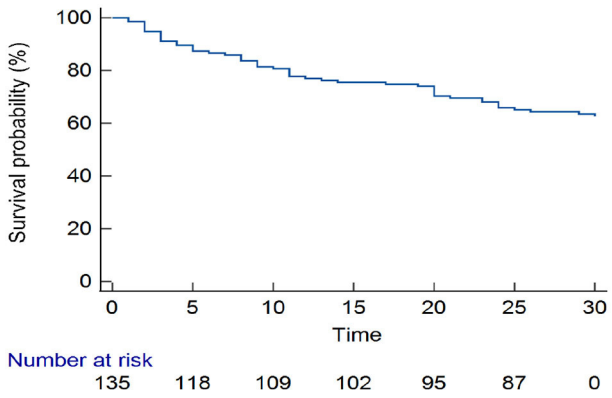

FIGURE 3
Kaplan–Meier curve estimating overall survival experience.

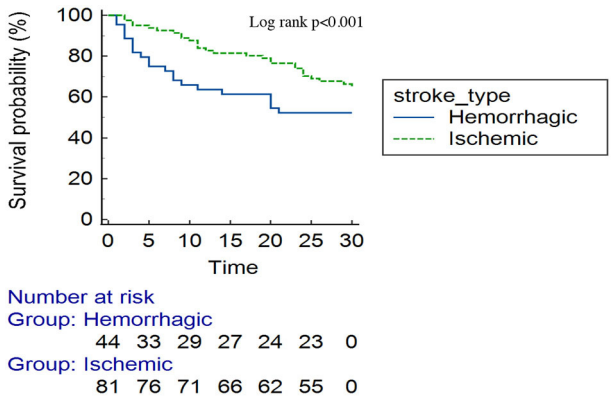

FIGURE 4
Kaplan–Meier survival curve by stroke subtype.

global data, where the hemorrhagic stroke is associated with poor outcomes compared to ischemic stroke (25). Hypertensive hemorrhagic stroke results from the rupture of the thinner-walled perforating arteries, which supply the sub-cortical regions of the brain (26). Untreated, ongoing hypertension increases the risk of further bleeding, hematoma expansion, and intraventricular extension. In the present study, more than half (56.4%) had a hematoma size of ≥30 ml, and intraventricular extension was observed in 63.6% of patients. Similarly, elevated blood pressure readings were observed in more than two-thirds of patients (80.7%). Uncontrolled hypertension appears to be a major etiology for stroke, particularly the hemorrhagic stroke subtype in the current study, as almost one-half of the bleeds were located in the sub-cortical regions (particularly the basal ganglia and the thalamus), signifying a hypertensive etiology. Hypertension is a leading risk factor for stroke in Tanzania, and its early detection, treatment, and control cannot be overemphasized (5). This is a call for increasing community awareness for screening and treating hypertension in SSA. Furthermore, our findings also highlight the need for multidisciplinary stroke management on dedicated stroke units, with input from neurosurgeons for consideration of hematoma evacuation, decompressive surgery, or placement of an external ventricular drain.

TABLE 5 Predictors of 30-day mortality.

Variable	HR (95%CI)	*p*-value	aHR (95%CI)	*p*-value
Age				
<60	1		1	
≥60	1.14 (0.64–2.05)	0.654	0.57 (0.22–1.50)	0.261
Gender				
Female	1		1	
Male	1.04 (0.60–1.82)	0.886	2.25 (0.83–6.05)	0.109
Smoking				
No	1			
Yes	1.10 (0.60–2.02)	0.756		
Alcohol				
No	1			
Yes	1.00 (0.54–1.84)	0.987		
Waist-hip ratio				
Normal	1			
Increased	1.05 (0.59–1.89)	0.859		
BP on admission ≥140/90				
No	1			
Yes	1.53 (0.69–3.40)	0.299		
Fever				
No	1			
Yes	1.18 (0.18–7.30)	0.861		
mRS				
mRS 0–3	1		1	
mRS 4–5	3.52 (1.80–6.89)	<0.001	5.50 (2.02–15.04)	0.001
Medical complications				
No complications	1		1	
Aspiration pneumonia	13.95(6.84–28.45)	<0.001	3.69 (1.51–13.69)	0.005
Others	1.91 (0.92–3.96)	0.081	1.15 (0.36–2.41)	0.815
NIHSS on admission	3.52 (1.80–6.89)	<0.001	1.09 (1.02–1.18)	0.012
Stroke subtype				
Hemorrhagic	1		1	
Ischemic	0.59 (0.33–1.04)	0.066	1.61 (0.61–4.26)	0.335
ECG abnormalities				
No	1		1	
Yes	2.21 (1.24–3.95)	0.007	2.28 (1.86–5.86)	0.044
Previous stroke				
No	1		1	
Yes	0.65 (0.12–3.51)	0.622	0.43 (0.08–2.28)	0.335
Hyperglycemia				
No	1			
Yes	1.31 (0.53–3.24)	0.608		

Our study found that severe stroke, severe neurological impairment, aspiration pneumonia, and ECG abnormalities were independent predictors for 30-day mortality. High NIHSS and mRS scores are known predictors for 30-day mortality (27, 28). Notably, patients with stroke in the current study presented late to the hospital from the time of stroke onset (mean time of 2.67 days). This delay could be a major contributor to high admission NIHSS scores, stemming from a lack of community awareness of early recognition of stroke symptoms and signs and challenges in the overall healthcare infrastructure in Tanzania. Similarly, the relatively high admission NIHSS may also indicate that patients with milder strokes are not routinely accessing healthcare. Patients with severe neurological deficits have a high risk of medical complications such as aspiration pneumonia. This is in line with other studies and is a leading cause of early mortality in SSA (11). This is a call to advocate for specialized stroke units in Tanzania to help manage patients with stroke to prevent or reduce stroke-related complications.

Overall, ECG abnormalities were observed in more than one-third (46%) of the patients. The most common patterns in both stroke subtypes were as follows: ST changes (46.9 vs. 29.6%) and T-wave inversion (38.3 vs. 34.1%) for ischemic and hemorrhagic stroke, respectively, in line with previous studies (29). ECG abnormalities are a result of bidirectional interaction between the brain and the heart. This is thought to be caused by the over-activation of sympathetic activity and the hypothalamic–pituitary–adrenal axis, as well as immune and inflammatory responses resulting in brain dysregulation following an acute stroke. Similarly, the release of catecholamines causes vasoconstriction of peripheral and coronary vessels, which leads to myocardial ischemia (29). Therefore, there is a need for continuous electrocardiographic monitoring among patients with stroke to reduce early mortality.

Our study is limited by the following: It was a single center with a small sample size. Therefore, the results may not be generalizable. Most patients did not have a baseline ECG, so the changes observed might be attributed to other medical conditions. There may be other unmeasured or unknown confounders responsible for the observed data, including interdisciplinary and nursing input and timing of antithrombotic or anti-hypertensive therapy. We did not collect data on the final causes of death, which limits our ability to draw further inferences. Continuous ECG (telemetry) was not performed due to limited resources.

Conclusion

In this present study, stroke is associated with a high 30-day mortality rate in Northwestern Tanzania. The hemorrhagic stroke subtype had the highest mortality, and independent predictors of death included higher NIHSS on admission with severe disabilities, aspiration pneumonia, and ECG abnormalities. Concerted efforts are warranted in the prevention and management of patients with stroke to reduce the associated morbidity and mortality.

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 joint Catholic University of Health and Allied Sciences-Bugando Medical Center Institutional Review Board approval number CREC/528/2022. The study was carried out in accordance with the tenets of the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization of the study: SSM, KM, FK, and GM. Data collection: GM, JS, LA, and JN. Interpretation of the results: GM, SSM, and PN. Data analysis: GM and SSM. Writing the initial manuscript: SSM. Critically reviewing and revising the manuscript: RA, FK, PN, BT, KK, RM, MM, FS, and KM. 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.

Publisher's note

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One-stop stroke management platform reduces workflow times in patients receiving mechanical thrombectomy

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Background and purpose: Clinical outcome in patients who received thrombectomy treatment is time-dependent. The purpose of this study was to evaluate the efficacy of the one-stop stroke management (OSSM) platform in reducing in-hospital workflow times in patients receiving thrombectomy compared with the traditional model.

Methods: The data of patients who received thrombectomy treatment through the OSSM platform and traditional protocol transshipment pathway were retrospectively analyzed and compared. The treatment-related time interval and the clinical outcome of the two groups were also assessed and compared. The primary efficacy endpoint was the time from door to groin puncture (DPT).

Results: There were 196 patients in the OSSM group and 210 patients in the control group, in which they were treated by the traditional approach. The mean DPT was significantly shorter in the OSSM group than in the control group (76 vs. 122 min; $P < 0.001$). The percentages of good clinical outcomes at the 90-day time point of the two groups were comparable ($P = 0.110$). A total of 121 patients in the OSSM group and 124 patients in the control group arrived at the hospital within 360 min from symptom onset. The mean DPT and time from symptom onset to recanalization (ORT) were significantly shorter in the OSSM group than in the control group. Finally, a higher rate of good functional outcomes was achieved in the OSSM group than in the control group (53.71 vs. 40.32%; $P = 0.036$).

Conclusion: Compared to the traditional transfer model, the OSSM transfer model significantly reduced the in-hospital delay in patients with acute stroke receiving thrombectomy treatment. This novel model significantly improved the clinical outcomes of patients presenting within the first 6 h after symptom onset.

KEYWORDS

stroke, time, puncture, thrombectomy, outcome

Introduction

Thrombectomy recanalization of large-vessel occlusion in acute ischemic stroke is strongly time-dependent, and thus shortening the time between onset and recanalization can improve the prognosis of patients (1, 2). Hence, several pre-hospital and in-hospital measures for improvement have been implemented to shorten the time from onset to recanalization (ORT) (3, 4).In this regard, the development of a green channel that integrates medical specialists in multidisciplinary cooperation for hospital treatment of acute stroke considerably shortened the time from the door admission to recanalization treatment (5).

In most stroke centers, patients undergo imaging examination at an imaging center and are then transferred to the emergency department to receive intravenous thrombolysis and then transferred to the catheter room for thrombectomy. However, the long transfer times often cause delays in the treatment of patients with stroke. The one-stop stroke management (OSSM) platform combines computed tomography (CT), magnetic resonance imaging (MRI), and digital subtraction angiography (DSA) equipment in one space, using the same track to transfer patients from one device to another without switching beds. Thus, preoperative imaging examination and thrombectomy procedure are completed in the same space. Such an integrated combination of examination and treatment can dramatically shorten in-hospital transportation time. Our center, in collaboration with Siemens Healthcare, developed an OSSM platform to reduce the in-hospital delays of patients with acute stroke. In this study, we compared the data of patients who received thrombectomy *via* the OSSM platform with those who underwent the procedure *via* the traditional workflow to establish the effect of the OSSM platform model on the reduction of the delay of in-hospital stroke treatment.

Methods

The data of patients who received thrombectomy through the OSSM platform and those of patients who underwent thrombectomy through the protocol transshipment pathway, defined as the control group, were retrospectively analyzed.

Inclusion criteria

All the included patients received thrombectomy treatment at our center between January 2017 and December 2021. The data were retrieved from a prospectively maintained database. The inclusion criteria were as follows: (1) aged ≥18 years; (2) treated within 24 h from onset; (3) the National Institutes of Health Stroke Scale (NIHSS) ≥6; (4) Alberta Stroke Program Early Computed Tomography Score (ASPECT) ≥6; (5) pre-stroke Modified Rankin Scale (mRS) score ≤1; (6) the treatment protocols were consistent with those in the OSSM group or the control group; (7) the follow-up data were completed; and (8) informed consent was received from the patient or a family member.

Treatment protocol

In our center, patients with acute stroke typically arrive at the emergency department are then evaluated by a stroke physician, and receive the necessary treatment. The patients in the OSSM group were transferred to the OSSM platform for imaging examination, followed by thrombectomy. Patients who met the criteria of intravenous thrombolytic therapy (IVT) received IVT in the platform before thrombectomy therapy (Figure 1). Patients in the traditional protocol transshipment pathway group were transported from the emergency department to the imaging center for pretreatment imaging examination. Which imaging modality to choose was a comprehensive consideration based on the patient's actual condition, including the time from onset, severity, and the presence or absence of contraindications. Imaging examinations included noncontrast CT, CTP, MRI, and PWI, as well as noninvasive vascular imaging including CTA and MRA. Patients were then transferred to the catheter unit for thrombectomy therapy. Mechanical thrombectomy was performed under local or general anesthesia; heparin was used selectively. Endovascular thrombectomy methods included stent retrieval, aspiration thrombectomy, balloon dilatation, stent implantation, or other treatments based on the specific subject's clinical conditions.

Relevant efficacy observation and follow-up examinations

The primary efficacy endpoint was the time from door to groin puncture (DPT). The secondary efficacy endpoints were the time from imaging to groin puncture (IPT), the time from door to successful reperfusion (DRT) or final angiographic results, the time from symptom onset to successful reperfusion (ORT), and the time from groin puncture to successful reperfusion (PRT). The rate of successful reperfusion of target vessels (modified thrombolysis in cerebral infarction, mTICI ≥ 2b) was defined as successful reperfusion; the rate of good functional independence was defined as an mRS score from 0 to 2 for a postoperative follow-up period of 90 ± 14 days. Safety outcomes included the rate of symptomatic intracranial hemorrhage (sICH) and all-cause mortality during the follow-up period. The complications of sICH were defined as any form of intracranial hemorrhage with an increase in NIHSS score of ≥ 4 points during the perioperative period.

Statistical analysis

Statistical analysis was performed using the SPSS version 22.0 software. Continuous data that met normal distribution were represented with interquartile range or means with SD, comparison between groups used independent sample *t*-test, whereas categorical data were expressed as several cases and percentages. $P < 0.05$ was considered to be statistically significant.

Results

A total of 406 patients were included in the analysis, of whom 196 were in the OSSM group and 210 were in the control group. The mean age was 63 years, the mean NIHSS score was 15, and the mean ODT was 407 min. The baseline data of the two groups of patients are presented in Table 1. No statistically significant differences were observed between the baseline data of the two groups, such as the baseline NIHSS score, gender, ASPECT, occlusion location, and the time from symptom onset to hospital admission. Furthermore, no significant differences were detected in the risk factors, such as a history of hypertension, diabetes, stroke history, and atrial fibrillation.

The mean DPT was 76 min in the OSSM group and 122 min in the control group ($P < 0.001$). Notably, a 44 min reduction was observed in the OSSM group compared to the control group. The median DIT was significantly shorter in the OSSM group than in the control group (30 vs. 36 min; $P < 0.001$). The mean DPT was 47 in the OSSM group, whereas it was 86 min in the control group ($P < 0.001$). The mean

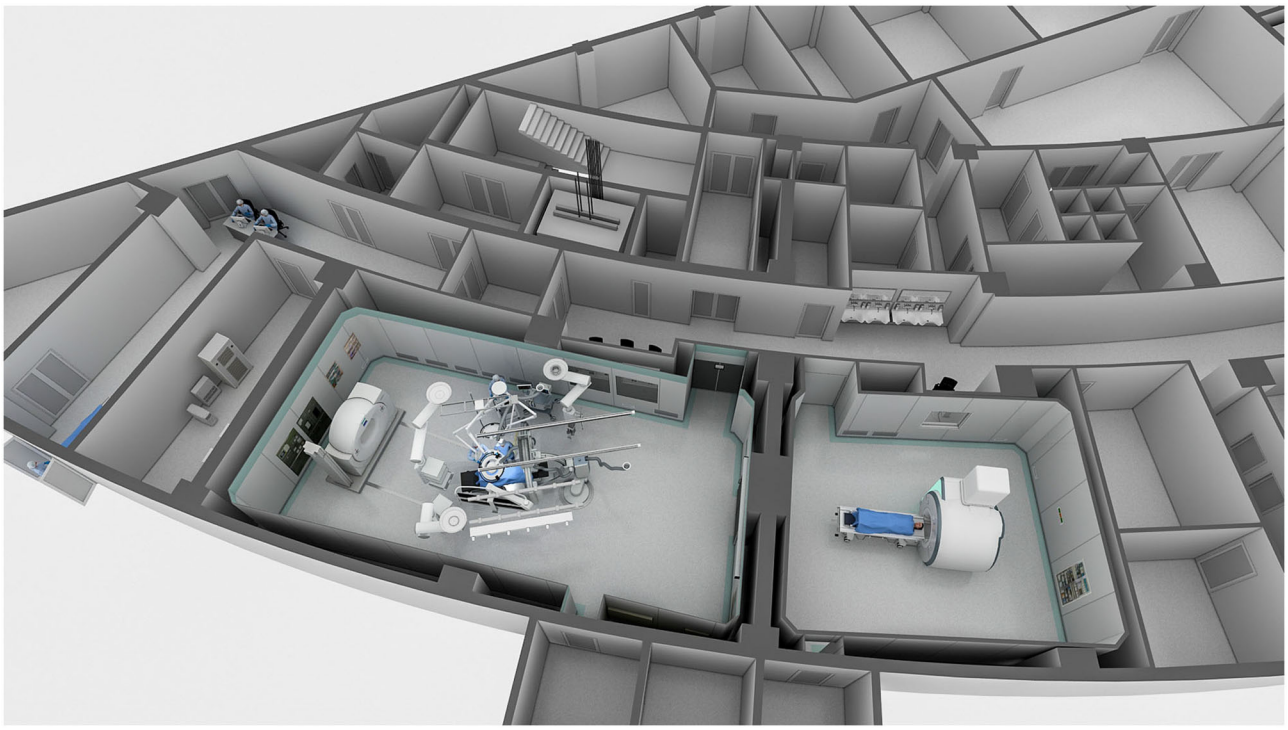

FIGURE 1
This overview of the one-stop stroke management (OSSM) platform shows the layout of the computed tomography (CT), magnetic resonance imaging (MRI), and digital subtraction angiography (DSA) equipment rooms.

DRT was 165 min in the OSSM group, whereas it was 212 min in the control group, and the difference between the two groups was statistically significant ($P < 0.001$). The mean PRT was comparable between the two groups (88 vs. 89 min; $P = 0.701$). The mean ORT was shorter in the OSSM group (572 min) than in the control group (618 min) but with no significant difference between the two groups ($P = 0.114$).

There was no significant between-group difference in the rate of successful reperfusion (90.82 vs. 89.05%, $P = 0.554$). The percentages of good clinical outcomes at the 90-day follow-up examination were 47% in the OSSM group and 40% in the control group. No statistical differences were present between the two groups ($P = 0.110$). The rate of all-cause 90-day mortality was 17% in the OSSM group and 20% in the control group ($P = 0.526$), the rate of symptomatic hemorrhage was 8% in the OSSM group and 10% in the control group, and the rate did not differ significantly across groups in pairwise comparisons ($P = 0.606$).

Subgroup analyses

A total of 245 patients arrived at the hospital within 360 min from symptom onset, of whom 121 were in the OSSM group and 124 were in the control group (Table 2). There were no statistically significant differences in the baseline data between the two groups. Their mean ODT and PRT were comparable. In the OSSM group, the mean DPT, IRT, DRT, and ORT were significantly shorter than those in the control group. There was no significant between-group difference in the rate of successful reperfusion (90.82 vs. 89.05%, $P = 0.554$. A higher rate of good functional outcomes was achieved in the OSSM group than in the control group (53.71 vs. 40.32% $P = 0.036$). The rate of all-cause 90-day mortality and sICH were comparable between the two groups.

Discussion

This retrospective cohort study showed that the OSSM platform incorporating CT, MRI, and DSA equipment can significantly reduce in-hospital delays in the thrombectomy treatment of patients with stroke compared to traditional workflow. The patients who arrived in the OSSM platform of the hospital within 6 h from symptom onset had a significantly shorter DPT and a better clinical outcome than those in the traditional protocol transshipment pathway.

Early ischemic brain reperfusion is critical for achieving good clinical outcomes in patients with stroke. A meta-analysis of five randomized clinical trials showed that of every 1,000 patients in whom substantial endovascular reperfusion was achieved; for every 15-min faster emergency department door-to-reperfusion time, an estimated 39 patients would have had a less-disabled outcome at 3 months (1). In the ESCAPE trial, the shorter imaging-to-reperfusion time significantly improved the chance of achieving a functionally independent outcome (6). A number of measures have been taken to reduce delays in the treatment of patients with stroke, particularly in improving the process of in-hospital imaging examination (7, 8).

Compared with multidetector CT, the latest generation of flat-panel CT (FPCT) is of high diagnostic value in the detection of ischemic changes. In addition, occluded vessels and cerebral collaterals can be detected with FP-CTA, and make it possible to be

TABLE 1 Comparison of data between the two groups of patients underwent thrombectomy therapy.

	All (406)	OSSM group (196)	Control group (210)	*P*
Age (y)	63.3 ± 12.5 63.5 (21–91)	63.5 ± 12.6 63.5 (30–91)	63.0 ± 12.5 63 (21–89)	0.529
Male	271 (66.75%)	129 (65.92%)	142 (67.62%)	0.752
Hypertension	247 (60.84%)	120 (61.22%)	127 (60.48%)	0.919
Diabetes	104 (25.62%)	50 (25.51%)	54 (25.71%)	1.000
Stroke history	88 (21.67%)	46 (23.47%)	42 (20%)	0.402
Smoking history	87 (21.43%)	43 (21.94%)	44 (20.95%)	0.810
History of atrial fibrillation	168 (41.38%)	81 (41.33%)	87 (41.43%)	0.100
Baseline NIHSS	15.2 ± 5.0 15 (6–32)	15.3 ± 5.2 15 (6–32)	15.2 ± 4.8 15 (6–32)	0.858
Intravenous thrombolysis	57 (14.04%)	25 (12.75%)	32 (15.24%)	0.479
ASPECT score	7.606 ± 0.98 (6–10)	7.597 ± 0.9953 8 (6–10)	7.614 ± 0.9678 8 (6–10)	0.859
General anesthesia	393 (96.80%)	191 (97.45%)	202 (96.19)	0.472
Occlusion location				
ICA	105 (25.86%)	52 (26.53%)	53 (25.24%)	0.821
MCA	173 (42.61%)	84 (42.86%)	89 (42.38%)	1.000
ICA-MCA	40 (9.85%)	21 (10.71%)	19 (9.05%)	0.303
VA-BA	88 (21.67%)	39 (19.90%)	49 (23.33%)	0.470
Time intervals (min)				
ODT	406.6 ± 289.2	407.3 ± 297.2	405.9 ± 282.2	0.961
DPT	99.8 ± 3 0.5 99 (32–236)	76.4 ± 20.7 76 (32–191)	121.6 ± 20.2 121 (81–236)	0.001
DIT	32.9 ± 10.9 32 (13–88)	29.8 ± 11.0 28 (13–88)	35.8 ± 10.0 34 (19–76)	0.000
IPT	66.9 ± 28.3 63 (13–211)	46.7 ± 18.2 43 (13–121)	85.8 ± 22.3 85.5 (39–211)	0.000
PRT	88.7 ± 44.0 79 (15–320)	88.2 ± 43.7 78.5 (15–320)	89.3 ± 44.4 79 (15–320)	0.801
DRT	189.1 ± 52.8 188 (80–431)	164.6 ± 48.2 156.5 (80–396)	211.9 ± 46.4 202 (116–431)	0.000
ODT	406.6 ± 289.2 300 (30–1,440)	407.3 ± 297.2 300 (30–1,440)	405.9 ± 282.2 300 (30–1,440)	0.961
ORT	595.7 ± 292.1 496.5 (196–1,726)	571.9 ± 299.7 471.5 (196–1,726)	617.9 ± 283.8 526 (230–1,639)	0.114
mTICI2b/3	365 (89.90%)	178 (90.82%)	187 (89.05%)	0.554
mRS (0-2) at 90 days	176 (43.35%)	93 (47.45%)	83 (39.52%)	0.110
sICH	37 (9.11%)	16 (8.16%)	21 (10%)	0.606
Mortality	76 (18.72%)	34 (17.35%)	42 (20%)	0.526

ODT, time from symptom onset to door; DPT, time from door to groin puncture; DIT, time from door to imaging; IPT, time from imaging to groin puncture; PRT, time from groin puncture to reperfusion; DRT, time from door to reperfusion; ODT, time from symptom onset to door; ORT, time from symptom onset to reperfusion.
NIHSS, The National Institutes of Health Stroke Scale; ASPECT, Alberta Stroke Program Early Computed Tomography Score; ICA, Internal carotid artery; MCA, Middle cerebral artery, VA, vertebral artery; BA, Basilar artery. mTICI, modifiied thrombolysis in cerebral infarction; sICH, symptomatic intracranial hemorrhage; mRS, modified Rankin scale.

used as a peri-interventional diagnostic tool. Bypassing the CT suite, with direct transfer to angiosuite (DTAS), has been implemented in many centers and has contributed to a significant reduction in hospital workflow times (9). Marios-Nikos reported the case of a first mothership patient who was transported directly to the angiography suite and received nonenhanced FPCT (10). This patient was diagnosed with large-vessel occlusion, based on the FPCT-angiography results, and treated by endovascular thrombectomy, with a door-to-groin time (DNT) reduction to 23 min (10). A prospective, randomized trial showed that patients with acute stroke who were directly transferred to the angiosuite (DTAS) and received noncontrast-enhanced FPCT had a significantly reduced time from

TABLE 2 Comparison of data between the two groups of patients underwent thrombectomy therapy within 6h from symptom onset.

	All (245)	OSSM group (121)	Control group (124)	*P*
Age (y)	62.9 ± 12.7 63 (30–91)	63.9 ± 12.6 65 (30–91)	61.9 ± 12.7 62 (30–89)	0.205
Male	163 (66.53%)	78 (64.46%)	85 (68.55%)	0.498
Hypertension	149 (60.82%)	74 (61.15%)	75 (60.48%)	1.000
Diabetes	58 (23.67%)	28 (23.14%)	30 (24.19%)	0.846
Stroke history	52 (21.22%)	25 (20.67%)	27 (21.77%)	0.831
Smoking history	46 (18.78%)	21 (17.35%)	25 (20.16%)	0.574
History of atrial fibrillation	105 (42.86%)	51 (42.15%)	54 (43.55%)	0.825
Baseline NIHSS	15.2 ± 4.7 15 (6–32)	15.4 ± 5.1 15 (6–32)	15.0 ± 4.3 15.5 (6–32)	0.51
Intravenous thrombolysis	38 (15.51%)	17 (14.04%)	21 (16.94%)	0.533
ASPECT score	7.6 ± 1.0 8 (6–10)	7.7 ± 1.0 8 (6–10)	7.6 ± 1.0 8 (6–10)	0.305
General anesthesia	235 (95.92%)	117 (96.69%)	118 (95.16%)	0.544
Occlusion location				
ICA	73 (29.80%)	36 (29.75%)	37 (29.84%)	0.988
MCA	98 (40%)	50 (43.12%)	48 (38.71%)	0.676
ICA-MCA	20 (8.16%)	9 (7.44%)	11 (8.87%)	0.682
VA-BA	54 (22.04%)	26 (21.49%)	28 (22.58%)	0.837
Time intervals (min)				
ODT	214.7 ± 82.7 240 (30–360)	216.2 ± 83.7 240 (30–360)	213.3 ± 82.0 240 (30–360)	0.782
DPT	99.6 ± 31.9 99 (36–236)	76.8 ± 23.6 76 (36–191)	121.8 ± 21.6 121 (90-236)	0.000
DIT	32.6 ± 11.2 32 (13–88)	29.3 ± 11.3 27 (13–88)	35.8 ± 10.2 31 (21–76)	0.000
IPT	67.0 ± 29.1 63 (13–211)	67.0 ± 29.1 44 (13–121)	86.0 ± 23.2 84 (39–211)	0.000
PRT	88.1 ± 41.8 79 (12–320)	87.2 ± 41.6 79 (15–320)	88.9 ± 42.1 81 (12-220)	0.762
DRT	188.5 ± 52.3 186 (81–369)	164.0 ± 46.9 156 (81–369)	212.3 ± 46.1 202 (116–320)	0.000
ODT	212.3 ± 46.1 240 (30–360)	216.2 ± 83.7 240 (30–360)	213.3 ± 82.0 240 (30–360)	0.782
ORT	403.2 ± 93.9 406 (169–619)	380.2 ± 91.9 381 (169–587)	425.6 ± 90.7 436 (230–619)	0.000
mRS (0–2) at 90 days	115 (46.94%)	65 (53.71%)	50 (40.32%)	0.036
sICH	23 (9.39%)	11 (9.09%)	12 (9.68%)	0.875
Mortality	40 (16.33%)	18 (14.88%)	22 (17.74%)	0.544

ODT, time from symptom onset to door; DPT, time from door to groin puncture; DIT, time from door to imaging; IPT, time from imaging to groin puncture; PRT, time from groin puncture to reperfusion; DRT, time from door to reperfusion; ODT, time from symptom onset to door; ORT, time from symptom onset to reperfusion.
NIHSS, The National Institutes of Health Stroke Scale; ASPECT, Alberta Stroke Program Early Computed Tomography Score; ICA, Internal carotid artery; MCA, Middle cerebral artery, VA, vertebral artery; BA, Basilar artery. mTICI, modifified thrombolysis in cerebral infarction; sICH, symptomatic intracranial hemorrhage; mRS, modified Rankin scale.

stroke imaging to groin puncture (by ∼7 min) in comparison with the CT transit (CTT) pathway (11). Another study showed that DTAS protocols significantly increased the odds ratio of achieving a favorable outcome (12).

However, it should be pointed out that patients with DTAS directly transported to the angiography suite and received a noncontrast enhanced FPCT as preoperative imaging examination but not a CT-angiography or perfusion imaging, and as a result, patients evaluated by FPCT did not have large-vessel occlusion. Mendez reported the cases of patients, $n = 7/97$ (7.2%), whose initial angiogram did not show a treatable occlusion (12). The study of Psychogios included patients with NIHSS score > 10, but large-vessel occlusion was detected in the angiograms of only 18 of 30 patients (13). In addition, blindly transferring patients directly to

the angiography room for plate CT examination also interferes with the normal operation of the C-arm for patients undergoing endovascular treatment. In our study, we creatively integrated CT, MR, and C-arm into the same space, and noninvasive vascular examinations such as CTA and MRA were performed to determine whether the patients were suitable for thrombectomy. For patients beyond 6 h from symptom onset, multimodal imaging was to be employed for treatment guidance, which was hard to be completed by FPCT.

In the present study, the OSSM model significantly shortened DPT compared to the traditional model. However, the time from admission to treatment was still long in comparison with those reported in previous publications, since more than 97% of the patients received general anesthesia during the operation, which requires a longer time than local anesthesia. For the majority of the patients, the preoperative imaging evaluation of patients in our center was based mainly on MR results, and thus a longer time was needed to complete an MR scan than a CT examination. This was also one of the main reasons that might have led to a longer delay.

It should be pointed out that with the extension of the thrombectomy time window to 24 h (14, 15), part of the thrombectomy patients arrived in the hospital beyond the 6-h time point. In this investigation, the median time from symptom onset to hospital admission was 6.7 h. The model described here did not significantly shorten the time from symptom onset to treatment; it did not significantly improve the clinical outcomes of the patients either. For patients who arrive at the hospital for treatment within a short time from onset, the OSSM transport mode is more likely to reduce the time from symptom onset to treatment, which can improve the prognosis of these patients compared with those in whom the traditional transport mode has been implemented.

Our study has some limitations. First of all, this was a retrospective analysis and comparison with a retrospective patient cohort. Certain selection biases in the baseline data might have been present, although the primary baseline data did not differ significantly between the two groups. Second, the study was carried out in a single center, and the equipment distribution, personnel allocation, and patient treatment process might have been different from those employed in other centers. Thus, the results of this study may not be applicable to other centers.

In conclusion, compared to the traditional transfer model, the OSSM platform significantly reduced the in-hospital delay in patients with acute stroke who received thrombectomy treatment. Furthermore, this model significantly improved the clinical outcomes in patients presenting within the first 6 h after symptom onset.

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 Henan Provincial People's Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

TZ: conception and design, drafting the article, and revising it critically for important intellectual content. TL: conception and design and final approval of the version to be published. LZ, ZL, QL, ZW, LW, YH, YL, ZZ, MG, ZM, Xp, SM, YF, GZ, WZ, XL, and MW: acquisition of data, analysis, and interpretation of data. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

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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Serum uric acid to serum creatinine ratio predicts neurological deterioration in branch atheromatous disease

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Background and objective: Branch atheromatous disease (BAD) makes patients prone to early neurological deterioration (END), resulting in poor prognosis. The aim of this study was to investigate the association between SUA/SCr and END in BAD stroke patients.

Methods: We conducted a retrospective study that included 241 patients with BAD-stroke within 48 h of symptom onset. We divided the patients into the END group and the no END group. END was defined as an NIHSS score increase of more than 2 points within 1 week. SUA/SCr was calculated by the concentration of serum uric acid and creatine (serum uric acid/serum creatine) on admission. Univariate and multivariate analyses were used to identify independent predictors of END in BAD-stroke patients.

Results END was observed in 24.1% (58/241) of the patients in our study. Multiple logistic regression analyses showed that SUA/SCr (aOR, 0.716; 95% CI, 0.538–0.952; $P = 0.022$) and female sex (aOR, 0.469; 95% CI, 0.245–0.898; $P = 0.022$) were associated with END after adjusting for confounding factors. The predicted value of SUA/Scr for END was a sensitivity of 79.3%, a specificity of 44.8%, and an AUC of 0.609 (95% CI, 0.527–0.691, $P < 0.05$). The optimal cut-off value was 4.76.

Conclusion: SUA/SCr was negatively associated with the risk of END in BAD stroke patients.

KEYWORDS

branch atheromatous disease, early neurological deterioration, SUA/SCr, uric acid, prognosis

1. Introduction

Branch atheromatous disease (BAD), which was initially put forward by Caplan in 1989, is a specific type of stroke caused by atheromatous occlusion at the orifice of large caliber penetrating arteries, is characterized by special MRI manifestations and makes patients prone to neurological deterioration in the early phase (1–4). Although the definition of BAD has not been fully set up yet, it is universally accepted that BAD is a single subcortical infarction and lack of severe stenosis of the parent artery that supplies the regions of deep perforators (5). The study indicated that BAD might show a larger lesion size and a greater tendency of neurologic worsening than lacunar infarction, although both disorders are forms of intracranial deep brain infarction (6). One large study found that the incidence of BAD in ischaemic stroke was 9.74%; however, the incidence of END was as high as 39.4% (7). Previous studies have shown that early neurological deterioration (END) in BAD patients is associated with various factors, such as infarct size, infarct location, female sex, severe neurological deficit, and platelet parameters (4, 8–12). Given the strong association between END and long-term clinical outcome, the END had been the most attentive clinical problem in BAD. So it is significant to assess the risk of END.

Serum uric acid (SUA) was reported to be associated with the development and prognosis of cerebrovascular disease (13–17). Some studies showed that hypouricaemia was related to reduced neurological deterioration, improved outcome and lower in-hospital mortality in patients with cerebral infarction (13, 15, 18). A tertiary analysis of the URICO-ICTUS trial suggested that uric acid therapy significantly reduced the incidence of early ischaemic worsening compared with placebo in patients treated with alteplase within 4.5 h of onset (13). Some studies have indicated that hyperuricaemia is a significant protective factor in ischaemic stroke (19, 20). In addition, a recent meta-analysis showed that there was no significant correlation between SUA levels and the prognosis of ischaemic stroke (21). At present, the conclusion remains controversial. Studies have also suggested that the effect of SUA on ischaemic stroke is affected by the renal function of patients (22, 23). A recent study used renal function-normalized SUA (SUA/SCr) to reflect the endogenous uric acid levels in order to avoid the effect of kidney function, assessed the associations between SUA and stroke prognosis and showed that a lower level of SUA/SCr was associated with poor function (14). However, the effect of SUA on patients with BAD-stroke has rarely been reported. The purpose of this study was to explore the relationship between the SUA/SCr ratio and early neurological deterioration (END) in BAD stroke patients.

2. Materials and methods

2.1. Research subjects

We retrospectively analyzed the data of BAD-patients who were admitted to department of Neurology in Chengdu Second People's Hospital between January 2020 to June 2022. The inclusion criteria were as follows: (1) patients presenting within 48 h of onset; (2) patients who met the diagnostic criteria for BAD-related stroke, which was defined as follows: (1) diffusion-weighted imaging (DWI) showing that the infarct was more than three slices in the lenticulostriate artery blood supply or that the lesions extended to the surface of the pontine base; (2) computed tomography angiography (CTA)/ magnetic resonance angiography (MRA) did not demonstrate evidence of responsible vessel stenosis (>50%); and (3) various embolic mechanisms were excluded (1–3, 24). The imaging evaluation was completed 48 h after admission. The exclusion criteria included the following: (1) patients with chronic renal failure (Cr >2 mg/dL) or who required dialysis; (2) patients receiving thrombolytic therapy. At present, thrombolysis therapies possible efficacy in BAD patients remains inconclusive. A retrospective study indicated that intravenous alteplase after stroke onset reduced the incidence of END while some studies showed intravenous thrombolysis seemed to have no preventative effect on END (1, 25, 26). In order to avoid confounder bias, we chose to exclude patients receiving thrombolytic therapy. (3) patients with a contraindication to antiplatelet drugs such as various bleeding diseases or coagulation dysfunction; (4) patients with atrial fibrillation or who took coagulation medications; (5) patients with poor cardiopulmonary function or severe liver insufficiency or liver failure; (6) patients with malignant tumors; (7) patients with incomplete follow-up at 3 months post-stroke; (8) patients with a modified Rankin scale (mRS) score >1 before admission; and (9) patients with incomplete clinical data. All the patients included in this study received oral antiplatelet treatment after admission for 21 consecutive days (100 mg aspirin and 75 mg clopidogrel daily) and then were treated with long-term single antibody therapy (100 mg aspirin or 75 mg clopidogrel daily) after discharge.

2.2. Clinical information and assessment

The clinical data were collected by two clinicians who reviewed the electronic medical record system from our hospital. The clinical information collected included the following factors: age, sex, hypertension, diabetes, smoking, drinking, history of stroke, and history of taking antiplatelet drugs. The clinical data included the time from onset to arrival, blood pressure at admission, National Institutes of Health Stroke Scale (NIHSS) score at admission, presence of END, infarct site, and NIHSS score at discharge. END was defined as an NIHSS score increase of more than 2 points within 1 week (1, 26). All the included subjects were followed up at 3 months after the onset by telephone or face-to-face interviews to determine their mRS at 90 days. The laboratory data we collected were as follows: random blood sugar, blood lipids, urea nitrogen, creatinine, uric acid, and creatinine clearance.

The study was approved by the Medical and Health Research Ethics Committee of the Second People's Hospital of Chengdu (Chengdu, China).

2.3. Sample collection and assessment of the SUA/SCr ratio

We collected non-fasting blood samples for some urgent laboratory tests (including serum creatinine, serum uric acid, urea nitrogen, and random blood sugar) for all patients with acute cerebral infarction who were admitted to our hospital, and the samples were submitted for examination immediately. Total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were tested in the fasting blood samples. All blood samples were analyzed by a Hitachi 7,600 automatic biochemistry analyzer (Hitachi, Tokyo, Japan). Laboratory physicians were responsible for reviewing the results. Renal function-normalized SUA was calculated using the SUA/SCr ratio.

2.4. Statistical analyses

We analyzed the data using SPSS Version 25.0 software (IBM Corp, Armonk, NY, USA). Continuous variables are expressed as the mean ± standard deviation (SD) or as the median and interquartile range (IQR). Student's *t*-test was used to compare normally distributed variables and Mann-Whitney *U* test was used to compare non-normally distributed variables. Categorical data are presented as frequencies (percentages), and the differences between the groups were compared using the chi-squared test or Fisher's exact test. The variables associated with END that had a low *p* value in the univariate analyses ($P < 0.20$) were included in the multivariate analysis. Multivariate logistic regression analysis was

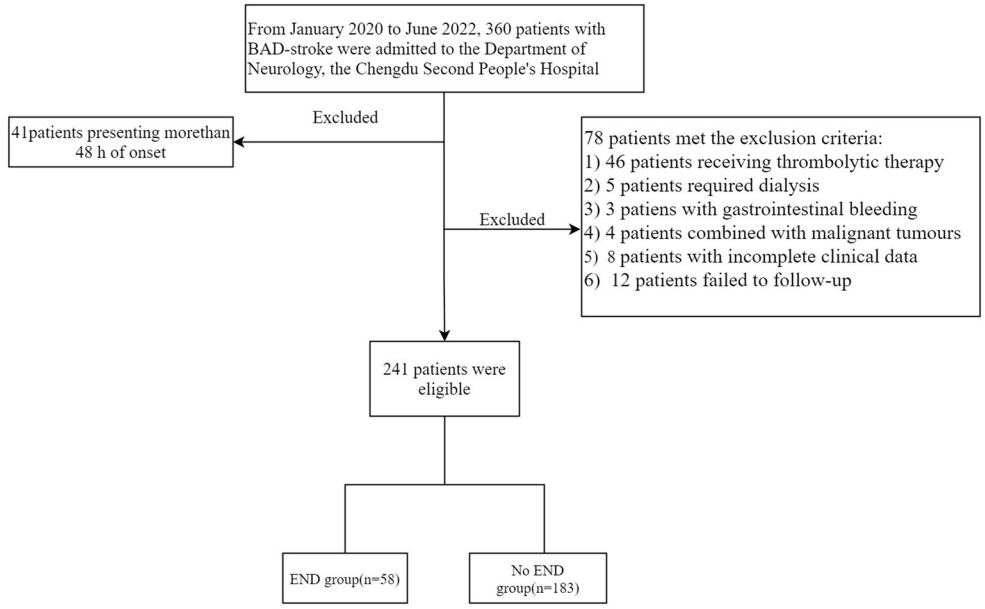


FIGURE 1
A flow chart for the patients' screening.

performed to identify risk factors associated with END. Receiver operating characteristic (ROC) analysis was used to assess the value of SUA/SCr for predicting END. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Flow chart of the study

As shown in Figure 1, a total of 360 patients with BAD-stroke were admitted to our hospital from January 2020 to June 2022. A total of 119 patients were excluded, including 41 patients with an onset time of more than 48 h on admission, and 78 patients met the exclusion criteria (detailed information is shown in Figure 1). Finally, the remaining 241 patients were included in the final study.

3.2. Comparison of the clinical baseline data in the END and No-END groups

The baseline characteristics of the patients are summarized in Table 1. The median age was 66.0 years, and 35.7% were female. Twenty-four percent (58/241) of all patients exhibited END after admission. In the univariate logistic regression analysis, the SUA ($P < 0.0001$), Scr ($P < 0.0001$), and SUA/Scr ($P = 0.012$) in the END group were significantly lower than those in the non-END group (Table 2). There were also statistically significant differences in sex between the two groups ($P < 0.05$; Table 1). The patients who experienced END had higher NIHSS scores at discharge and mRS scores at 90 days after discharge than the patients without END ($P < 0.0001$; Table 1), and a comparison of the mRS scores between the END group and the non-END group at 3 months is shown in Figure 2.

3.3. Multivariate logistic regression analysis results of the factors related to END

When the factors associated with END in the univariate analyses ($P < 0.20$) were entered into the multivariate logistic regression analysis, SUA/Scr (aOR, 0.716; 95% CI, 0.538–0.952; $P = 0.022$) remained a significant factor in the final regression analysis (Table 3). The multivariate logic analysis also showed that females seemed to be more prone to END (aOR, 0.469; 95% CI, 0.245–0.898; $P = 0.022$) (Table 3). The predicted value of SUA/Scr for END was a sensitivity of 79.3%, a specificity of 44.8%, and an AUC of 0.609 (95% CI, 0.527–0.691, $P < 0.05$) (Figure 3). The optimal cut-off value was 4.76. It indicated that patients with the SUA/SCr $\leq$ 4.76 are more likely to experience END. Figure 4 shows the SUA/SCr levels in both groups.

4. Discussion

The purpose of our study was to explore the association between SUA/SCr and END in BAD stroke patients. Our research found that SUA/SCr was an independent risk factor for END in BAD-stroke patients, and patients with a lower SUA/SCr were more prone to END. To the best of our knowledge, few analyses have investigated the relationship between SUA/SCr and END in BAD stroke patients.

BAD is a specific type of ischaemic stroke caused by the occlusion of the orifice of the penetrating artery (3), which is different from lacunar infarction (LI), whose pathological characteristic is fibrinoid degeneration or lipohyalinosis of penetrating artery (27). Both of them are intracranial deep brain infarction. LI was defined as an intracerebral lesion with a diameter of <15 mm and fewer than 3 slices or a lesion within the pontine parenchyma while BAD was defined as an intracerebral lesion of ≥15 mm in diameter and more than 3 slices or a lesion extending to the surface of the pontine base observed on DWI (24). zBAD stroke patients have been

TABLE 1 The baseline characteristics of the patients with END and the No-END group.

	END ($n = 58$)	No-END ($n = 183$)	*P*
Age, year	67.0 (57.8, 76.3)	66.0 (57.0,76.0)	0.775
Female, sex, *n* (%)	29 (50.0)	57 (31.1)	0.009
Hypertension, *n* (%)	46 (79.3)	138 (75.4)	0.542
Diabetes, *n* (%)	17 (29.3)	65 (35.5)	0.384
Hyperlipidaemia *n* (%)	15 (25.9)	61 (33.3)	0.286
Smoking, *n* (%)	15 (25.9)	53 (29.0)	0.648
Drinking, *n* (%)	5 (8.6)	30 (16.4)	0.143
History of ischaemic stroke, *n* (%)	2 (3.4)	4 (2.2)	0.595
History of taking antiplatelet drugs, *n* (%)	1 (1.7)	4 (2.2)	0.830
History of taking statin drugs, *n* (%)	1 (1.7)	4 (2.2)	0.830
Blood pressure at admission			
SBP, mmHg	154.1 ± 22.3	154.1 ± 21.6	0.574
DBP, mmHg	84.5 (76.0,97.0)	87.0 (78.0,97.0)	0.512
Arrival time, hours	24.0 (12.0,31.5)	24.0 (11.0,48.0)	0.998
NIHSS score at admission, hours	3.0 (2.0,4.0)	3.0 (2.0,4.0)	0.249
Infarct site, *n* (%)			
LSA	31 (53.4)	117 (63.9)	0.153
PPA	27 (46.6)	66 (36.1)	
NIHSS at discharge	4 (2,6)	2 (1,2)	<0.0001
mRS at 90 days	3 (2,4)	1 (0,1)	<0.0001

SBP, systolic blood pressure; DBP, diastolic blood pressure; NIHSS, National Institutes of Health Stroke Scale; LSA, lenticulostriate artery; PPA, paramedian pontine artery.

TABLE 2 Laboratory results in both outcome groups.

	END ($n = 58$)	No-END ($n = 183$)	*P*
Random blood sugar, mmol/L	5.8 (4.9,8.9)	5.7 (4.9,7.5)	0.839
TC, mmol/l	1.42 (1.02,1.91)	1.41 (1.02,2.21)	0.567
TG, mmol/l	4.75 (3.81,5.82)	4.73 (3.98,5.65)	0.923
HDL, mmol/l	1.16 (0.93,1.39)	1.09 (0.90,1.29)	0.296
LDL, mmol/l	2.93 (2.19,3.68)	2.78 (2.21,3.50)	0.451
SCr, mmol/l	64.0 (52.0,72.5)	73.0 (63.0,85.0)	0.000
Urea nitrogen	4.90 (4.19,5.92)	5.30 (4.30,6.30)	0.092
Creatinine clearance	77.53 (68.01,97.04)	77.02 (59.49,95.11)	0.534
SUA	258.0 (218.5,325.8)	327.0 (277.0,394.0)	0.000
SUA/Scr	4.08 (3.69,4.75)	4.61 (3.90,5.48)	0.012

TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low density lipoprotein; SUA,Serum uric acid; Scr, serum creatinin.

reported to have a tendency of neurologic worsening compared with lacunar infarction patients (2, 6). In our study, we observed that approximately 24.1% of the BAD-stroke patients experienced END, which resulted in more severe disability on discharge and higher 3-month MRS scores than the patients without END. Most of the previous studies on predictions of END in patients have focused on imaging features (8–12, 28). Studies have shown that larger infarct size or lower pons lesions may be associated with a higher probability of progressive motor deficits in patients with basilar artery branch disease (11, 28, 29). The longitudinal length of the infarcted lesion along the perforating artery was also reported to be associated with END in single subcortical infarction (10). However, there are few studies on the serum markers of END in BAD-stroke patients. Oji et al.'s small sample study showed that high mean platelet volume values on admission may be an independent biomarker for END in BAD patients (4). The effect of uric acid on END in BAD-stroke patients is currently unknown.

END after stroke has been reported to be associated with poor outcomes (30). In recent years, there have been a few studies dedicated to the relationship between uric acid and the development

TABLE 3 Multivariate logistic regression analysis results of the factors related to END.

Risk factors	OR	95% CI	*P*
Sex	0.469	0.245–0.898	0.022
Drinking, *n* (%)	1.441	0.497–4.174	0.501
Infarct site, *n* (%)	0.997	0.987–1.008	0.639
urea nitrogen	0.814	0.656–1.010	0.062
SUA/Scr	0.716	0.538–0.952	0.022

SUA,Serum uric acid; Scr, serum creatinine.

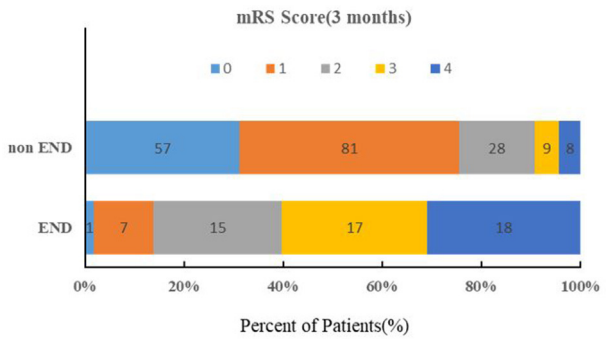
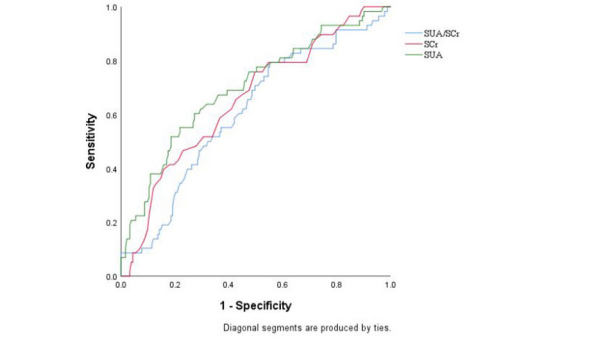


FIGURE 2
Comparison of the mRS scores at 3 months between the END group and No-END group.



Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
SUA/SCr	.609	.042	.012	.527	.691
SCr	.654	.041	.000	.575	.734
SUA	.698	.041	.000	.618	.778

The test result variable(s): SUA/SCr, SCr, SUA has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

FIGURE 3
ROC analysis of SUA/SCr in predicting END in BAD patients.

and prognosis of stroke. Whether uric acid is a protective or destructive factor in ischaemic stroke is still controversial. A meta-analysis by Lei et al. in 2019, which included a total of 15 high-quality studies with 12,739 acute ischaemic stroke patients, suggested that there was a significant positive association between SUA level and the outcome of ischaemic stroke (31). A retrospective study highlighted that a high SUA level (>237 mmol/L) was a protective

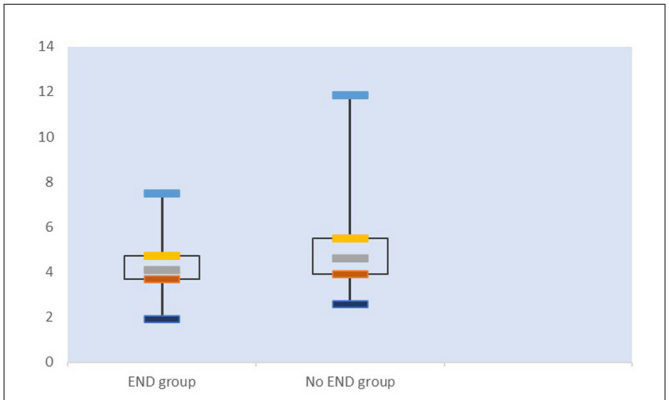


FIGURE 4
Whisker plots of SUA/SCr in the END and No-END groups.

factor for neurological functional outcome only in males and in the patients with the large-artery atherosclerosis subtype but not in females nor in the patients with other stroke subtypes (32). Chamorro et al. showed that each milligram per decilitre increase in serum uric acid was associated with a 12% increase in the odds of good clinical outcome in patients with acute ischaemic stroke (19). Wang et al.'s and Amaro's studies also support that uric acid is a protective factor for stroke (17, 20). In contrast, a meta-analysis in 2021 showed that there was no significant association between serum uric acid levels and functional outcome (21). A tertiary analysis of the URICO-ICTUS trial, which was a double-blind, placebo-controlled, phase 2b trial, suggested that uric acid therapy significantly reduced the incidence of early ischaemic worsening (EIW) compared with placebo in patients treated with alteplase within 4.5 h of AIS onset (13). As 90% of SUA is filtered and reabsorbed by the kidney, the uric acid concentration is largely influenced by kidney function. Some studies have shown that the effects of SUA on ischaemic stroke are regulated by kidney function (22, 23). A study including 3,284 AIS patients from the CATIS suggested that a high SUA concentration was a protective factor only in ischaemic stroke patients with normal renal function but not in those with abnormal renal function (22), while Falsetti et al. showed that high SUA was associated with higher in-hospital mortality for ischaemic stroke patients with kidney disease but not in patients with normal kidney function (23). Therefore, it is necessary to consider the impact of renal function when assessing the relationship between SUA and stroke. SUA/SCr has been considered to be a superior biomarker of endogenous uric acid levels (14, 16).

Our study found that BAD-stroke patients with a lower SUA/Scr had a higher risk for END and therefore poorer outcomes. A recent prospective cohort study enrolled 8,169 ischaemic stroke or transient ischaemic patients and found that a lower SUA/SCr was independently related to poor functional outcomes in patients at 3 months and 1 year after AIS. Lin et al.'s prospective observational study including 196 patients found that a BUN/Cr higher than 15 was an independent predictor of SIE (33). Then, they proceeded with a prospective interventional study that included 189 AIS patients (hydration group, $n = 92$; control group, $n = 97$), and the hydration group received an intravenous bolus (300–500 mL) of saline followed by a maintenance saline infusion, while the control group received a maintenance saline infusion. Finally, they concluded that hydration therapy may help reduce the occurrence of SIE and therefore improve

prognosis (34). Our findings indicated that SUA/SCr may be a useful marker to predict END and guide the individual treatment regimen for BAD-stroke patients with high END risk. Additionally, we found that females were less likely to experience END than males.

The precise mechanism for the effect of the SUA/SCr ratio on stroke development and prognosis remains unclear. SUA is the end product of purine metabolism, and it is also well known as an endogenously generated antioxidant during hypoxia (35). The brain is a highly oxygen-demanding organ, and it is extremely dangerous for the patient when the brain suffers from oxidative stress. However, oxidative stress is one of the mechanisms that contributes to neuronal damage in AIS patients (36). It has been reported through animal experiments that uric acid, as an antioxidant, can remove free radicals and inhibit oxidative stress to protect brain tissue (35). Therefore, the antioxidant properties of uric acid may be one of the mechanisms. Therefore, we speculate that when the renal function of the two patients is the same, patients with lower levels of uric acid may be more prone to END and poor prognosis.

BAD-stroke patients are prone to END, so it is crucial to explore the factors associated with END. To the best of our knowledge, this was the first study to investigate the relationship between SUA/SCr and END in BAD stroke patients, which was a strength of our research. However, our study also has the following limitations. First, this is a retrospective study with a small sample size. Second, our study only assessed SUA/SCr at admission without continuous observations to further analyse the impact of fluctuating ratio levels. Third, we did not investigate the association between SUA/SCr and END in patients with normal and abnormal renal function separately. Fourth, Our research showed that the SUA/SCr has a good sensitivity in predicting END, but its specificity is relatively low. In daily practice, it is necessary to combine multiple factors to evaluate the risk of END. In conclusion, our study suggested that SUA/SCr was negatively associated with the risk of END in BAD-stroke patients. In clinical practice, the occurrence of END should be monitored in BAD stroke patients with a low SUA/SCr.

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.

Author contributions

YL participated in the whole process of the study, including designing the study, collecting data, analyzing the data, and drafting the original manuscript. HW participated in the data collection and collation. RX and LH participated in the statistical analysis. KW and YX participated in data management, provided professional guidance in the evaluating the laboratory results, and imaging review. JW and FX designed the study and revised 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

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S100b in acute ischemic stroke clots is a biomarker for post-thrombectomy intracranial hemorrhages

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Background and purpose: Post-thrombectomy intracranial hemorrhages (PTIH) are dangerous complications of acute ischemic stroke (AIS) following mechanical thrombectomy. We aimed to investigate if S100b levels in AIS clots removed by mechanical thrombectomy correlated to increased risk of PTIH.

Methods: We analyzed 122 thrombi from 80 AIS patients in the RESTORE Registry of AIS clots, selecting an equal number of patients having been pre-treated or not with rtPA (40 each group). Within each subgroup, 20 patients had developed PTIH and 20 patients showed no signs of hemorrhage. Gross photos of each clot were taken and extracted clot area (ECA) was measured using ImageJ. Immunohistochemistry for S100b was performed and Orbit Image Analysis was used for quantification. Immunofluorescence was performed to investigate co-localization between S100b and T-lymphocytes, neutrophils and macrophages. Chi-square or Kruskal-Wallis test were used for statistical analysis.

Results: PTIH was associated with higher S100b levels in clots (0.33 [0.08–0.85] vs. 0.07 [0.02–0.27] mm^2, H1 = 6.021, $P = 0.014$*), but S100b levels were not significantly affected by acute thrombolytic treatment ($P = 0.386$). PTIH was also associated with patients having higher NIHSS at admission (20.0 [17.0–23.0] vs. 14.0 [10.5–19.0], H1 = 8.006, $P = 0.005$) and higher number of passes during thrombectomy (2 [1–4] vs. 1 [1–2.5], H1 = 5.995, $P = 0.014$*).

S100b co-localized with neutrophils, macrophages and with T-lymphocytes in the clots.

Conclusions: Higher S100b expression in AIS clots, higher NIHSS at admission and higher number of passes during thrombectomy are all associated with PTIH. Further investigation of S100b expression in AIS clots by neutrophils, macrophages and T-lymphocytes could provide insight into the role of S100b in thromboinflammation.

KEYWORDS

S100b, stroke biomarkers, thrombus, acute ischemic stroke, post-thrombectomy intracranial hemorrhages

Introduction

Post-thrombectomy intracranial hemorrhages (PTIH) are the most serious complication of endovascular procedures following acute ischemic stroke (AIS). Intracranial hemorrhage can take a wide range of different forms, including extraparenchymal (subdural hematoma and subarachnoid hemorrhage) and intraparenchymal (1). Intracranial hemorrhage occurs when the blood-brain barrier (BBB) is sufficiently damaged to permit extravasation of blood components into the brain parenchyma, increasing stroke morbidity and mortality (2). There are several factors associated with increased risk of PTIH, such as stroke severity (3), recanalization therapy (both thrombolysis and thrombectomy) (4), hypertension (5), hyperglycemia (3, 5) and age (6).

In the last few years, there have been several efforts to predict intracranial hemorrhage after AIS (3, 6–9). Also, many studies looking for novel biomarkers for stroke diagnosis and prognosis have converged on the crucial role of inflammation (8), focusing on a panel of proteins potentially useful for this purpose (10–12). Several proteins have been explored as potential biomarkers of hemorrhagic transformation after acute ischemic stroke, including matrix metalloproteinase-9 (MMP9), neuron-specific enolase (NSE), cellular-fibronectin (c-Fn), plasminogen activator inhibitor (PAI-1), thrombin-activated fibrinolysis inhibitor (TAFI) and S100b (10), which was the main focus of this manuscript. The glial protein S100b can be produced by several peripheral cell subtypes (13) including T-lymphocytes (14, 15) and it is not a specific indicator for stroke, as its levels are increased also in other neurological conditions (16). However, it is among the most interesting candidates that have been investigated as stroke biomarkers, showing potential in discriminating between ischemic and hemorrhagic stroke (17, 18). Nonetheless, there is evidence that increased levels of S100b in blood of AIS patients are associated with increased intracranial hemorrhage rate following thrombolytic therapy (19). However, the involvement of S100b in stroke has not yet been fully investigated.

We evaluated the expression of S100b in 122 thrombi retrieved from 80 AIS patients, with equal numbers with or without acute thrombolytic administration. In this study, we investigated if there was a difference in S100b expression in AIS clots extracted from patients with or without PTIH, to further explore the role of S100b as a possible biomarker for PTIH. Furthermore, the possibility that white blood cell subtypes are the source of S100b in AIS clots was investigated.

Materials and methods

Patient cohort

Eighty acute ischemic stroke cases from the RESTORE registry of AIS clots were included in this study. The RESTORE registry is registry of thrombotic material extracted *via* mechanical thrombectomy from 1,000 AIS patients during the period February 2018 to December 2019 from four stroke centers in Europe (20). Two of the four participating hospitals (Sahlgrenska University Hospital, Gothenburg and Metropolitan Hospital, Athens) provided information on PTIH (472 patients). Of these, 81 patients developed PTIH, i.e., 17%. In this study, we analyzed clot samples from an equal number of cases in the two subgroups PTIH yes and PTIH no, closely matched for factors such as age, sex, etiology, and thrombolysis yes/no. PTIH was identified by two experienced radiologists at each clinical site on a CT scan 24-36h after thrombectomy and classified according to the European Cooperative Acute Stroke Study II (ECASS II) classification system (21). The experimental plan is illustrated in Figure 1. This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and its amendments (22), by approval of the regional hospital ethics committees and National University of Ireland Galway research ethics committees (16-SEPT-08). We included only patients >18 years, having been treated with mechanical thrombectomy for AIS whose thrombus material was available for analysis and having information whether the patients suffered (or not) PTIH. For each patient we collected an anonymized data

abstraction form containing pertinent procedural data, such as rtPA administration, NIHSS score at admission, occlusion location, stroke etiology, number of passes for clot removal, final mTICI score and hemorrhagic transformation incidence. Suspected stroke etiology was reported according to the TOAST classification system (23). As it has been reported that rtPA administration may be associated with a higher risk of hemorrhagic transformation following AIS, therefore we included equal numbers ($n = 40$) of patients treated with bridging-therapy (rtPA and mechanical thrombectomy) and 40 patients treated with mechanical thrombectomy alone. For each subgroup we included equal numbers ($n = 20$) of patients with PTIH after mechanical thrombectomy and 20 closely matched controls without PTIH. Controls were AIS patients treated with mechanical thrombectomy but with no sign of hemorrhage and matched as closely as possible to the PTIH yes subgroup for age, sex, etiology and thrombolysis yes/no.

Thrombi collection, size measurement and processing

Thrombotic material extracted *via* mechanical thrombectomy was collected *per pass* at the hospital venue in separate pots containing 10% formalin and shipped to NUI Galway. A gross photo of each thrombus was taken with a Canon EOS 1300D Camera and the relevant Extracted Clot Area (ECA) was measured and used as an estimate of the extracted clot size, by drawing around each fragment with a specific tool using ImageJ software, as previously reported (24–27). In brief, to measure the Extracted Clot Area (ECA), the gross photo of the extracted clot was opened using ImageJ software, the scale was set and the Polygon tool was used to draw a region of interest around each clot fragment in the gross photo, and summed to give overall ECA for the sample. Following gross photos, thrombi were placed in histological cassettes for tissue processing and paraffin embedding. We analyzed a total of 122 thrombi, collected per pass from the 80 cases in this study.

Immunohistochemistry staining

After paraffin embedding, 3 μm sections were cut from each block with a microtome and S100b staining was performed by Immunohistochemistry (IHC) on a Leica Bond-III autostainer using a BOND Polymer Refine Red Detection kit (Leica Biosystems #DS9390). Antigen retrieval with tris-EDTA (Leica Biosystems #AR9640) was performed for 10 min. Primary antibody rabbit anti-S100b (abcam, ab41548, 1:100 dilution) incubation time was 15 min, followed by 30 min of incubation with an anti-rabbit secondary antibody. Counterstaining of tissue using haematoxylin was performed for 5 min. Sections were then washed with a washing solution (Leica Biosystems #AR9590) and rinsed in distilled water. Sections were then dehydrated in alcohol, cleared in xylene, and mounted with DPX. Negative controls were performed by omission of the primary antibody step. Entorhinal cortical brain tissue (BioIVT) was used as positive control tissue for S100b expression.

Slide scanning and quantification

Immunohistochemically stained slides were scanned on an Olympus vs120 slide scanner at 20× magnification and digital whole slide scan images were generated. Quantification was performed on the digital slides using Orbit Image Analysis Software (www.orbit.bio) (28), as previously described (29). In brief, we created exclusion and inclusion models to distinguish regions to be excluded (e.g., background and artifact) and regions containing the component of interest, S100b, enabling its quantitative assessment within each clot.

We quantified the expression of S100b in each case as area (in mm^2) by multiplying the component percent by the relevant ECA. For cases involving multiple passes, we summed the values of S100bXECA for all passes.

Immunofluorescence

Immunofluorescence staining was performed on a subset of samples in order to evaluate co-localization of S100b and three WBC markers, respectively CD3 staining for T-lymphocytes, CD68 staining for macrophages and CD66b staining for neutrophils. We used inflamed tonsil tissue (BioIVT), as positive control tissue for CD3, CD68, and CD66b. The primary antibodies used were the following: rabbit anti-S100b (abcam ab41548, 1:100 dilution); mouse anti-CD3 (abcam ab17143, 1:10); mouse anti-CD68 (abcam ab955, 1:50) mouse anti-CD66b (Novus biological NB100-77808, 1:100). Secondary antibodies were: Goat anti-mouse IgG H&L (Alexa Fluor 594, abcam ab150116, 1:200) and Goat anti-rabbit IgG H&L, (Alexa Fluor 488, abcam ab150077, 1:200).

After deparaffinization with xylene and following rehydration with 100, 95, 70 and 50% alcohol, 3 μm sections of thrombus tissue were incubated for 20 min with Tris-EDTA buffer in a microwave at 98°C. Sections were washed with phosphate-buffered saline (PBS) followed by PBS containing 0.2% Tween 20 (PBS-Tx) and incubated with blocking buffer (3% normal goat serum, NGS, in PBS-Tx) for 1 h at room temperature under agitation. Incubation with S100b and one of the WBC primary antibodies per slide followed for 1 h at 37°C, then over night at 4°C. After washing, sections were incubated with secondary antibodies

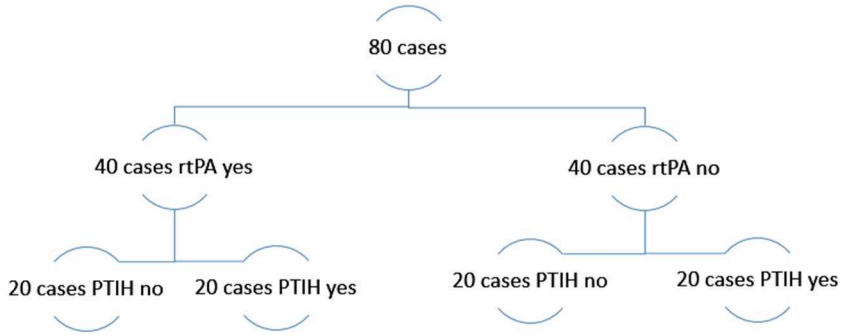

FIGURE 1
Experimental plan of the study.

for 1 h at 37°C and then cover slipped with 4',6-diamidino-2-phenylindole (DAPI) mounting medium for nucleic acid staining.

Immunostaining images were captured by using the objective of 60× in a FV3000 Confocal Laser Scanning microscope (Olympus) and analyzed with FIJI software (ImageJ).

Statistical analysis

Statistical analysis was performed with IBM SPSS-25 software and graphs were created with GraphPad Prism 9.2.0. Quantitative variables did not follow a standard normal distribution as indicated by Kolmogorov-Smirnov test. Therefore, the Chi square test or Kruskal-Wallis test were used to assess statistically significant differences among the groups, respectively for nominal or continuous variables. Correlation analysis was also performed (Spearman's Rho). The level of statistical significance was set at $p < 0.05$ (two-sided). Results are reported as median [IQ1–IQ3] or number and percentage (%) of cases.

Results

Baseline characteristics of the patients

Baseline clinical and procedural characteristics of the 80 patients selected are reported in Table 1, for the overall population analyzed and according to whether PTIH occurred or not. Main types of PTIH defined according to ECASS II classification are the following: small petechial haemorrhagic infarction (HI1), confluent petechial haemorrhagic infarction (HI2), small parenchymal hemorrhage (PH1) (<30% of infarct, mild mass effect), and large parenchymal hemorrhage (PH2, >30% of infarct, marked mass effect). In our cohort we found 14 cases of HI1 (35% of HT), 7 cases of HI2 (17.5% of HT), 7 cases of PH1 (17.5%) and 7 cases of PH2 (17.5%). We also found 4 cases (10%) of subarachnoid hemorrhage (SAH) and 1 case (2.5%) of subdural hematoma (SDH). There was no significant difference between the two subgroups in terms of sex ($P = 0.822$), age ($P = 8.885$), stroke etiology ($P = 0.966$) and occlusion location ($P = 0.461$). Also, no difference was found in terms of onset to groin puncture time ($P = 0.787$), onset to recanalization time ($P = 0.953$) and final mTICI score ($P = 0.086$). However, patients with no signs of hemorrhage had lower rate of mTICI 2b (17.5 vs. 32.5%) and higher rate of mTICI 3 (52.5 vs. 30%) compared to PTIH yes subgroup. NIHSS at admission was significantly higher for PTIH yes subgroup ($P = 0.005^*$). The total number of passes for clot removal was also significantly higher for the PTIH yes subgroup ($P = 0.014^*$); Table 1.

S100b expression in AIS clots is associated with WBC

We distinguished clots with different expression of S100b (Figure 2). Interestingly, as depicted in panels A–B, we noticed that S100b was closely associated with nucleated cells in the clots, leading us to perform further analysis with immunofluorescence co-staining to assess sub-types of WBC associated with S100b expression.

S100b expression in AIS clots is higher in clots from patients with PTIH regardless of rtPA administration preceding mechanical thrombectomy, NIHSS on admission and age

S100b expression in clots from patients with PTIH was statistically significantly higher than those from patients without

TABLE 1 Baseline clinical and procedural characteristics of the overall cohort of patients and divided according whether they had or not post-thrombectomy intracranial hemorrhage (PTIH).

Sex	Overall cohort of patients ($N = 80$)	PTIH YES ($N = 40$)	PTIH NO ($N = 40$)	Statistical analysis
Male	45 (56.3%)	23 (57.5%)	22 (55.0%)	$X^1 = 0.051$, $P = 0.822$
Female	35 (43.8%)	17 (42.5%)	18 (45.0%)	
Age (years)	75.0 [64.5–83.0]	75.0 [63.0–82.0]	74.5 [65.5–83.0]	H1 = 0.021, $P = 0.885$
Stroke etiology				
Patients with cardioembolic etiology	32 (40%)	15 (37.5%)	17 (42.5%)	$X^3 = 0.268$, $P = 0.966$
Patients with large artery atherosclerosis etiology	16 (20%)	8 (20.0%)	8 (20.0%)	
Patients with other etiology[a]	4 (5%)	2 (5.0%)	2 (5.0%)	
Patients with cryptogenic etiology	28 (35%)	15 (37.5%)	13 (32.5%)	
NIHSS admission	18.0 [11.0–21.5]	20.0 [17.0–23.0]	14.0 [10.5–19.0]	H1 = 8.006, $P = 0.005$*
Occluded vessel (s)[b]				
MCA, M1	31 (38.8%)	14 (35.0%)	17 (42.5%)	H1 = 0.543, $P = 0.461$
MCA, M2	8 (10.0%)	4 (10.0%)	4 (10.0%)	
MCA, M3	1 (1.3%)	0 (0.0%)	1 (2.5%)	
MCA (multiple branches/segments)	6 (7.5%)	3 (7.5%)	3 (7.5%)	
ICA&ICA terminus	14 (17.5%)	7 (17.5%)	7 (17.5%)	
ACA	1 (1.3%)	1 (2.5%)	0 (0.0%)	
VB	6 (7.5%)	4 (10.0%)	2 (5.0%)	
PCA	1 (1.3%)	0 (0.0%)	1 (2.5%)	
Tandem occlusion	6 (7.5%)	4 (10.0%)	2 (5.0%)	
Other dual	1 (1.3%)	1 (2.5%)	0 (0.0%)	
3 or more occluded vessels	5 (6.3%)	2 (5.0%)	3 (7.5%)	
Median number of passes performed during the endovascular procedure	2 [1–3]	2 [1–4]	1 [1–2.5]	$H_1 = 5.995$, $P = 0.014$*
Onset to groin puncture time (minutes)	145 [53–265]	145 [45–300]	142.5 [54.5–262.5]	H1 = 0.073, $P = 0.787$
Onset to recanalization time (minutes)	236 [88–345]	235 [99–355]	258 [88–328]	H1 = 0.003, $P = 0.953$
Final mTICI score				
mTICI 0	4 (5.0%)	2 (5.0%)	2 (5.0%)	H1 = 2.948, $P = 0.086$
mTICI 1	1 (1.3%)	0 (0.0%)	1 (2.5%)	
mTICI 2a	8 (10.0%)	5 (12.5%)	3 (7.5%)	
mTICI 2b	20 (25.0%)	13 (32.5%)	7 (17.5%)	
mTICI 2c	14 (17.5%)	8 (20.0%)	6 (15.0%)	
mTICI 3	33 (41.3%)	12 (30.0%)	21 (52.5%)	

[a]Other etiology included arterial dissection.

[b]MCA, Middle Cerebral Artery; ICA, Internal Carotid Artery; ACA, Anterior Cerebral Artery; VB, Vertebro-basilar; PCA, Posterior Cerebral Artery.

Data given as *N* (%) of cases or median [IQ1, IQ3].

*Statistically significant.

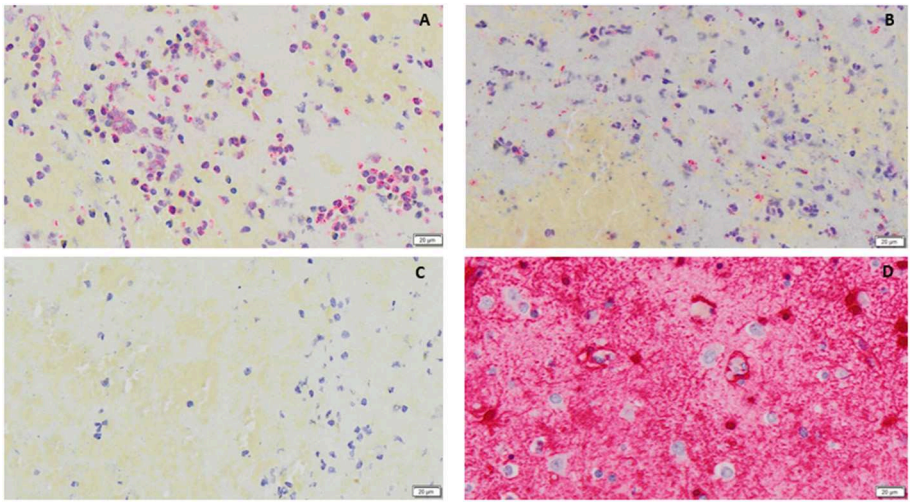

FIGURE 2
(A, B) examples of S100b (red immunostaining) in clots, respectively from a patient with **(A)** and without **(B)** Post-thrombectomy Intracranial Hemorrhage are shown. In **(C)** a negative control is shown, while in **(D)** positive control tissue (human entorhinal brain cortex) is shown. Nuclei are stained blue (counterstaining with haematoxylin). All images were captured using the 20× objective (scale bar 20 μm).

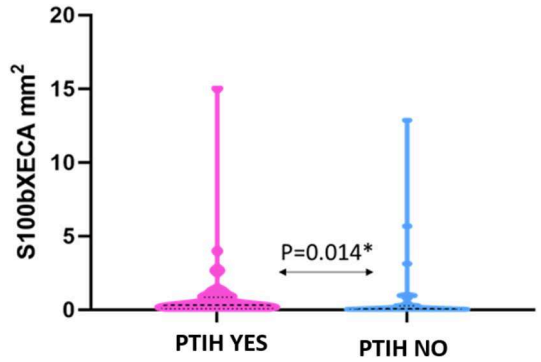

FIGURE 3
Violin plot showing that S100b expression in clots from patients with PTIH is significantly higher than patients without signs of hemorrhage. Dashed lines represent the median while dotted lines represent the interquartile ranges, Q1 **(lower dotted lines)** and Q3 **(upper dotted lines)**. *Statistically significant.

HT (0.33 [0.08–0.85] vs. 0.07 [0.02–0.27] mm^2, $P = 0.014$, when expressed as area, Figure 3. A similar trend was apparent when S100b was expressed as overall percentage (0.53 [0.21–1.48]% for PTIH yes vs. 0.32 [0.09–0.80]% for PTIH no, although not statistically different ($P = 0.100$).

We did not find any significant difference between expression of S100b in clots from patients pre-treated with rtPA and those of patients treated with mechanical thrombectomy alone ($P = 0.386$). Additionally, we did not find any significant correlation between S100b levels and NIHSS on admission (Spearman's rho = 0.213, $P = 0.058$), or age (Spearman's rho = −0.120, $P = 0.290$). The different types of hemorrhagic transformation observed and S100b expression in extracted thrombus material is described in Supplementary Table 1. We did not find any statistically significant difference in terms of S100b expression among the several types of PTIH, although we acknowledge that S100b expression in PH was higher than in HI. This could be worthy of further future investigation.

S100b expression is associated with macrophages, neutrophils and T-lymphocytes in clots

Immunofluorescence staining revealed association of S100b with the three WBC subtypes we studied, i.e., T-lymphocytes (CD3), Figure 4A, neutrophils (CD66b), Figure 4B and with macrophages (CD68), Figure 4C.

Discussion

Emergent reperfusion therapy is the cornerstone of treatment in AIS, aiming to restore cerebral blood flow to salvageable ischemic tissue to reduce patient disability. However, PTIH is the most feared complication following endovascular procedures (30). Hemorrhagic infarction following arterial thrombosis and embolism has been suggested as a natural progression of ischemic stroke (31–34). The incidence of this phenomenon greatly varies depending on several risk factors (3–6) and has been reported to range from 0 to 85% in different studies (35).

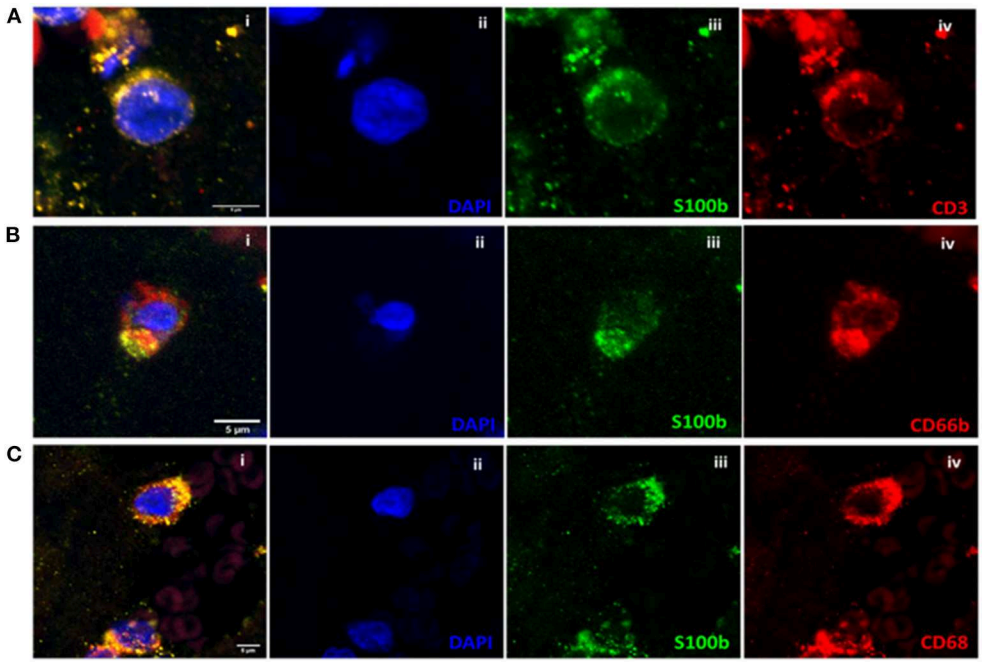

FIGURE 4
(A) Co-localization analysis of S100b and CD3 in clot with the three channels together (i) and separately (ii–iv). **(B)** Co-localization analysis of S100b and CD66b in clot with the three channels together (i) and separately (ii–iv). **(C)** Co-localization analysis of S100b and CD68 in clot with the three channels together (i) and separately (ii–iv). All images were captured using the 60× objective (scale bar 5 μm).

Severe stroke, expressed as high NIHSS score at admission, is generally associated with a higher probability of intracranial hemorrhage (3, 8), which is in line with the findings in the present study. Both thrombolysis and mechanical thrombectomy have been associated with increased risk of intracranial hemorrhage (4). According to some studies, intracranial hemorrhage following thrombolytic administration can be directly linked to rtPA activity, resulting from reperfusion of cerebral vessels whose integrity has been disrupted by severe ischemia (36, 37). It has been also suggested that alteplase may promote intracranial hemorrhage through non-fibrinolytic mechanisms, such as activation of the immune system (38), neutrophil degranulation and release of matrix metalloproteinase-9 (MMP-9) (39) favoring BBB disruption (40). Mechanical thrombectomy may increase intracranial hemorrhage rate due to direct vessel wall damage during endovascular procedures. The degree of damage has been shown to be proportional to the number of passes required for clot retrieval (41). Our results support these findings, as we observed a significantly higher number of passes during mechanical thrombectomy in the cases with PTIH.

Due to the high morbidity and mortality associated with PTIH, many efforts have been made to find new biomarkers and predictors of intracranial hemorrhage following AIS (3, 6–9). In this regard, S100b might have some potential. S100b is a calcium-binding protein belonging to the S100 family, which comprises more than 20 family members (13). At nanomolar concentrations, S100b has been shown to promote neurite outgrowth in cerebral cortex neurons *in vitro* and to enhance survival of neurons during development (42), neuronal maturation and to stimulate glial cell proliferation (43). Furthermore, S100b reduces cell death and protects against mitochondrial loss of function resulting from glucose deprivation (42, 44). However, at micromolar concentrations, S100b can cause deleterious effects. At these concentrations, it has been shown that extracellular S100b promotes its neurotoxic effects by stimulating the expression of proinflammatory cytokines and inducing apoptosis *in vitro* (44). Inflammation is a common characteristic of many neurological disorders. Elevated levels of S100b protein in biological fluids are observed in several neurological disorders, such as multiple sclerosis (45), Alzheimer's disease (46), Parkinson's disease (47), amyotrophic lateral sclerosis (48) and stroke (49).

S100b has been investigated as a possible biomarker to distinguish hemorrhagic stroke from ischemic stroke and some studies have shown that S100b concentrations in blood were higher for hemorrhagic stroke compared to ischemic stroke (50, 51). Also, a previous study highlighted how increased S100b levels in serum of AIS patients treated with thrombolysis might be a predictor of further hemorrhagic transformation (19). These results are in line with the findings of the present study. We have shown higher S100b expression in

AIS thrombi from patients with PTIH compared to those from the non-PTIH group. We also demonstrated that S100b expression in clots is not significantly affected by acute thrombolytic administration.

Whether the original source of S100b in the clots is peripheral or of central origin is still unclear, but worthy of further study.

In this study, immunohistochemistry and Immunofluorescence staining revealed that S100b expression in clots was associated with WBC nuclei, CD68+, CD66b+, and CD3+. The association of S100b with phagocytic cells such as macrophages and neutrophils is interesting, since S100 proteins can work as Damage-Associated Molecular Pattern (DAMP) molecules (52). DAMPs are biomolecules that are released from damaged or stressed cells and could act as endogenous danger signals to induce a rapid inflammatory response (53). Furthermore, S100 proteins play an important role as regulators of macrophage-mediated inflammation (54). In particular, S100b can up-regulate macrophage production of pro-inflammatory cytokines and worsen severity of inflammation (55), therefore, we could hypothesize a similar role also in stroke inflammation. Association between S100b and neutrophils has also been observed since it is known that S100b induces neutrophil migration to sites of inflammation (56, 57). It has been shown that neutrophils can induce damage in the ischemic area by causing neuronal death, destruction of the BBB, and brain edema (58, 59). Neutrophil extracellular traps can further activate platelets and thrombotic processes (60).

Presence of S100b in T-lymphocytes was first detected by Kanamori et al. (61) in 1984. Further studies proved that a cytotoxic T subtype (CD3+ CD8+ CD16-) and a natural killer subtype (CD3- CD8- CD16+) of lymphocytes is able to produce S100b upon stimulation (14, 15). Our results are in line with these studies. The association of S100b with CD3+ lymphocyte subtypes suggests that this protein acts as an interface to immunological processes in various physiological and pathological conditions although further studies are necessary to better clarify its function. The connection between inflammation and thrombosis in cardiovascular diseases is becoming more and more evident. Immunothrombosis is activated in the setting of bacterial and viral infection. Targeting inflammation to prevent cardiovascular events is an emerging concept as it is known that inflammation increases thrombotic tendency. Main cellular drivers of this process are platelets and innate immune cells, primarily neutrophils and monocytes which interplay with platelets and flanked by the activation of the complement system promote coagulation (62). T-lymphocytes play a major role in the initiation and perpetuation of inflammatory cascades as well, involving crosstalk with other immune cells, especially by modulating macrophage response (63), although their specific role in thrombus formation is still unclear (64).

Study limitations

Our study design focused on comparing two closely matched cohorts, respectively having, or not having experienced PTIH. We are aware that our cohort design approach may be prone to biases. A further study with a retrospective case control design, group comparisons adjusted for multiple testing and calculation of odds ratios would be useful. We did not assess S100b levels in serum in this study. S100b serum levels are known to be higher in stroke cases with larger lesion volumes (65, 66). It would be of interest to assess if clot S100b content reflects serum levels in future work. Also, as S100b levels can increase in cases of pre-stroke trauma or very recent surgery and this should be considered in further studies. Furthermore, it would be of interest to probe further if the source of S100b in the clots is astroglial or entirely extracerebral by using a second glial marker such as Glial Fibrillary Acidic Protein (GFAP) in future work. Finally, we did not take into account other factors that might influence occurrence of PTIH, such as pre-treatment with antiplatelet or anticoagulant medications and elevated blood pressure during and after the endovascular procedure. Also, because of the extensive thrombus heterogeneity, it is possible that thrombus composition might affect S100b expression. It would be of interest to consider these factors in further studies.

Conclusion

From our observations, we can conclude that a higher expression of S100b in the retrieved clots is associated with PTIH regardless of thrombolytic administration. We also found other factors directly correlating with PTIH, such as higher NIHSS score at admission and higher number of passes during mechanical thrombectomy. Furthermore, from co-localization studies we observed that S100b in retrieved AIS clots was associated with macrophages, neutrophils and some T-lymphocytes, suggesting it may have an effect on thrombo-inflammatory activity, although we acknowledge that further investigation is necessary to confirm our results.

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 regional ethics committee of Gothenburg (approval numbers 233-17 and T017-18 dates 06-04-2017 and 16-01-2018 respectively), the 'Epitropi Iatrikis Ithikis

Kai Deontologias Therapeutiriou Metropolitan' - Metropolitan Hospital Ethics Comittee (approval number 2430, 01-10-2018) and by the National University of Ireland Galway Research Ethics Committees (16-SEPT-08) following the ethical standards of the Declaration of Helsinki and its amendments. The patients/participants provided their written informed consent to participate in this study.

Author contributions

KD obtained the funds for the study, coordinated study design implementation and supervised the writing of the manuscript. RR participated in samples collection, developed the study design, composed the manuscript, performed the IHC and the IF analysis, performed the statistical analysis and wrote the results and discussion. AD participated in samples collection, performed IHC quantification and IF staining. SG performed the ECA measurements, the IF staining and the confocal analysis. JP and DJ participated in the sample collection and in the slide scanning and quantification. KJ, PR, AN, EC, DD, JC, GT, KP, and AR performed the thrombectomy at the several hospitals, collected samples and procedural data. IS, JT, KP, GT, TT, and AR contributed to the study design and were responsible thrombus collection at the relevant stroke center. AP, MG, and RM contributed to develop the study design and funding acquisition. All the authors have read and reviewed the manuscript.

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Conflict of interest

MG and RC were employed by Cerenovus.

The remaining 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.2022.1067215/full#supplementary-material

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Sex differences and risk factors in recurrent ischemic stroke

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Introduction: Recurrent ischemic stroke (RIS) is associated with increased mortality and poor outcomes. Therefore, secondary prevention is critical for reducing the risk of recurrent stroke. Previous studies have found sex differences in risk factors in patients with first-ever stroke; however, the results have been inconsistent for recurrent stroke. Therefore, this study aimed to investigate whether there are significant sex differences in the clinical characteristics and risk factors for recurrent ischemic stroke.

Methods: We retrospectively studied 787 patients with recurrent ischemic stroke after first-ever stroke confirmation using magnetic resonance imaging (MRI) after visiting a regional tertiary hospital between 2014 and 2020. Demographic characteristics, laboratory findings, and risk factors were compared between the male and female patients. In addition, multivariate logistic regression was performed to identify the independent factors associated with stroke recurrence in male patients.

Results: Among the 787 patients, 466 (59.2%) were males. Males were younger than females (67.6 vs. 71.9 years). Females had higher rates of hypertension, diabetes mellitus, dyslipidemia, and overweight than those of males. However, the alcohol drinking and smoking rate were significantly higher in males than that in females. There were no statistically significant sex-based differences in the laboratory findings. Among males, hypertension, alcohol drinking, smoking and dyslipidemia was a significant risk factor for ischemic stroke recurrence.

Conclusion: Hypertension and dyslipidemia were significant risk factors of recurrent ischemic stroke in both genders. Smoking and alcohol drinking were significant risk factors associated with ischemic stroke recurrence in males. Therefore, smoking cessation and alcohol abstinence are recommended after the first stroke to prevent recurrent ischemic stroke especially for males. Diabetes was a significant risk factor of ischemic stroke recurrence in females. More extensive studies are needed to understand the causal relationship of each factors with ischemic stroke recurrence according to sex differences and specification of preventive management is needed.

KEYWORDS

ischemic stroke, recurrent infarction, sex difference, smoking, secondary prevention

1. Introduction

Stroke is a fatal condition with a high mortality rate, and an appropriate and prompt response to acute stroke is crucial (1). To lower the mortality rate, preventing primary or secondary stroke is essential to identifying and controlling the risk factors for ischemic stroke in advance (2). Known risk factors for stroke include older age (a demographic factor), diabetes, hypertension, dyslipidemia, smoking, transient ischemic attack (TIA), and heart disease (e.g., coronary artery disease and atrial fibrillation). Moreover, stroke tends to recur, and the prognosis worsens when it recurs (2). Although previous studies have presented different results, studies on the general population have shown that the probability of recurrence within 1 year was between 3 and 22%, which increased to 10–53% within 5 years (3–5).

Modifying the risk factors associated with ischemic stroke recurrence is critical in preventing recurrence. Risk factors for stroke recurrence can be classified into non-modifiable and modifiable. Age, sex, and race are non-modifiable risk factors for stroke, while hypertension, diabetes, dyslipidemia, smoking, diet therapy, and lack of physical activity are modifiable risk factors. Although many studies on modifiable risk factors have been continuously reported, there are few reports on non-modifiable risk factors, especially sex, and the results obtained so far have been inconsistent (6–8).

Multiple studies have reported differences in the initial onset of ischemic stroke between males and females. Although males generally have a higher incidence rate than that of females, some risk factors are known to be associated with the incidence of stroke in females (9). This reportedly has been due to differences in the immune system, genetic background, endocrine system, social factors, and oral contraceptive use (10, 11); females are known to have a higher incidence of disability and dysfunction. Due to biological characteristics and female-specific risk factors, female have a higher risk of stroke, a higher chance of experiencing recurrence, and higher severity of stroke symptoms than that of males (12–14). Males and females showed differences in various risk factors for ischemic stroke, including age, carotid artery disease, hormonal changes, and cardiac arrhythmias (12). However, there are few studies on the differences in risk factors for recurrent ischemic stroke between males and females (13, 14). Based on recent reports, no significant differences in the recurrence rate of ischemic stroke and recurrence-related risk factors between males and females were found (13, 14).

Therefore, in this study, we retrospectively investigated the recurrence rate of ischemic stroke and the risk factors for recurrence. The purpose of this study was to investigate the risk factors for recurrence of ischemic stroke in each gender, and to find out whether these risk factors differed between males and females. This study targeted patients diagnosed with ischemic stroke who regularly received outpatient treatment and took appropriate medications according to doctors' instructions.

2. Materials and methods

2.1. Study subjects

This study included patients diagnosed with ischemic stroke for the first time and more than second event among patients with acute ischemic stroke (within 7 days after the onset of ischemic stroke) confirmed using magnetic resonance imaging (MRI) after visiting a regional tertiary hospital between January 2014 and December 2020. Patients with insufficient data, those not taking antiplatelets, and undocumented stroke diagnoses were excluded. The final subjects in this study were patients with recurrent ischemic stroke who visited the outpatient department regularly and could be followed up among the patients who were hospitalized due to ischemic stroke that occurred two or more times. Patients with initial (or first) onset of ischemic stroke and recurrent ischemic stroke who visited the hospital around the same time and around same ages were compared and analyzed separately in males and females.

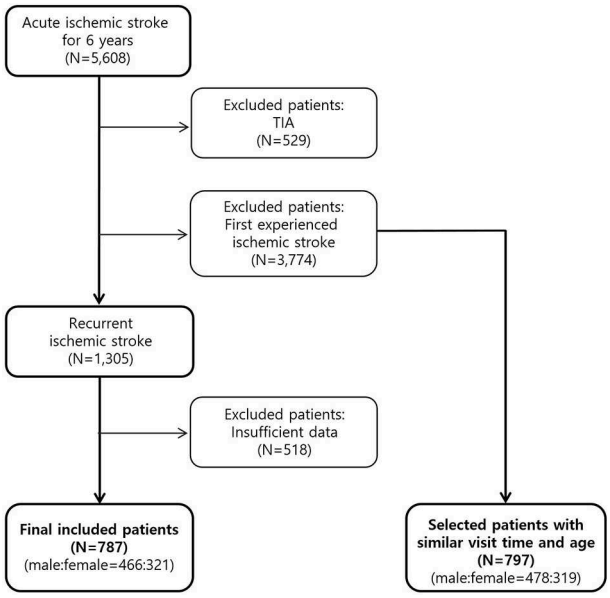

FIGURE 1
Flow diagram of recurrent ischemic stroke patient selection. First onset patients were set as a control group and both groups were compared divided by male and female.

2.2. Methods

This study examined risk factors by retrospectively reviewing the medical records of 797 patients with first onset ischemic stroke and 787 patients with recurrent ischemic stroke. Ischemic stroke was diagnosed by a neurologist when there was a neurological abnormality confirmed using MRI. This study excluded cases with other causes (e.g., tumor, epilepsy, and toxicity) and TIA. The subtype classification of ischemic stroke was based on the TOAST classification criteria (15). Recurrent ischemic stroke was defined as patients who developed a new neurological abnormality that persisted for 24 h after 21 days from the onset of the first ischemic stroke, patients whose existing symptoms worsened, or patients who developed a lesion at a location different from the location of the original lesion (if it occurred within 21 days among patients who were initially diagnosed with ischemic stroke and received treatment such as taking medications through regular outpatient treatment). Even if a new lesion was detected using computed tomography (CT) or MRI, asymptomatic patients were excluded (4).

This study investigated age, sex, height, weight, body mass index (BMI), abdominal circumference, peripheral arterial disease, hypertension, diabetes, dyslipidemia, coronary artery disease (CAD), TIA, smoking, and overweight as risk factors for cerebrovascular disease. Each item is defined as follows. Hypertension was defined in patients who had already been diagnosed with hypertension before hospitalization or had a systolic blood pressure of 140 mmHg or higher and a diastolic blood pressure of 90 mmHg or higher after the acute phase of ischemic stroke had passed or had already been

Abbreviations: BMI, body mass index; CAD, coronary artery disease; CT, computed tomography; HDL, high-density lipoprotein; LAA, large artery atherosclerosis; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; RIS, Recurrent ischemic stroke; TIA, transient ischemic stroke.

diagnosed. If white-coat hypertension was suspected, patients were recommended to maintain a blood pressure diary at home. Diabetes was defined in patients who were diagnosed with diabetes before hospitalization, those who had fasting blood glucose of 126 mg/dL or higher (8 h after admission), those who had a blood glucose level of 200 mg/dL or higher on the random blood glucose test along with diabetes symptoms, and those who had a blood glucose level of 200 mg/dL or higher. Dyslipidemia was defined as a total cholesterol level ≥200 mg/dL or a low-density lipoprotein (LDL)-cholesterol level ≥130 mg/dL in a fasting blood test. The results of the blood test performed on the first day of hospitalization were investigated. CAD was defined as a case diagnosed by a cardiologist before or after admission or a history of percutaneous coronary intervention or bypass surgery. TIA was investigated by recording the medical history of patients or guardians using the medical records only for paroxysmal local brain dysfunctions that were caused by cerebral blood flow disorders that completely recovered within 24 h. Smokers were defined as those who smoked five cigarettes per day regularly. Persistent smokers who continued to smoke at the time of ischemic stroke recurrence after the initial onset of ischemic stroke were classified as smokers. Those who quit or had stopped smoking for more than 1 year were considered non-smokers. Alcohol consumption was classified as a risk factor when the daily alcohol intake was ≥20 mg for 3 months or longer. Overweight was defined as BMI ≥25.

This study was approved by the ethics review committee of our center. Informed consent was waived due to the retrospective nature of the study (CUH 2022-03-015). All the procedures were performed in accordance with the ethical standards of the institutional and national research committees and the Declaration of Helsinki.

2.3. Statistical analysis

First, demographics and laboratory findings were compared between female and male patients with first and recurrent ischemic stroke. Pearson's chi-square or Fisher's exact test was used for categorical variables, and the *t*-test was used for continuous variables. Second, multivariate analysis was performed to identify independent factors associated with stroke recurrence in each gender. To avoid variable selection caused by spurious correlations, only variables showing a potential association ($p < 0.1$) in the univariate analysis were included as potential factors associated with stroke recurrence in male patients in the multivariate logistic regression model. Statistical significance was set as $p < 0.05$ (two-tailed). All statistical analyses were performed using SPSS 21.0 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Age and sex distribution

During the study period, 5,608 patients with acute ischemic stroke were hospitalized and treated. This study excluded 3,774 patients who experienced ischemic stroke for the first time and 529 patients diagnosed with TIA. This study also excluded 518 patients with insufficient data were excluded. Finally, this study evaluated the data obtained from 787 patients (Figure 1). Among the excluded

TABLE 1 Comparison of baseline characteristics between males and females patients with recurrent ischemic stroke.

	Males ($n = 466$)	Females ($n = 321$)	*P-value*
Age	67.55 ± 10.51	71.87 ± 8.80	<0.001
Height (m)	166.79 ± 5.55	154.43 ± 5.44	<0.001
Weight (kg)	67.40 ± 9.87	58.52 ± 9.67	<0.001
BMI (kg/m^2)	24.19 ± 3.04	24.55 ± 3.66	0.148
Abdominal circumference	88.79 ± 10.02	88.62 ± 11.27	0.948
TOAST			
LAA	231 (49.6)	135 (42.1)	0.038
SVO	235 (50.4)	186 (57.9)	
ICAS	304 (65.2)	199 (62.0)	0.352
NIHSS (admission)	4.67 ± 3.79	4.88 ± 4.05	0.465
NIHSS (discharge)	3.89 ± 4.24	4.18 ± 4.50	0.354
mRS (discharge)	2.09 ± 1.33	2.34 ± 1.29	0.087
Previous TIA or Stroke Hx	234 (50.4)	146 (45.5)	0.173
Peripheral artery disease	5 (1.1)	1 (0.3)	0.41
Coronary heart disease	66 (29.5)	32 (24.4)	0.306
HTN	388 (83.3)	292 (91.0)	0.002
DM	213 (45.7)	179 (55.8)	0.006
Dyslipidemia	305 (65.7)	237 (73.8)	0.016
Alcohol drinking	310 (66.5)	23 (7.2)	<0.001
Smoking	330 (71.1)	24 (7.6)	<0.001
Overweight (BMI > 25)	162 (34.8)	136 (42.4)	0.031
Thrombolysis	31 (6.7)	10 (3.1)	0.028
WBC ($10^3/\mu l$)	8.38 ± 3.15	7.97 ± 2.92	0.067
Hb (g/dL)	14.10 ± 1.73	12.89 ± 1.63	<0.001
Platelet ($10^3/\mu l$)	237.03 ± 71.50	264.59 ± 75.68	<0.001
BUN (mg/dL)	18.42 ± 8.44	17.61 ± 7.33	0.168
Creatinine (mg/dL)	1.01 ± 0.59	0.80 ± 0.76	<0.001
Total cholesterol (mg/dL)	162.74 ± 39.73	177.39 ± 47.37	<0.001
Triglyceride (mg/dL)	133.28 ± 79.33	126.20 ± 83.78	0.237
HDL-cholesterol (mg/dL)	40.44 ± 17.66	45.11 ± 11.62	<0.001
LDL-cholesterol (mg/dL)	102.93 ± 37.94	113.21 ± 42.09	0.001
HbA1c (%)	6.66 ± 1.46	6.76 ± 1.54	0.348
CRP (mg/dL)	1.42 ± 8.73	1.27 ± 2.73	0.763
Fibrinogen	320.25 ± 83.49	342.61 ± 98.45	0.001

Results expressed as number (% column) or mean (standard deviation).
BMI, body mass index; LAA, large artery atherosclerosis; SVO, small vessel occlusion; ICAS, intracranial arterial stenosis; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale; TIA, transient ischemic attack; HTN, hypertension; DM, diabetes mellitus. WBC, white blood cell; Hb, hemoglobin; BUN, blood urea nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; CRP, C-reactive protein.

TABLE 2 Comparison of baseline characteristics between first ischemic stroke and recurrent ischemic stroke patients in male patients.

	First stroke ($n = 478$)	Recurrent stroke ($n = 466$)	*P-value*
Age	68.47 ± 12.03	67.55 ± 10.51	0.213
BMI (kg/m^2)	24.18 ± 3.18	24.19 ± 3.04	0.943
TOAST			
LAA	183 (61.8)	231 (49.6)	0.001
SVO	113 (38.2)	235 (50.4)	
ICAS	204 (80.6)	304 (65.2)	<0.001
NIHSS (admission)	3.87 ± 3.98	4.67 ± 3.79	0.002
NIHSS (discharge)	3.13 ± 5.70	3.89 ± 4.24	0.025
mRS (discharge)	2.36 ± 1.49	2.09 ± 1.33	0.009
Coronary heart disease	37 (7.7)	66 (29.5)	<0.001
HTN	247 (51.8)	388 (83.3)	<0.001
DM	155 (32.5)	213 (45.7)	<0.001
Dyslipidemia	106 (22.2)	305 (65.7)	<0.001
Alcohol drinking	168 (35.4)	310 (66.5)	<0.001
Smoking	145 (30.6)	330 (71.1)	<0.001
Overweight (BMI > 25)	174 (36.6)	162 (34.8)	0.566
Thrombolysis	84 (17.6)	31 (6.7)	<0.001
Hb (g/dL)	13.53 ± 2.15	14.10 ± 1.73	<0.001
Platelet ($10^3/\mu l$)	220.57 ± 72.82	237.03 ± 71.50	<0.001
BUN (mg/dL)	18.32 ± 10.00	18.42 ± 8.44	0.869
Creatinine (mg/dL)	1.06 ± 0.77	1.01 ± 0.59	0.274
Total cholesterol (mg/dL)	175.11 ± 48.12	162.74 ± 39.73	<0.001
Triglyceride (mg/dL)	155.78 ± 129.91	133.28 ± 79.33	0.002
HDL-cholesterol (mg/dL)	43.55 ± 12.31	40.44 ± 17.66	0.002
LDL-cholesterol (mg/dL)	106.09 ± 39.85	102.93 ± 37.94	0.219
HbA1c (%)	7.44 ± 23.33	6.66 ± 1.46	0.475
Fibrinogen	323.40 ± 83.57	320.25 ± 83.49	0.571

BMI, body mass index; TOAST, trial of org 10,172 in aucte stroke treatment; LAA, large artery atherosclerosis; SVO, small vessel occlusion; ICAS, intracranial arterial stenosis; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale; TIA, transient ischemic attack; HTN, hypertension; DM, diabetes mellitus. WBC, white blood cell; Hb, hemoglobin; BUN, blood urea nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; CRP, C-reactive protein.

3,774 first onset ischemic stroke, 797 patients were selected with a similar visit time and age to the 787 recurrent patients and set as a control group.

3.2. Differences in risk factors between male and female patients with recurrent ischemic stroke

Among the 787 patients, 466 (59.2%) were males. The mean age of the males was 67.6 years, which was lower than that of the females (71.9 years) (Table 1). Regarding the TOAST classification of patients with recurrence, the proportion of large artery atherosclerosis (LAA) was 49.6% in males and 42.1% in females, showing that the proportion was significantly higher in males (p=0.038). Females had significantly more cases of hypertension (83.3 vs. 91.0%, $p = 0.002$), diabetes mellitus (45.7 vs. 55.8%, $p = 0.006$), dyslipidemia (65.7 vs. 73.8%, $p = 0.016$), and overweight (BMI >25: 34.8 vs. 42.4%, $p = 0.006$) than those of males. In contrast, 71.1 and 7% of males and females had a smoking history, respectively, indicating that the smoking rate was significantly higher among males ($p < 0.001$).

3.3. Differences in laboratory findings between male and female patients with recurrent ischemic stroke

Hemoglobin, platelet, creatinine, total cholesterol, high-density lipoprotein (HDL), LDL, fibrinogen, and clopidogrel resistance levels were significantly different between males and females (Table 1). In addition, hemoglobin, creatinine, and clopidogrel resistance rates were significantly higher in males, while PLT, TC, HDL, LDL, and fibrinogen levels were significantly higher in females (Table 1). However, other laboratory findings were not significantly different between males and females.

3.4. Differences in risk factors and laboratory findings between first and recurrent ischemic stroke in males

ICAS was significantly higher in first stroke, and coronary heart disease, HTN, DM, dyslipidemia, alcohol drinking, smoking were significantly higher in recurrent ischemic stroke. Hemoglobin, platelet, total cholesterol were significantly higher in recurrent ischemic stroke in males (Table 2).

3.5. Differences in risk factors and laboratory findings between first and recurrent ischemic stroke in females

BMI, ICAS, coronary heart disease, HTN, DM, dyslipidemia were significantly higher in recurrent ischemic stroke. Hemoglobin, platelet, HbA1c were significantly higher in recurrent ischemic stroke in females (Table 3).

3.6. Risk factors for ischemic stroke recurrence in males

This study identified significant ($p < 0.1$) variables in the univariate analysis and confirmed factors associated with ischemic stroke recurrence in males using multivariate analysis to identify factors associated with the ischemic stroke recurrence by sex. The results showed that HTN, alcohol drinking, smoking, dyslipidemia were risk factors for ischemic stroke recurrence in males (Table 4).

TABLE 3 Comparison of baseline characteristics between first ischemic stroke and recurrent ischemic stroke patients in female patients.

	First stroke ($n = 319$)	Recurrent stroke ($n = 321$)	*P-value*
Age	73.52 ± 12.62	71.87 ± 8.80	0.056
BMI (kg/m^2)	23.38 ± 3.72	24.55 ± 3.66	<0.001
TOAST			
LAA	103 (55.7)	135 (42.1)	0.003
SVO	82 (44.3)	186 (57.9)	
ICAS	158 (94.6)	199 (62.0)	<0.001
NIHSS (admission)	5.10 ± 4.70	4.88 ± 4.05	0.538
NIHSS (discharge)	4.83 ± 7.45	4.18 ± 4.50	0.213
mRS (discharge)	2.77 ± 1.62	2.34 ± 1.30	0.001
Coronary heart disease	24 (7.5)	32 (24.4)	<0.001
HTN	200 (62.9)	292 (91.0)	<0.001
DM	92 (28.9)	179 (55.8)	<0.001
Dyslipidemia	68 (21.4)	237 (73.8)	<0.001
Alcohol drinking	27 (8.5)	23 (7.2)	0.518
Smoking	7 (2.2)	24 (7.6)	0.002
Overweight (BMI > 25)	1 (20.0)	136 (42.4)	0.403
Thrombolysis	63 (19.9)	10 (3.1)	<0.001
Hb (g/dL)	12.09 ± 1.69	12.89 ± 1.63	<0.001
Platelet ($10^3/\mu l$)	233.71 ± 79.12	264.59 ± 75.68	<0.001
BUN (mg/dL)	17.65 ± 9.37	17.61 ± 7.33	0.960
Creatinine (mg/dL)	0.80 ± 0.51	0.80 ± 0.76	0.982
Total cholesterol (mg/dL)	174.99 ± 49.12	177.39 ± 47.37	0.537
Triglyceride (mg/dL)	133.14 ± 104.98	126.20 ± 83.78	0.368
HDL-cholesterol (mg/dL)	47.82 ± 13.07	45.11 ± 11.62	0.007
LDL-cholesterol (mg/dL)	106.89 ± 40.85	113.21 ± 42.09	0.060
HbA1c (%)	6.22 ± 1.26	6.76 ± 1.54	<0.001
Fibrinogen	324.34 ± 80.08	342.61 ± 98.45	0.012

BMI, body mass index; TOAST, trial of org 10,172 in aucte stroke treatment; LAA, large artery atherosclerosis; SVO, small vessel occlusion; ICAS, intracranial arterial stenosis; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale; TIA, transient ischemic attack; HTN, hypertension; DM, diabetes mellitus. WBC, white blood cell; Hb, hemoglobin; BUN, blood urea nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; CRP, C-reactive protein.

3.7. Risk factors for ischemic stroke recurrence in females

This study identified significant ($p < 0.1$) variables in the univariate analysis and confirmed factors associated with ischemic stroke recurrence in females using multivariate analysis to identify factors associated with the ischemic stroke recurrence by sex. The results showed that HTN, DM, dyslipidemia were risk factors for recurrence of ischemic stroke in females (Table 4).

4. Discussion

Undoubtedly, recurrent stroke is a dangerous and frightening event in stroke patients. In particular, as medical sciences develop, the proportion of the elderly population and mean age have rapidly increased. Simultaneously, much attention has been given to improving the quality of life of the elderly population. Many studies have evaluated primary and secondary prevention programs for stroke in various countries owing to the surge in socioeconomic costs for stroke patients. Many studies on stroke recurrence have reported different results, depending on the methods, subjects, and analyses (6–8).

Although there have been many studies on the recurrence rate, risk factors, and incidence rates between males and females with ischemic stroke, they have shown different inconsistent results (6–8, 16). Most studies have reported no significant difference in recurrence factors between males and females (6–8, 16). However, Jung et al. (16) revealed that males had a higher overall recurrence rate and females had a higher chance of earlier recurrence. The males-to-females ratio was 1.45:1. Since the results between the two groups were not statistically significant, it was impossible to conclude that males were more likely to experience recurrence than females in this study. Basu et al. (13) reported in 2021 that risk factors associated with recurrence did not differ between males and females with ischemic stroke and transient ischemic stroke.

Although hypertension has been established as one of the causative factors of stroke, various views regarding its effect on recurrence have been discussed. Sacco et al. (2), Lai et al. (3), Jorgensen et al. (5), and Hier et al. (17) reported that hypertension was significantly associated with recurrence, whereas Petty et al. (18) reported no significant relationship. This study showed significant association between higher risk of recurrence with a history of hypertension and hypertension diagnosed during hospitalization in both genders, which is similar to previous findings (2, 3, 5, 17).

Diabetes is a strong risk factor for stroke, with evidence from previous meta-analyses to suggest that the risk of stroke associated with diabetes is greater in women than men, independently of other stroke risk factors (19), but the relationship between diabetes and stroke recurrence is controversial. Although many studies reported that the incidence rate of stroke is high in diabetic patients, Alter et al. (20) revealed that the follow-up of diabetes patients and recurrence rates for 2 years were not significantly different compared to those of the non-diabetic group. Alter et al. (20) also showed that in a 4-year follow-up, diabetes did not affect the recurrence rate of ischemic stroke despite blood glucose dysregulation. In this study, although not statistically significant in the male group, diabetes was identified as a risk factor for ischemic stroke recurrence in females (Table 1).

Dyslipidemia is an important risk factor for ischemic stroke. In this study, dyslipidemia was significantly associated with higher risk of recurrent ischemic stroke in both genders. 65% of males and 73% of females with recurrent ischemic stroke had a history of dyslipidemia, which showed that the proportion was higher in females than that in males. Furthermore, total cholesterol and LDL cholesterol levels were also significantly higher in females than those in males, consistent with that reported by Chen et al. (21), who showed that females with recurrent ischemic stroke had significantly higher cholesterol levels than those in males with recurrent ischemic stroke. This is attributed

TABLE 4 Factors related to recurrent ischemic stroke in males and females.

	Univariate analysis		Multivariate analysis	
	Crude OR (95% CI)	*P-value*	Adjust OR (95% CI)	*P-value*
Male patients				
HTN	4.63 (3.42–6.27)	<0.001	4.86 (3.34–7.08)	<0.001
DM	1.75 (1.34–2.28)	<0.001	1.11 (0.79–1.56)	0.552
Alcohol drinking	3.62 (2.77–4.74)	<0.001	2.41 (1.73–3.34)	<0.001
Smoking	5.59 (4.22–7.39)	<0.001	5.11 (3.65–7.16)	<0.001
Dyslipidemia	6.71 (5.03–8.96)	<0.001	5.03 (3.61–7.02)	<0.001
Female patients				
BMI	1.09 (1.04–1.14)	<0.001	1.02 (0.96–1.07)	0.553
HTN	5.94 (3.81–9.26)	<0.001	3.71 (2.20–6.25)	<0.001
DM	3.09 (2.23–4.30)	<0.001	2.01 (1.23–3.28)	0.006
Dyslipidemia	10.37 (7.19–14.95)	<0.001	8.15 (5.49–12.09)	<0.001
HbA1c	1.34 (1.18–1.52)	<0.001	1.03 (0.87–1.23)	0.726
Smoking	3.62 (1.54–8.52)	0.003	2.76 (0.98–7.80)	0.055

Non-parametric tests were performed for continuous variables that did not show normal distribution and are presented as median (25–75 percentile range).
Results are expressed as odds ratios (ORs) and 95% confidence intervals (CIs). Variables with $p < 0.1$ by univariate analysis were entered into the multivariate analysis model. HTN, hypertension; DM, diabetes mellitus; HbA1c, hemoglobin A1c, LDL, low-density lipoprotein; BMI, body mass index.

to an increase in LDL cholesterol, associated with a decrease in estrogen levels in postmenopausal females (22).

Although smoking is recognized as a risk factor for ischemic stroke, there is insufficient evidence suggesting that it is a causal factor of recurrence. In this study, the smoking rate among patients with recurrent ischemic stroke was significantly higher in males than that in females ($p < 0.001$, Table 1). Since smoking among females continues to be perceived negatively in many countries in East Asia due to social norms, smoking among males may be very high. However, smoking affects the recurrence of ischemic stroke in males significantly. Several studies have reported smoking as a risk factor for stroke. Smoking after the onset of the initial stroke would increase the risk of stroke recurrence, and a dose-response relationship was ascertained with the amount of smoking (21). However, Boiten and Lodder (23) and the World Health Organization (24) reported that smoking was not significantly associated with recurrence. Owing to these contrary results, the effect of smoking on the recurrence of ischemic stroke remains unclear and difficult to explain. However, the results of this study confirmed smoking as an important risk factor for recurrent ischemic stroke. Since this study only investigated whether patients smoked within 1 year from the onset of stroke without considering the duration and amount of smoking to identify smoking history, further detailed research is needed.

Alcohol is known to reduce stroke risk when consumed in moderation, but increases risk of ischemic stroke when consumed in excess. In particular, it is known that there is a close relationship between the occurrence of hemorrhagic stroke and alcohol (2). However, the association with ischemic stroke recurrence is not well-known. In this study, similar with the smoking rate, the rate of alcohol intake among patients was higher in males significantly and was associated with higher risk of ischemic stroke recurrence in males, but not in females.

This study had several limitations. First, since the study was conducted retrospectively on a group of patients who regularly visited a university hospital rather than a community-based group, there was a possibility of selection bias because the risk factors for ischemic stroke could be controlled to some extent for these patients. Furthermore, because this study was based on outpatients, patients had different follow-up intervals and different numbers and intervals of examinations. Consequently, evaluating the effects of treatment methods and efficacy was difficult. Although this study identified that smoking was one of risk factors for recurrent ischemic stroke in males, we did not know the type of cigarette, age since smoking, attempts to quit, duration of cessation, duration of smoking, amount of cigarette consumption, and depth of inhalation. Future studies should examine the dose-response relationship between smoking quantity and stroke recurrence in the group that continued to smoke after the onset of the initial ischemic stroke. We also believe that comparing differences between a group that continued to smoke after the first stroke and one that stopped smoking after the incident and comparing the smoking rate between first stroke and recurrent stroke would yield more solid evidence for recommending smoking cessation for the secondary prevention of ischemic stroke. Since the rate of smoking among young females is on the rise in recent years, if the smoking rate between males and females becomes similar in the future, it is highly likely that similar results will be obtained not only for males but also for females.

Since the results of this study revealed that smoking and alcohol drinking is distinct risk factors for the recurrence of ischemic stroke in males, it is believed that smoking cessation and alcohol abstinence will be especially beneficial for the secondary prevention of ischemic stroke in males who have experienced an onset of ischemic stroke. Although the American Heart Association announced in 2014 that smoking cessation was a class I recommendation in secondary prevention guidelines for stroke, the level of evidence was only C (25). There is yet not enough evidence to support this recommendation. Therefore, more extensive studies are needed to understand the effect of

smoking cessation on secondarily preventing ischemic stroke in males.

In this study, it was found that hypertension and dyslipidemia are significant risk factors for recurrence of ischemic stroke in both genders. Factors differed between genders were smoking and drinking in males and diabetes in females. Therefore, as a secondary prevention of ischemic stroke, hypertension and dyslipidemia should be controlled importantly, and factors differed between genders should be intensively controlled according to sex differences.

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

This study protocol was reviewed and approved by the Institutional Review Board of Chosun University Hospital (approval number: CUH 2022-03-015). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JC and BL contributed to the study concept and design, data collection and interpretation, manuscript drafting, and revision. YK and B-SS contributed to the data interpretation and revised the manuscript. HK contributed to the study concept and design, data interpretation, manuscript drafting, and revision. 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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Predictors and long-term outcome of intracranial hemorrhage after thrombolytic therapy for acute ischemic stroke—A prospective single-center study

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Introduction: Acute ischemic stroke (AIS) is a potentially devastating disease with high disability and mortality. Recombinant tissue plasminogen activator (rt-PA) is an effective treatment with a 2–8% possible risk for symptomatic intracranial hemorrhage (sICH). Our aim was to investigate the risk factors and long-term clinical outcomes of ICH in patients after rt-PA treatment.

Methods: Consecutive patients with AIS, thrombolysed at the Department of Neurology, University of Debrecen, between 1 January 2004 and 31 August 2016 were enrolled prospectively. Risk factors, stroke severity based on the National Institute of Health Stroke Scale (NIHSS), functional outcome using the modified Rankin scale, and mortality at 1 year were compared in patients with and without ICH following rt-PA treatment. We evaluated clinical characteristics and prognosis by hemorrhage type based on the Heidelberg Bleeding Classification. Descriptive statistics, the chi-square test, the Mann–Whitney *U*-test, ANOVA, the Kruskal–Wallis test, a survival analysis, and logistic regression were performed as appropriate.

Results: Out of 1,252 patients with thrombolysis, ICH developed in 138 patients, with 37 (2.95%) being symptomatic. Mean ages in the ICH and non-ICH groups differed significantly ($p = 0.041$). On admission, the 24-h NIHSS after thrombolysis was higher in patients with ICH ($p < 0.0001$). Large vessel occlusion was more prevalent in patients with ICH ($p = 0.0095$). The ICH risk was lower after intravenous thrombolysis than intra-arterial or combined thrombolysis ($p < 0.0001$). Both at 3 months and 1 year, the outcome was worse in patients with ICH compared to patients without ICH group ($p < 0.0001$). Mortality and poor outcome were more prevalent in all hemorrhage types with a tendency for massive bleeding associated with unfavorable prognosis. At 3 months with the logistic regression model, the worse outcome was detected in patients with ICH after thrombolysis, at 1 year in patients with ICH after thrombolysis and smoking.

Discussion: Older age, higher NIHSS, large vessel occlusion, and intra-arterial thrombolysis may correlate with ICH. The unfavorable outcome is more common in patients with ICH. Precise scoring of post-thrombolysis bleeding might be a useful tool in the evaluation of the patient's prognosis. Our findings may help to identify predictors and estimate the prognosis of ICH in patients with AIS treated with rt-PA.

KEYWORDS

thrombolysis, risk factors, intracranial hemorrhage, outcome, ischemic stroke

Introduction

Acute ischemic stroke (AIS) is a common and potentially devastating disease causing death in one-third of patients, leaving another third permanently disabled (1). Intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (rt-PA) is a standard treatment for AIS and has been proven as an effective and safe therapy within 3–4.5 h from the onset of stroke (2, 3). Local intra-arterial thrombolysis (IAT) was a possible therapeutic option in selected patients whose treatment could be started within 3–6 h after the onset of symptoms caused by the occlusion of the middle cerebral artery or within 12 h by occlusion of the basilar artery (4, 5). The significance of IAT changed over the years and nowadays. Intra-arterial thrombolysis can be used as a treatment of primary distal occlusions, as rescue after proximal occlusion thrombectomy, and/or as an adjunct therapy to primary mechanical thrombectomy (6). According to a recent summary, there is no clear consensus on best practices or criteria for the administration of IA rt-PA, although IAT is used in clinical practice (6). Nevertheless, trials concluded that IAT after thrombectomy is safe (7, 8). In mild strokes (NIHSSS $\leq$5) with LVO, the ICH rate in the case of IAT alone was better than after mechanical thrombectomy, emphasizing the importance of IAT (9).

Hemorrhagic complications, especially symptomatic intracranial hemorrhage (sICH), are the most feared and least treatable consequences of thrombolytic therapy, which may limit the use of rt-PA in patients with AIS. The risk of sICH varies from 2 to 8% depending on the definition used (based on NINDS, ECASS-II, III, ATLANTIS, and SITS-MOST studies) (10), while asymptomatic hemorrhagic transformation (HT) occurs in 18% (11). Several studies have demonstrated that HT after AIS is associated with poor functional outcomes and higher mortality rates (12). HT is a complex and multifactorial phenomenon that is most likely related to the disruption of the blood–brain barrier (BBB) and reperfusion injury of ischemic tissues (13). Several risk factors for sICH after thrombolysis have been identified. They include older age, greater stroke severity assessed by the National Institute of Health Stroke Scale (NIHSS), higher blood pressure on admission, history of diabetes mellitus, atrial fibrillation and baseline antithrombotic use, and the presence of acute ischemic changes in the computed tomography (CT) scan, all of which are proven poor prognostic factors (14). Knowledge of these predictors is important and may help clinicians to select the most suitable patients for treatment and improve the safety of thrombolysis.

The aim of our single-center prospective study was to evaluate the predictors and outcomes of ICH in patients having received thrombolytic therapy for AIS. In addition to the well-known risk factors, we analyzed the impact of large vessel occlusion (LVO) and the route of rt-PA administration for the incidence of ICH. We also evaluated the clinical characteristics and prognosis by hemorrhage type based on Heidelberg Bleeding Classification (15).

Methods

Subjects, patients

We performed a single-center prospective study. We analyzed 1,252 consecutive patients with AIS treated with rt-PA, of whom 1,124 had IVT, 61 patients underwent IAT, and 67 were given bridging therapy. Data were collected between 1 January 2004 and 31 August 2016. Our center receives patients within 90 km, in a catchment area of 600,000 inhabitants and 600–700 acute stroke hospitalizations per year. All of the patients were treated at the Neurological Intensive Care Unit, Department of Neurology, University of Debrecen, and we monitored the parameters recommended in the European Stroke Organization (ESO) guideline (5). Treatment for AIS with IV rt-PA started within 4.5 h after symptom onset was one of the inclusion criteria. The patients were categorized into two subgroups: patients with ICH and patients without ICH. In the latter group, 37 patients (2.95%) had symptomatic ICH, while 101 patients (8.06%) had asymptomatic ICH. Figure 1 shows a flowchart of participants.

Database

The collected data included baseline characteristics, common stroke risk factors, prestroke anticoagulation, occurrence and location of large vessel occlusion, type of ICH, treatment modality, stroke severity, clinical outcome at 3 months, and mortality at 1 year.

We observed the prevalence of stroke risk factors as follows: hypertension, diabetes mellitus, previous or current smoking, history of stroke, atrial fibrillation, congestive heart failure, and alcohol abuse. Upon admission, we also checked every patient's blood glucose, cholesterol and triglyceride levels, and systolic and diastolic blood pressure. Blood pressure and laboratory parameters were expressed as mean $\pm$ standard deviation.

Stroke severity was assessed by the neurologist in the stroke unit, based on NIHSS on admission and 24 h later. NIHSS scores were presented as medians (1; 3 quartile).

The 3-month outcome was evaluated using the modified Rankin scale (mRS). A good clinical outcome was defined as a score of 0–2 (16). The assessment of mRS was performed during follow-up clinical visits by certified neurologists. At 1 year, we dichotomized patients into groups "dead" and "alive."

We have chosen the risk factors of stroke (Table 1) and, on admission, the most important vital signs and stroke outcome for comparison.

Imaging

All patients underwent non-contrast CT on admission. Arterial occlusion (trunk or at least one branch of any large artery) was identified by CT angiography. Where available (93.7%), the Alberta Stroke Programme Early CT Score (ASPECTS) was used to assess early ischemic signs on admission (17). The CT was repeated 24 h after treatment in the case of clinical relapse to evaluate hemorrhagic changes. Any hemorrhage detected intracranially with imaging within 24 h after treatment was defined as post-thrombolysis ICH (18). We rated all follow-up CT scans based on the anatomical description of ICH according to the Heidelberg Bleeding Classification, where hemorrhagic infarction (HI) and parenchymatous hematoma (PH) were used as basic: *HI-1* refers to the hemorrhagic transformation of infarcted tissue as scattered small petechiae without mass effect (class 1a); *HI-2* is more confluent petechiae within the infarcted area but without space-occupying

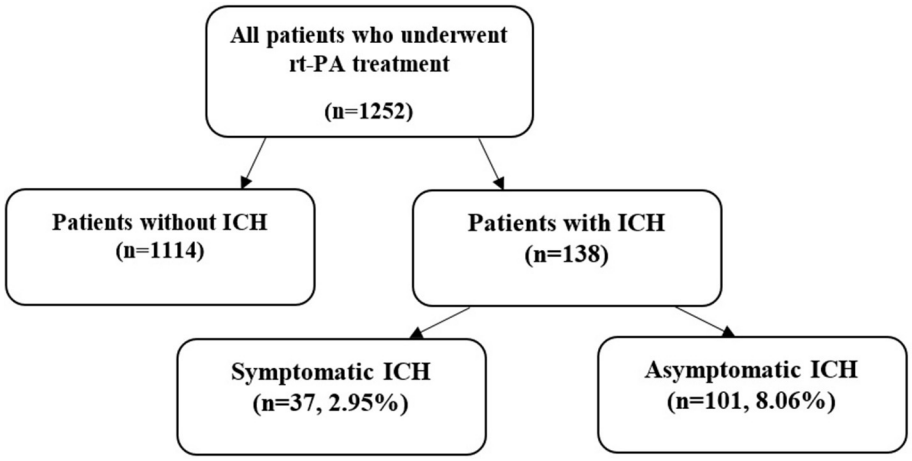

FIGURE 1
Flowchart of participants (rt-PA, recombinant tissue plasminogen activator; ICH, intracranial hemorrhage).

TABLE 1 Baseline characteristics, risk factors, and clinical parameters of patients.

Variable	Patient groups			
	Total ($n = 1252$)	ICH ($n = 138$)	no ICH ($n = 1114$)	*P*-value
Age (years), mean ± SD	67.7 ± 12.9	70 ± 10.3	67.5 ± 13.2	0.041
Gender, male, *n* (%)	702 (56)	84 (60)	618 (55.5)	NS
Risk factors				
Hypertension, *n* (%)	956 (76.3)	99 (71.7)	857 (77)	NS
Smoking, *n* (%)	318 (25.4)	38 (27.5)	280 (25.2)	NS
Diabetes mellitus, *n* (%)	248 (19.8)	24 (17.3)	224 (20.1)	NS
Alcohol abuse, *n* (%)	167 (13.3)	20 (14.5)	147 (13.2)	NS
History of stroke, *n* (%)	274 (21.9)	24 (17.4)	230 (20.7)	NS
Atrial fibrillation, *n* (%)	231 (18.4)	26 (18.8)	205 (18.4)	NS
Congestive heart failure, *n* (%)	165 (13.2)	19 (13.8)	146 (13.1)	NS
Pre-stroke anticoagulation, *n* (%)	122 (9.7)	17 (12.3)	105 (9.4)	NS
Vital parameters on admission				
Systolic blood pressure (mmHg), mean ± SD	156.7 ± 20.7	155 ± 25.54	158 ± 20.68	NS
Diastolic blood pressure (mmHg), mean ± SD	86.8 ± 13.27	86 ±17	113.5 ± 13.35	NS
Serum glucose level (mmol/l), mean ±SD	7.8 ± 1.8	7.25 ± 3.17	7.4± 2.7	NS
Cholesterol level (mmol/l), mean ± SD	5.17 ± 2.75	4.7 ± 0.49	4.9 ± 1.04	0.053
Triglyceride level (mmol/l), mean ± SD	1.27 ± 0.91	1.15 ± 0.34	1.56 ± 0.91	NS
NIHSS score on admission, median (1; 3 quartile)	10 (6; 16)	14 (10; 18)	10 (5; 15)	<0.0001

lesion (class 1b); *PH-1* is defined as a hematoma not exceeding 30% of the infarcted area but with some mild space-occupying lesion (class 1c); *PH-2* represents hematoma occupying 30% or more of the infarcted tissue, with an obvious mass lesion (class 2). Other bleeding types are classified as ICH outside the infarcted tissue (class 3a), intraventricular (class 3b), subarachnoid (class 3c), or subdural (class 3d) hemorrhage (15). In Table 3, we have chosen the variables according to our preliminary data that might have had an impact on the form of bleeding and the outcome in different groups was also of interest. The following parameters were compared in the categories of the Heidelberg Bleeding Classification score: age, ASPECTS on admission and at 24 h, NIHSS score on admission and at 24 h, serum glucose level, mRS at 3 months, and sICH.

Patients with ICH were categorized into sICH and asymptomatic ICH groups. We used three definitions for sICH: SITS, ECASS, and RCT NINDS criteria (2, 19, 20), while asymptomatic ICH was defined as the presence of any hemorrhage without neurological worsening (21).

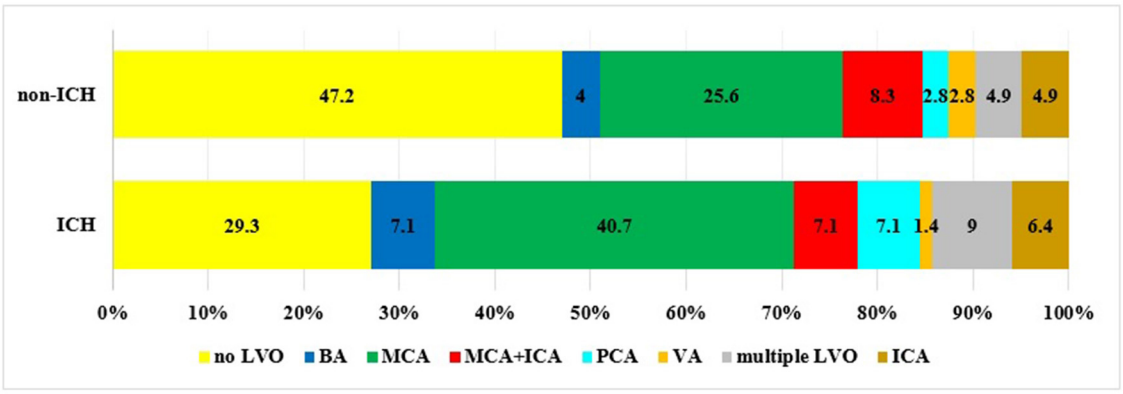

FIGURE 2
Incidence of LVO in patients with ICH compared to patients without ICH (BA, basilar artery; MCA, middle cerebral artery; ICA, internal carotid artery; PCA, posterior cerebral artery; VA, vertebral artery).

Treatment

Intravenous thrombolysis was performed in accordance with the ESO guidelines (5, 22). In the case of IVT, the total amount of rt-PA was 0.9 mg/kg of body weight (maximum 90 mg), with 10% of the dose given as a bolus followed by an infusion over 60 min using a syringe pump. Continuous monitoring of neurological status, pulse, blood pressure, body temperature, and oxygen saturation was performed according to guideline recommendations. Patients diagnosed with large vessel occlusion were started on intravenous treatment which was followed by intra-arterial administration ("bridging" therapy). IAT was used alone in patients who were not candidates for IV rt-PA. A microcatheter (Progreat TERUMO) was used for endovascular intervention. The microcatheters used were compatible with "0.017 and 0.021" guide wires. We navigated it at the site of the occlusions until the occlusion, and repeated doses of 5 mg rt-PA were given by electric syringe (1 mg/min) until the artery opened up or the maximum weight-adjusted dose was reached. After every 5 mg of rt-PA contrast material was given, and if the vessel did not open, we continued the procedure. The study and the intra-arterial use of rt-PA were approved by the Local Research Ethics Committee of the University of Debrecen (23). In 11 cases—among which only one patient had ICH—another therapeutic approach, mechanical thrombectomy, was used, when it was already available. This was negligible compared to other interventions. The type of treatment was chosen according to the actual guidelines and individually decided which treatment modality was used by the treating physician who consulted with the neuroradiologists.

Statistical analysis

Statistical analysis was carried out using the SPSS for Windows 19.0 program suite (SPSS Inc. Chicago, USA). Descriptive statistics were performed. Correlations between categorical variables were identified using Pearson's chi-squared test, and correlations between continuous variables were determined using the Mann–Whitney *U*-test. To compare each hemorrhagic transformation group, we used the Kruskal–Wallis test for non-parametric variables and the one-way ANOVA test for metric variables. The binary logistic regression analysis was used to assess outcomes at 3 months and at 1 year. Logistic regression models were used to identify the independent predictors of 3-month disability and 1-year case fatality. The analysis was performed with the multivariate general linear model (GLM). In the models, disability at 3 months (mRS >2) and case fatality at 1 year were the dependent variables, and those factors that were found to be associated with the outcome by univariate analyses were entered as confounding variables. The variables were excluded from the analysis one by one, and the variable with $p > 0.05$ and closest to 1.0 dropped out, until all features left in the model had $p < 0.05$. Survival analyses were done (Kaplan–Meier curve and logrank).

All tests were performed at a *p*-value of < 0.05 significance level.

Results

Baseline characteristics

The baseline characteristics, risk factors, and clinical parameters of the patients are summarized in Table 1. A total of 1,252 patients, 702 male patients (56%) and 550 (44%) female patients, with AIS received rt-PA treatment (aged 17–99 years; mean age, 67.7 ± 12.9). ICH was detected in 138 patients (11%); sICH occurred in 37 patients (2.95%), while asymptomatic ICH affected 101 patients (8.06%). Out of these 138 patients, 94 had ischemia-related HT, six patients had intracerebral hemorrhage outside the infarcted tissue, 26 had subarachnoid hemorrhage, 11 had intraventricular hemorrhage, and only one patient had subdural hematoma. The patients with ICH were significantly older than those without ICH (70 ± 10.3 vs. 67.5 ± 13.2, $p = 0.041$).

None of the analyzed stroke risk factors showed significant differences between the two groups. Compared with the non-ICH group, patients with ICH presented with lower serum cholesterol levels on admission (4.9 ± 1.04 vs. 4.7 ± 0.49 mmol/l), but the difference was not significant ($p = 0.053$). Baseline stroke severity was significantly higher ($p < 0.0001$) in patients with ICH compared to patients without ICH [median NIHSS scores on admission were 14 (10, 18) and 10 (5, 15), respectively].

TABLE 2 Clinical outcome of patients with and without post-thrombolysis intracranial hemorrhage.

	ICH ($n = 138$)	non-ICH ($n = 1114$)	*P*-Value
NIHSS score at 24 h, median (1; 3 quartile)	15 (9; 20)	7 (3; 14)	<0.0001
mRS score at 3 months			<0.0001
Favorable outcome (mRS: 0-2), *n* (%)	36 (26)	598 (53.6)	
Moderate/severe disability (mRS: 3-5), *n* (%)	51 (36.9)	333 (29.8)	
Death (mRS: 6), *n* (%)	49 (35.5)	162 (14.5)	
Mortality at one year	72 (52.2)	263 (23.6)	<0.0001

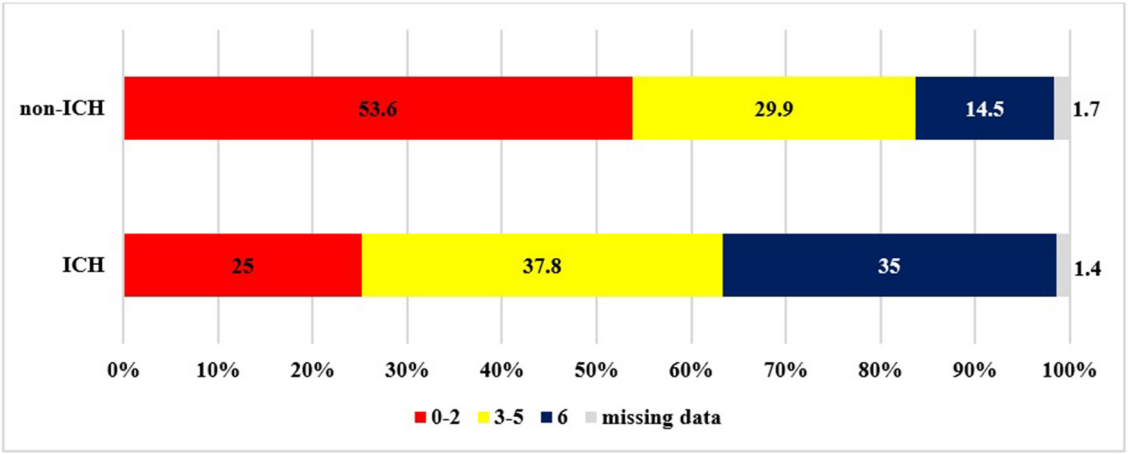

FIGURE 3
Outcome at 3 months based on mRS (ICH: intracranial hemorrhage).

Imaging

Figure 2 demonstrates the occurrence of arterial occlusion in patients with ICH and without ICH. For the entire patient population, LVO was detected in 688 patients (54.9%). The incidence of LVO was significantly higher in the ICH group compared to the non-ICH group (70.7% vs. 52.8%, $p = 0.0095$). ICH was more/most likely to develop as a result of an occlusion in the middle cerebral artery (40.7% vs. 25.6%, $p = 0.0062$), the basilar artery (7.1% vs. 4%, $p = 0.097$), and the posterior cerebral artery (7.1% vs. 2.8%, $p = 0.15$) or in the case of multiple arterial occlusions (9% vs. 4.9%, $p = 0.27$).

Treatment modality

Regarding the route of rt-PA administration, IVT occurred in 1,124 patients, 61 patients received IAT, while "bridging" therapy was done in 67 patients. In the ICH group, the distribution of treatment modalities was as follows: IVT and IAT were given to 100 patients (72.5%) and 24 patients (17.4%), respectively, while 14 patients (10.1%) received combined therapy. The relevant percentages in the non-ICH group were 89.7, 4.9, and 5.4%, respectively. The risk of ICH was 9.1% in intravenous thrombolysis, significantly lower than in intra-arterial (39.3%) or combined thrombolysis (20.9%) including mechanical thrombectomy. These data show that the intra-arterial use of rt-PA is associated with a significantly higher rate of ICH ($p < 0.0001$).

Outcome

Table 2 and Figures 3, 4 summarize the data concerning clinical outcomes. At 24 h, the patients in the ICH group had higher NIHSS scores than patients without ICH [median NIHSS scores 15 (9, 20) and 7 (3, 14)], the difference being statistically significant ($p < 0.0001$). At 3 months, only 26 % of the patients with ICH had favorable outcomes (mRS: 0–2), which was significantly worse ($p < 0.0001$) compared to the non-ICH group (53.6%). More than one-third of the patients with ICH (36.9%) had moderate or severe residual symptoms (mRS: 3–5) and, unfortunately, 35.5% of patients were dead at 3 months. In the non-ICH group, however, those rates were 29.8 and 14.5%, respectively. The differences also were significant ($p < 0.0001$). At 1 year, 52.2% of the patients with ICH had passed away, while 23.6% of the patients without ICH had passed away ($p < 0.0001$).

Table 3 and Figure 5 show the baseline characteristics, the functional outcome at 90 days, and the rate of sICH by the extent of thrombolysis-related hemorrhagic transformation. HI-2 was more frequent than HI-1, PH-1, and PH-2. Patients who experienced HT were of advanced age, especially in the PH-2 and class 3a groups ($p = 0.04$). Elevated serum glucose levels were more likely in patients with HT, especially in the HI-1 group and in patients with intraventricular

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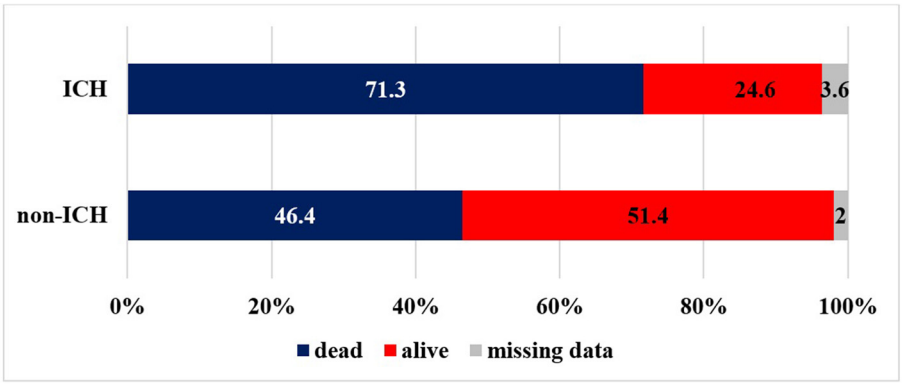

FIGURE 4
Mortality at year (ICH, intracranial hemorrhage).

TABLE 3 Baseline characteristics, functional outcome at 3 months, and rate of sICH in patient groups based on Heidelberg bleeding score.

	Patient groups based on Heidelberg bleeding score									
Variable	No HT	1a (HI-1)	1b (HI-2)	1c (PH-1)	2 (PH-2)	3a	3b	3c	3d	*P*-value
N	1114	13	31	27	23	6	11	26	1	**<0.001**
Age (years), mean ± SD	67.5 ± 13.2	70.2 ± 10.3	70.3 ± 11.6	67 ± 8.8	72.8 ± 8.7	72 ± 9	70 ± 10.3	69.8 ± 13.2	77	**0.040**
ASPECTS on admission, mean	9.1	9.5	8.1	8.6	9.4	10	8.13	9.13	10	0.965
ASPECTS at 24h, mean	7.1	5.3	5.9	5.0	4.7	4.5	5.2	4.78	3	**0.007**
NIHSS score on admission, median (1; 3 quartile)	10 (5; 15)	10.5 (6; 15)	10 (7.75; 16)	14 (12; 18.5)	16 (12.5; 18.5)	10 (10; 13.75)	16 (10.5; 17.5)	14 (11; 16.75)	14	**0.0004**
NIHSS score at 24h, median (1; 3 quartile)	7 (3; 14)	14 (8.75; 16.5)	11 (6.75; 17)	15 (5; 19)	19 (15; 22.5)	15.5 (12; 18.25)	17 (13.5; 20)	16.5 (11.25; 21)	45	**<0.0001**
Serum glucose level (mmol/l), mean ± SD	7.4 ± 2.7	9.47 ± 5.3	8.3 ± 3	8.1 ± 3.6	8.2 ± 2.8	8.3 ± 5	9.3 ± 3.7	7.5 ± 2.8	5.5	**0.019**
mRS score at 3 months, *n* (%)										
Favorable outcome (mRS: 0-2)	598 (53.6)	4 (30.8)	9 (29)	10 (37.1)	3 (13)	2 (33.3)	4 (36.4)	4 (15.4)	0 (0)	**<0.00001**
Moderate/severe disability (mRS: 3-5)	333 (29.8)	5 (38.4)	12 (38.7)	13 (48.1)	8 (34.8)	1 (16.3)	1 (9.3)	11 (42.1)	0 (0)	**0.003**
Death (mRS: 6)	162 (14.5)	4 (30.8)	10 (32.3)	4 (14.8)	12 (52.2)	3 (50.3)	6 (54.4)	10 (38.4)	1 (100)	**<0.00001**
sICH, *n* (%)	-	2 (15.4)	9 (28.1)	6 (22.2)	4 (17.4)	3 (50)	4 (36.4)	8 (30.8)	1 (100)	0.17

Abbreviations are defined in the text. The bold values indicate the values of $p < 0.05$.

hemorrhage ($p = 0.019$). The means of ASPECTS on admission did not differ significantly in the groups, but with follow-up CT scans at 24 h, significantly lower ASPECT scores were identified in all HT groups. PH-1 and PH-2 were more frequently associated with higher baseline NIHSS scores when compared with no HT ($p = 0.0004$). At 24 h, the median NIHSS was significantly higher in all HT groups. Mortality and poor outcome were more prevalent in all hemorrhage types with a tendency for heavy/massive bleeding associated with unfavorable prognosis. The rates of sICH did not differ significantly in the HT groups.

Survival analyses were conducted, but there were limitations to their interpretation (Figure 6). The 3-month survival rate in the group with intracerebral hemorrhage was significantly different from that of the group with no hemorrhage. Between 3 months and 1 year, the difference was not significant. The sample size was too small for a logrank test, so we could not perform a correct evaluation. The other factor limiting the interpretation was that the exact dates of deaths between 3 months and 1 year had not always been correctly recorded. The records only informed about whether a patient died within 3 months or within 1 year.

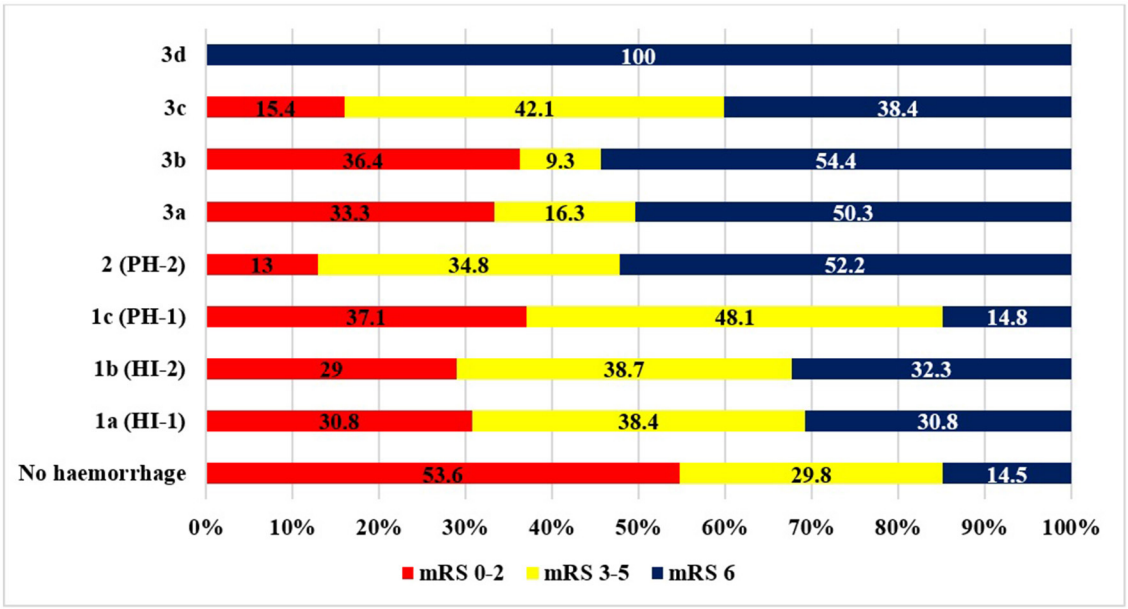

FIGURE 5
Outcome at 3 months on the mRS by hemorrhage type. Abbreviations are defined in the text.

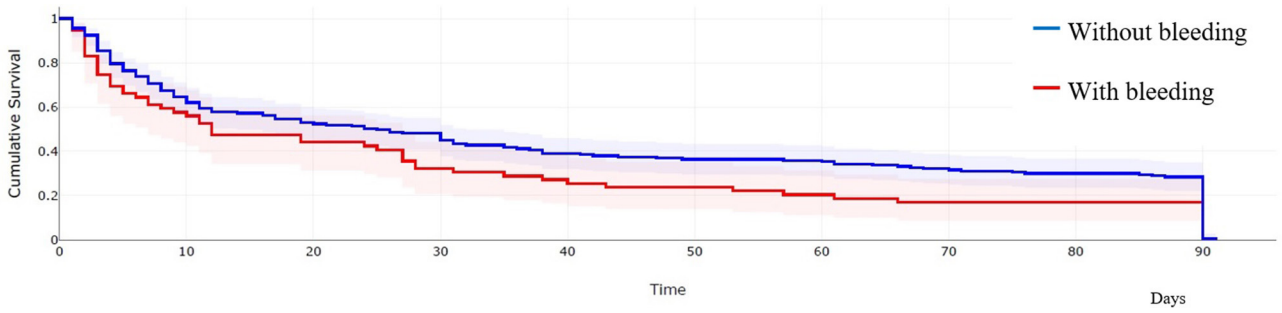

FIGURE 6
Kaplan–Meier curve of the death given in days in patients with and without bleeding after thrombolysis.

With the logistic regression model (Table 4) at 3 months, a significant difference was detected in patients with diabetes mellitus, previous atrial fibrillation, previous cardiac failure, and intracerebral bleeding after thrombolysis. From them, intracerebral bleeding after thrombolysis was the variable that had a major negative impact on the outcome at 3 months and at 1 year not only ICH but smoking also. Interestingly, diabetes mellitus, previous atrial fibrillation, and previous cardiac failure seem to have a positive effect in the logistic regression model, but this might be due to the relatively small sample size in some subgroups. Another possible explanation might be that these groups were treated previously, so their conditions were known and treated, and secondary prevention has been applied.

Discussion

Currently, rt-PA is the only approved and validated treatment for pharmacological revascularization in AIS. The treatment option for rt-PA has been selected according to the current guidelines for IV treatment; at the time of our study, IAT could be given according to the ESO 2008 guideline (5), but today IA is given after mechanical thrombectomy in the frame of studies (6–8) or alone in mild acute ischemic strokes (9). The majority of patients undergoing thrombolysis have a good prognosis compared with patients not receiving thrombolysis (3). However, the use of thrombolytic therapy is associated with an increased risk of ICH, which can reduce the chances of favorable outcomes. In this prospective single-center study, we analyzed the data of a total of 1,252 patients with AIS having undergone thrombolysis. We estimated the incidence, predictors, and outcome of ICH after treatment.

The rate of sICH in our study was 2.95%, while asymptomatic ICH occurred in 8.06%, which, in line with previous international studies, shows that it is one of the most common and serious complications of thrombolysis (20, 24, 25). Many of the underlying mechanisms for HT have not been completely evaluated yet, but studies have suggested that reperfusion injury, oxidative

TABLE 4 Factors that have a significant effect on the outcome (poor outcome means mRS > 2 points) at 3 months and at 1 year with the logistic regression model.

Factors	3 months			1 year		
	p	EXP(B)	95% Confidence interval CI	*p*	EXP(B)	95% Confidence interval CI
Diabetes mellitus	**0.019**	**0.692**	**(0.508; 0.942)**	0.141	0.781	(0.563; 1.085)
Smoking	0.075	1.078	(0.822; 1.414)	**<0.0001**	**1.703**	(1.239; 2.342)
Previous atrial fibrillation	**0.005**	**0.623**	**(0.448; 0.866)**	0.355	0.528	(0.379; 0.737)
Previous cardiac failure	**0.004**	**0.570**	**(0.387; 0.838)**	**0.001**	**0.513**	(0.350; 0.753)
Previous stroke	**0.008**	**0.661**	**(0.485; 0.899)**	0.355	0.856	(0.615; 1.190)
ICH after thrombolysis	**<0.0001**	**3.454**	**(2.262; 5.267)**	**<0.0001**	**3.168**	(2.144; 4.682)

The bold values indicate the values of $p < 0.05$.

stress, leukocyte infiltration, vascular activation, and dysregulated extracellular proteolysis are the principal triggers for the disruption of BBB which leads to blood extravasation (26). It is also suggested that the use of rt-PA may exacerbate BBB disruption by activating matrix metalloproteinases and altering endothelial function (27). A number of clinical, radiological, and laboratory variables have been shown to be associated with an increased risk of sICH following thrombolysis. In our study, the results of statistical analysis indicated that older age, higher NIHSS, LVO, and intra-arterial administration of rt-PA were risk factors for ICH after thrombolytic therapy.

Age is the most remarkable non-modifiable risk factor for stroke and a major predictor of clinical outcome (28). The literature on the risk of sICH after thrombolysis in the elderly is divisive. Previously, several studies have shown that advanced age is an independent risk factor for sICH (19, 29, 30) and was considered a relative contraindication for 3–4.5 h IVT by many guidelines. However, many other articles have reported that the incidence of sICH does not differ significantly between younger and older patients, and this age group still seems to benefit from treatment, as was mentioned in our previous publication (31–34).

In the present study, the patients with ICH were significantly older than those without ICH. At the same time, in our previous study, when patients with thrombolysis were dichotomized into those aged ≥ 80 years and those aged <80 years, statistically speaking, the risk of sICH was not significantly greater in the older group (34). These data suggest that patients over 80 and suffering from an acute stroke should not be excluded from treatment with rt-PA based on their risk for sICH.

Although a history of hypertension, being the most significant pre-existing risk factor, was present in 76.3% of the patients in the overall population, we could not find any significant correlation in either the presence of hypertension or the mean blood pressure values on admission between the ICH and non-ICH groups. Other comorbidities, including diabetes mellitus, previous stroke, smoking, atrial fibrillation, prestroke anticoagulation, congestive heart failure, and alcohol consumption, were not correlated with ICH in the present study. However, we found that elevated baseline blood glucose was more likely in the HI and PH subgroups, which was consistent with previous findings where it had been shown that serum glucose was a predictor of ICH in patients treated with rt-PA (35). Some reports suggest that lower serum total cholesterol and triglyceride levels are associated with an increased risk for ICH (36, 37). We also found a similar trend, although no statistical significance was reached ($p = 0.053$). A possible explanation for this relationship is that cholesterol plays an important role in the integrity of small cerebral vessels and the neurovascular unit (23).

It is an important finding of our study that the NIHSS scores at presentation and at 24 h are significantly higher in patients with ICH. NIHSS measures stroke severity, which is primarily associated with the size of cerebral infarction. The higher the NIHSS score values are, the more severe the strokes and the larger the infarcts may become. Most of the previous studies including NINDS and ECASS trials have also shown that the severity of a baseline stroke is one of the most important predictors of ICH after thrombolysis (38). In our study, subgroup analyses across the HT groups showed that PH-1 and PH-2 were associated with higher baseline NIHSS scores than the HI groups. It has been suggested that the underlying mechanism of HI and PH differs from each other. Previous studies revealed that HI frequently occurred without the use of rt-PA as a natural phenomenon in the course of ischemic stroke, while severe PH was mainly associated with treatment using alteplase (39, 40). It can be an explanation for our finding that severe ischemic stroke with higher baseline NIHSS is suggestive of larger areas of infarcted tissue, including injured blood vessels, which are more likely to bleed after thrombolytic treatment. However, in our study, the median NIHSS scores at 24 h were significantly higher in all HT groups compared to no HT. These data suggest that post-thrombolysis hemorrhage is associated with early deterioration of neurological symptoms regardless of the extent of bleeding.

In the present study, the incidence of LVO was found to be 54.9%, slightly higher than the rate (24–46%) reported in the literature (41). Our results showed that LVOs, especially occlusions in the middle cerebral artery (MCA), were significantly more prevalent in patients with ICH, as also found in previous studies (42, 43). The MCA is the largest intracranial artery and by far the most commonly affected vessel in AIS. The occlusion of this artery may lead to massive cerebral infarction which is one of the most dangerous factors of HT. The development of extensive brain edema and enhanced permeability of vascular walls caused by prolonged ischemia and hypoxia may be the possible explanation for the higher probability of HT (24). The use of rt-PA can aggravate BBB disruption and may further increase the risk of HT (27). Therefore, the presence

of an LVO shows a positive correlation with the incidence of ICH after thrombolysis.

Regarding the route of administering rt-PA, we found that the hemorrhage rate in patients treated with IVT was comparable to the rates reported in large thrombolysis trials (2, 20, 25). Another conclusion of our study is that intra-arterial use of rt-PA is associated with a significantly higher rate of ICH, which is also consistent with previous findings (24, 43). Large multicenter studies (PROACT II, MELT, and IMS II) have proven the efficacy of performing intra-arterial thrombolysis in LVO, although the indications and dosage of IAT remained less well-standardized (44). The rate of ICH in the aforementioned studies has ranged from 10 to 15%, which is in agreement with our results (11%). In the CHOICE trial, IAT was given after mechanical thrombectomy—this is different from our scenario because our patients with IAT did not undergo mechanical thrombectomy—in 61 patients (vs. 52 placeboes), sICH was 0% compared with placebo, but overall cerebral hemorrhage was 19% (6). A possible reason for the increased risk of ICH is the relatively high concentration of the thrombolytic agent at the site of application, which triggers a stronger activation of metalloproteinases and greater damage to the BBB (45); in addition, this is the larger infarct side that might occur in case of LVO. Nowadays, the first choice of LVO treatment is thrombectomy, but rt-PA treatment should be started, if needed before it, emphasizing the impact of our study in everyday use.

Finally, as far as the long-term outcome is considered, the functional status at 3 months has turned out to be significantly worse in the ICH group. This result is consistent with most previous studies which showed lower rates of favorable and independent outcomes in patients with ICH at day 90 (25, 29, 31, 46). Similarly, the 1-year mortality rate is also significantly higher in patients with ICH. Based on these studies, the fatality rates are between 50 and 80%, which is consistent with our result (50.7%). However, two factors need to be mentioned regarding the effect of ICH on the outcome. First, it is still unclear how the extent of HT influences the long-term functional outcome. An ECASS-II study reported that none of the radiological subtypes of hemorrhage have the same effect on the outcome (19). There is no doubt that massive HT is likely to be associated with clinical worsening/relapse, while HI might be a clinically irrelevant phenomenon of ischemic damage and reperfusion (39). Our results showed that both HI and PH have a negative impact on patient outcomes, which is consistent with some previous studies (46, 47) suggesting that HI grades of HT may not be benign. Second, there is an overlap between risk factors for thrombolysis-associated ICH and risk factors for poor outcomes following thrombolytic therapy in the absence of ICH. Thus, the observed poor outcomes are more probably related to the combination of the ICH and the underlying ischemic event itself (14).

The logistic regression model shows the importance of regular screening of the population suffering from diabetes mellitus, atrial fibrillation, cardiac failure, stroke, and quitting smoking. Intracerebral bleeding was a strong predictor of worse outcomes even in these models at 3 months and at 1 year as well. Our aim was to find the predictors of ICH after rt-PA treatment, including the treatment modality. Our findings show that not only LVO but IA treatment alone is also a risk factor. Many factors, such as technique and equipment, may be responsible for this, but our study was not designed to find this. Our study was a real-life scenario study with consecutive and not randomized patients, so the findings may be different from randomized clinical trials.

Our study has limitations. First, the size of the sample is relatively small for survival analysis. In the Heidelberg Bleeding Classification, some groups had few patients. Nevertheless, the most important and relevant risk factors have been identified in a real-world scenario. Second, we could not form a parallel non-treated group for obvious ethical reasons. If we had managed to, it could have ruled out the bias that, without rt-PA treatment, ICH might also occur in AIS. Despite the aforementioned, our study has highlighted that ICH after treatment may be a prognostic factor for poor outcomes.

Conclusion

In conclusion, we found that patients of older age, having higher NIHSS, suffering from an occlusion affecting a large intracranial artery, and treated with intra-arterial rt-PA were at an increased risk for ICH after thrombolysis. Those patients seemed to have worse long-term functional outcomes and higher mortality rates than patients without ICH, so ICH after thrombolysis was a strong predictor of worse outcomes in univariate and multivariate analyses as well. We found that precise scoring of post-thrombolysis bleeding might be a useful tool in the evaluation of the patient's prognosis. To our knowledge, this was the first study with a real-life scenario in our region on this patient population.

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 Local Research Ethics Committee of the University of Debrecen. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

KF, IF, and LH led the initiative and revised the drafted document. KF, LH, and MH selected the abstract, extracted data, and drafted the manuscript. KF, AH, and LH were involved in the creation of the database. JT was involved in the evaluation and conceptualization of the radiological part. SM, LH, KF, and MH were involved in the investigation, data curation, data analysis, and writing the original draft. IF and KF were involved in supervision. All authors were

involved in the conceptualization, methodology, review, editing and approved the final 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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General anesthesia vs. non-general anesthesia for vertebrobasilar stroke endovascular therapy

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Background: The optimal type of anesthesia for acute vertebrobasilar artery occlusion (VBAO) remains controversial. We aimed to assess the influence of anesthetic management on the outcomes in VBAO patients received endovascular treatment (EVT).

Methods: Patients underwent EVT for acute VBAO at 21 stroke centers in China were retrospectively enrolled and compared between the general anesthesia (GA) group and non-GA group. The primary outcome was the favorable outcome, defined as a modified Rankin Scale (mRS) score 0–3 at 90 days. Secondary outcomes included functional independence (90-day mRS score 0–2), and the rate of successful reperfusion. The safety outcomes included all-cause mortality at 90 days, the occurrence of any procedural complication, and the rate of symptomatic intracranial hemorrhage (sICH). In addition, we performed analyses of the outcomes in subgroups that were defined by Glasgow Coma Scale (GCS) score ($\leq$8 or >8).

Results: In the propensity score matched cohort, there were no difference in the primary outcome, secondary outcomes and safety outcomes between the two groups. Among patients with a GCS score of 8 or less, the proportion of successful reperfusion was significantly higher in the GA group than the non-GA group (aOR, 3.57, 95% CI 1.06–12.50, $p = 0.04$). In the inverse probability of treatment weighting-propensity score-adjusted cohort, similar results were found.

Conclusions: Patients placed under GA during EVT for VBAO appear to be as effective and safe as non-GA. Furthermore, GA might yield better successful reperfusion for worse presenting GCS score ($\leq$8).

Registration: URL: http://www.chictr.org.cn/; Unique identifier: ChiCTR2000033211.

KEYWORDS

vertebrobasilar occlusion, anesthesia, endovascular treatment, outcome, propensity score

Introduction

Despite remarkable advances in the endovascular treatment (EVT) of large artery occlusion in acute ischemic stroke, the clinical outcomes have not kept pace. Among the numerous studies of the reasons for this mismatch, perioperative management has received comparatively little attention in terms of affecting clinical outcomes. Prior observational studies have suggested that patients undergoing EVT without general anesthesia (non-GA) have a higher probability of good clinical outcomes than patients treated with general anesthesia (GA) (1, 2). Non-GA may lead to faster initiation of therapy and may avoid complications associated with intubation, however, the detrimental effect of GA was ultimately mediated through infarct growth (3). The well-known randomized trials (GOLIATH, SIESTA, and ANSTROKE) compared general anesthesia to conscious sedation (CS) during EVT, but the conclusions were inconsistent (4–6).

To our knowledge, most observational studies and prospective randomized controlled trials (RCTs) have been limited to enrolling patients with anterior circulation stroke (3, 4, 6, 7). Few studies have been conducted on the types of anesthetics that may impact functional outcomes in acute vertebrobasilar occlusion (VBAO) patients treated with EVT (8, 9). Unlike anterior circulation strokes, a considerable proportion of patients with posterior circulation strokes require emergency intubation for airway protection due to alterations in the level of consciousness. For patients with poorer clinical presentation and more severe stroke, it is worth exploring whether GA is more beneficial than non-GA. Therefore, the best anesthetic management for VBAO is still unclear.

We aimed to determine whether the use of GA for EVT of VBAO was safe and to compare the differences in clinical outcomes between GA and non-GA based on acute PostErior ciRculation iSchemIc Stroke regisTry (PERSIST), a retrospective multicenter EVT registry program of VBAO treated with EVT in China.

Methods

Data from this study are available from the corresponding author upon reasonable request.

Study population

The retrospective PERSIST recruited stroke centers within China to submit demographic, clinical presentation, procedural details, angiographic and clinical outcome data on consecutive patients who present with acute, symptomatic, radiologically verified VBAO treated with EVT at 21 stroke centers from Dec 2015 to Dec 2018 (Registration: URL: http://www.chictr.org.cn/; Unique identifier: ChiCTR2000033211). Previously published work described PERSIST methodology in detail (10, 11).

In this study, we further excluded patients whose anesthetic method was not recorded specifically. The remaining patients were divided into two groups based on the anesthetic choice at the beginning of each EVT procedure: (1) patients who had endotracheal intubation along with general anesthesia (GA); (2) patients who had local anesthesia with or without sedation, as long as they had no endotracheal intubation (non-GA). Patients converted to GA during MT procedures were scored as non-GA according to the intention-to-treat principle. As-treated analysis considered the treatment actually received, which was sensitivity analysis. Patients in the non-GA group received a subcutaneous injection of Xylocaine and, if necessary, low-dose short-acting analgesics and/or sedatives. Patients in GA received analgesics and/or sedatives at higher doses at the discretion of anesthetists. In patients treated under GA, extubation was aimed for at the earliest time. The study was approved by the Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China (USTC) in Hefei, China. Informed consent was waived by the Ethics Committee for this retrospective nature.

Outcomes

The primary outcome measure was the favorable outcome, defined as a modified Rankin Scale (mRS) score of 0–3 at 90 days. Secondary outcomes included functional independence (90-day mRS score 0–2) and the rate of successful reperfusion (modified Thrombolysis in Cerebral Infarction score [mTICI], 2b−3) (12). Safety outcomes included all-cause mortality at 90 days, the occurrence of any procedural complication (dissection, perforation, and embolus in a new territory), and the rates of symptomatic intracranial hemorrhage (sICH). sICH was diagnosed if the newly observed ICH on imaging was related to any of the following conditions: (1) an NIHSS score that increased more than 4 points; (2) an NIHSS score that increased more than 2 points in a category; (3) deterioration led to hemicraniectomy, external ventricular drain placement, intubation, or other major medical interventions (13). All the neuroimaging data were sent to the core laboratory in the First Affiliated Hospital of USTC and were evaluated in a blinded manner by two experienced neuroradiologists. If there was any disagreement, the final assessment was confirmed on the basis of consensus.

Statistical analysis

Continuous variables were described as the mean (SD) or median (IQR) as appropriate. Categorical variables were described as numbers (percentage). Normality of distributions was assessed using histograms and the Shapiro-Wilk test. To evaluate the magnitude of between-group differences for baseline characteristics, we calculated the absolute standardized difference, which interprets more than 10% as a meaningful difference (14). We compared the outcomes between the 2 groups after taking into account the potential confounding factors by using prespecified propensity score methods (PSM) (15).

The effects of the anesthetic approach were estimated by using propensity score matching as the primary analysis and by using the inverse probability of treatment weighting (IPTW) propensity score method (using stabilized inverse propensity score as weighty in regression models) as a secondary analysis. Patients in the GA group were matched 1:1 to patients in the non-GA group according to propensity score, using the greedy nearest neighbor matching algorithm, with a caliper of width equal to 0.2 of the standard deviation of the logit of the propensity score (16). A multivariable logistic regression model was used to compute the propensity score, with the anesthetic protocol as the dependent variable and all the baseline data in the table as covariates. Due to the lack of

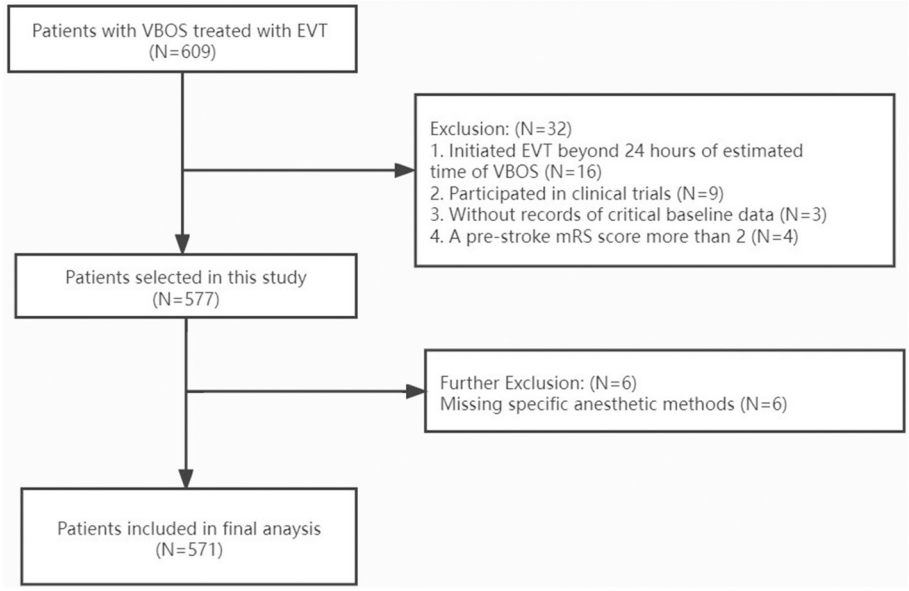

FIGURE 1
Flow chart of patient inclusion. VBAO indicates vertebrobasilar occlusion strokes; EVT, endovascular treatment; mRS, modified ranking scale.

baseline data (range from 0 to 8%), the missing covariate values are processed through multiple imputation (chained equations with $m = 5$ imputations obtained) (17). The imputation procedure was performed under the missing at random assumption with a predictive mean matching method for continuous variables and logistic regression model for categorical variables. In each multiply imputed dataset, we calculated the propensity score and assembled a matched cohort to provide both matched and IPTW-propensity score-adjusted effect sizes, which were subsequently combined by using Rubin's rules (18).

Univariate analysis was performed using Student's t-test for continuous variables and χ^2 or Fisher's exact test (for small cell size) for categorical variables. We compared the outcomes between groups by binary logistic regression analysis. Our initial analysis followed an intention-to-treat principle in which patients who converted from non-GA to GA during the procedure were included in the non-GA group. The as-treated analysis was also performed as a sensitive analysis. Statistical testing was conducted at the 2-tailed level of 0.05. All analyses were processed using SPSS version 26 (IBM Corp., Armonk, NY) and R version 4.0.5.

Results

The dataset of 609 patients with VBAO who received EVT during the study period included 571 patients [median age 64 (55–73) years, 71.5% male] who were ultimately eligible for analysis. The flow chart for the selection is presented in Figure 1. Of these, 451 patients underwent non-GA (80%) and 120 underwent GA (20%). The conversion from non-GA to GA occurred in 9/451 (2%) patients during the procedure because of severe movement or vomiting/aspiration. Both groups had similar medical histories, with the exception of atrial fibrillation and drinking history, which was more common in the GA group (Table 1). Patients in the GA group had a higher glucose level at admission, and more use of intravenous thrombolysis prior to EVT than patients in the non-GA group (Table 1). The admission systolic blood pressure, NIHSS score and GCS score were similar between the GA and non-GA groups, as well as the site of occlusion. The clinical characteristics and outcomes in the overall population without missing data imputation were shown in Supplementary Table 1.

One hundred and three matched pairs were found in the primary analysis. The baseline characteristics according to the 2 study groups before and after PSM are shown in Table 1. Before matching, sex, atrial fibrillation history, drinking history, admission glucose levels and prior use of intravenous thrombolysis showed stronger differences (ASD $>$ 10%). ASD decreased significantly after PSM with a maximum ASD of 2.3% for sex, 2.7% for atrial fibrillation history, 5.4% for drinking history, 2.8% for admission glucose levels, and 5% for prior use of intravenous thrombolysis (Table 1).

Procedural-related outcomes and complications

The time from estimated occlusion to groin puncture between the GA and non-GA groups was not significant ($p = 0.23$); however, the time from groin puncture to reperfusion was 35 min longer in the GA group than in the non-GA group ($p < 0.001$) (Table 2). The rate of aspiration pneumonia was 78.6% in the GA group, which was significantly different from that in the non-GA group (63.1%) ($p < 0.001$). The remaining complications did not differ between patients who received GA and those who did not (Table 2). The rate of procedural complications occurred in 8 (6.7%) of 120 patients who had GA vs. 15 (11.1%) of 451 patients who had non-GA ($p = 0.62$).

TABLE 1 Clinical characteristics according to anesthetic approach in VBAO patients admitted for thrombectomy before and after propensity score matching.

Characteristic	Before matching			After matching		
	GA ($n = 120$)	Non-GA ($n = 451$)	ASD, %	GA ($n = 103$)	Non-GA ($n = 103$)	ASD, %
Age, median (IQR), year	66 (55–73)	64 (55–74)	3.4*	64 (54–73)	64 (54–72)	3.7*
Sex, male	92 (76.7)	316 (70.1)	15.0	76 (73.8)	77 (74.8)	2.3
Medical history						
Hypertension	82 (68.3)	304 (67.4)	2.0	70 (68.0)	71 (68.9)	1.9
Dyslipidemia	41 (34.2)	168 (37.3)	6.3	39 (37.9)	37 (35.9)	4.2
Atrial fibrillation	19 (15.8)	108 (23.9)	20.4	18 (17.5)	17 (16.5)	2.7
Diabetes	24 (20.0)	103 (22.8)	6.8	24 (23.3)	27 (26.2)	6.8
Coronary heart disease	10 (8.3)	46 (10.2)	6.6	7 (6.8)	9 (8.7)	7.1
Smoking	37 (30.8)	143 (31.7)	2.0	32 (31.1)	37 (35.9)	10.0
Drinking	18 (15.0)	94 (20.8)	15.2	16 (15.5)	18 (17.5)	5.4
Clinical status						
Admission SBP, mean (SD), mmHg	149 (23.2)	151 (25.3)	4.4	151 (21.5)	151 (31.0)	0.8
GCS score, median (IQR)	7 (6–11)	8 (6–12)	9.4*	8 (6–11)	7 (6–11)	5.6*
NIHSS score, median (IQR)	23 (14–28)	23 (14–30)	4.0*	23 (14–28)	22 (14–31)	2.3*
Glucose, median (IQR), mmol/L	8.2 (6.3–10.4)	7.2 (5.8–9.5)	13.9*	7.9 (6.3–10.4)	7.4 (5.8–10.3)	2.8*
Site of occlusion						
Basilar artery	86 (71.1)	342 (75.8)	9.3	75 (72.8)	71 (68.9)	8.6
Treatment						
IV thrombolyis	26 (21.7)	77 (17.1)	11.7	22 (21.4)	20 (19.4)	5.0

Values expressed as numbers (%) unless otherwise indicated. Values were calculated after handling missing data using multiple imputation procedure. ASD indicates absolute standardized difference; GA, general anesthesia; GCS, glasgow coma scale; IQR, interquartile range; IV, intravenous; NIHSS, national institutes of health stroke scale; non-GA, without general anesthesia; SD, standard deviation, and SBP, systolic blood pressure.

*Estimated using the rank-transformed data.

Primary and secondary outcomes

In the propensity score matched cohort, the favorable outcome was not associated with any significant changes between the GA group and the non-GA group (aOR, 0.97, 95% CI 0.34–2.76, $p = 0.95$) (Figure 2). Similarly, the rate of functional independence (aOR, 0.92, 95% CI 0.32–2.66, $p = 0.87$) was not significantly different between the GA group and non-GA group, as well as the successful reperfusion (aOR, 2.19, 95% CI 0.59–8.06, $p = 0.23$). In the IPTW-propensity score-adjusted cohort, similar results were found in favorable outcome, functional independence and successful reperfusion (Figure 2). With respect to the safety outcomes, we found no significant differences in the PSM cohort, which showed the same outcomes in the IPTW-propensity cohorts (Figure 2). The sensitivity analysis restricted to the as-treated sample provided similar results across all studied outcomes in the PSM cohort as well as in the IPTW-propensity cohorts (Supplementary Tables 2, 3 and Supplementary Figure 1).

Subgroup analysis

Propensity score-matched patients in each group were divided into subgroups by Glasgow Coma Scale (GCS) score (≤8 or >8). Among patients with GCS score ≤8, the proportion of successful reperfusion was significantly higher in the GA group (88.5%) than in the non-GA group (79.6%) (aOR, 3.57, 95% CI 1.06–12.50, $p = 0.04$). The effect on favorable outcome (aOR, 1.23, 95% CI 0.43–3.57, $p = 0.70$), functional independence (aOR, 1.33, 95% CI 0.39–4.55, $p = 0.64$), and all safety outcomes remained no differences. In IPTW-propensity score-adjusted cohort, similar results were presented (Figure 3).

Discussion

After adjustment for baseline characteristics, our study showed that VBAO patients who underwent GA achieved similar rates of primary and secondary outcomes as those who had non-GA, meanwhile, without an increased risk of symptomatic intracranial hemorrhage or mortality. The results were similar using both intention-to-treat and as-treated analysis. Additionally, subgroup analysis based on GCS score revealed that in VBAO patients with a lower presenting GCS score (≤8), GA could yield a higher proportion of successful reperfusion.

Most early observational studies demonstrated that patients with acute ischemic stroke undergoing EVT appeared to show worse neurological outcomes and higher mortality when treated with GA

TABLE 2 Procedural-related outcomes and complication according to anesthetic approach in VBAO patients admitted for thrombectomy before and after propensity score matching.

	Before matching			After matching		
	GA ($n = 120$)	Non-GA ($n = 451$)	*P*-value	GA ($n = 103$)	Non-GA ($n = 103$)	*P*-value
Workflow						
Estimated occlusion to groin puncture, median (IQR), hours	6.4 (4.0–8.5)	5.4 (3.8–8.7)	0.16	6.5 (4.0–8.8)	5.5 (3.9–8.3)	0.23
Onset to groin puncture, median (IQR), min	390 (252–500)	330 (233–513)	0.17	390 (255–500)	300 (215–480)	0.10
Groin puncture to reperfusion, median (IQR), min	140 (104–190)	103 (68–143)	<0.001	140 (103–190)	105 (80–140)	<0.001
Procedural complication	8 (6.7)	50 (11.1)	0.16	8 (7.8)	10 (9.7)	0.62
Dissection	1 (0.8)	15 (3.3)	0.21	1 (1.0)	4 (3.9)	0.37
Perforation	3 (2.5)	11 (2.4)	1.00	3 (2.9)	2 (1.9)	1.00
Embolus in a new territory	4 (3.3)	24 (5.3)	0.48	4 (3.9)	4 (3.9)	1.00
Complication						
Pneumonia	95 (79.1)	268 (59.4)	<0.001	81 (78.6)	65 (63.1)	<0.001
Cerebral hernia	18 (15.0)	43 (9.5)	0.09	13 (12.7)	7 (6.8)	0.17
Acute heart failure	22 (18.3)	65 (14.4)	0.29	18 (17.5)	10 (9.7)	0.10
Gastrointestinal bleeding	9 (7.5)	37 (8.2)	0.80	9 (8.7)	11 (10.7)	0.64

Values expressed as numbers (%) unless otherwise indicated. Values were calculated after handling missing data using multiple imputation procedure. GA, general anesthesia; IQR, interquartile range; non-GA, without general anesthesia.

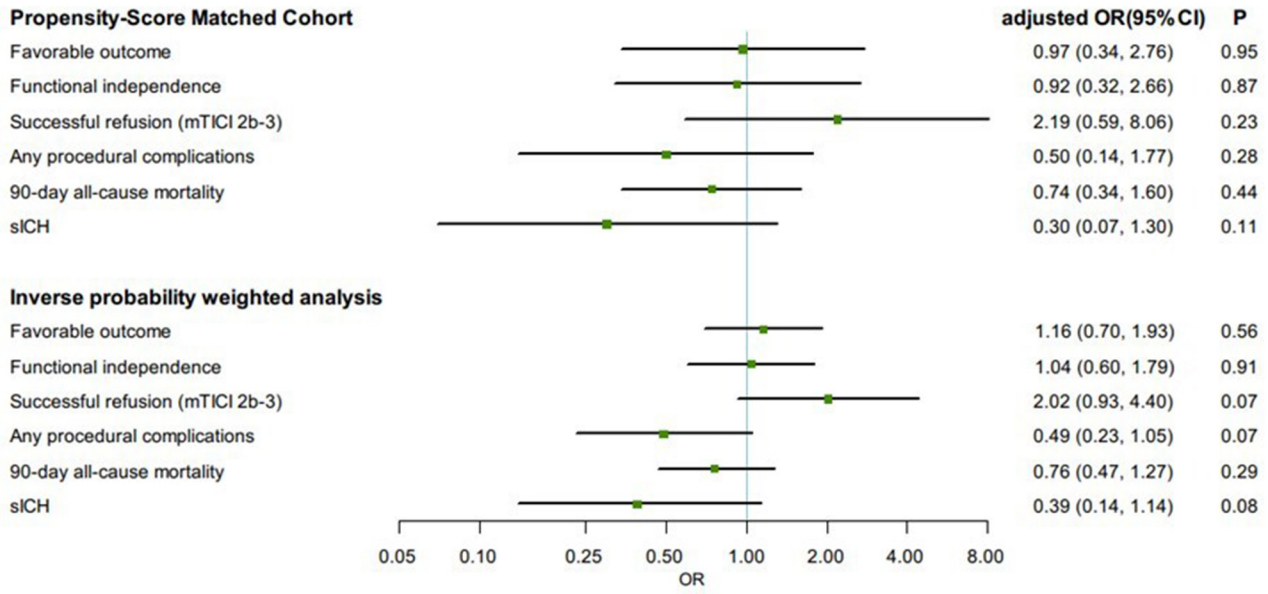

FIGURE 2
Comparisons in clinical and angiographic outcomes according to first-line anesthetic approach in patients treated with thrombectomy in matched and inverse probability of treatment weighting (IPTW) analyses. CI, confidence interval; mTICI, modified thrombolysis in cerebral infarction; OR, odds ratio; sICH, symptomatic intracranial hemorrhage. All regression analyses were adjusted for the following variables: age, sex, atrial fibrillation, smoking history, systolic blood pressure, glucose, site of occlusion, Glasgow Coma Scale score, baseline NIH Stroke Scale score, IV thrombolysis, pneumonia, time from estimated occlusion to groin puncture, door to groin puncture, and groin puncture to reperfusion.

compared with non-GA (19, 20). Delay in treatment initiation (due to the time required for GA induction, emergency endotracheal intubation, and an available experienced anesthesiologist) has been speculated to be a reasonable explanation as to why GA may be associated with poorer neurological outcomes after EVT. The highly effective reperfusion using multiple endovascular devices

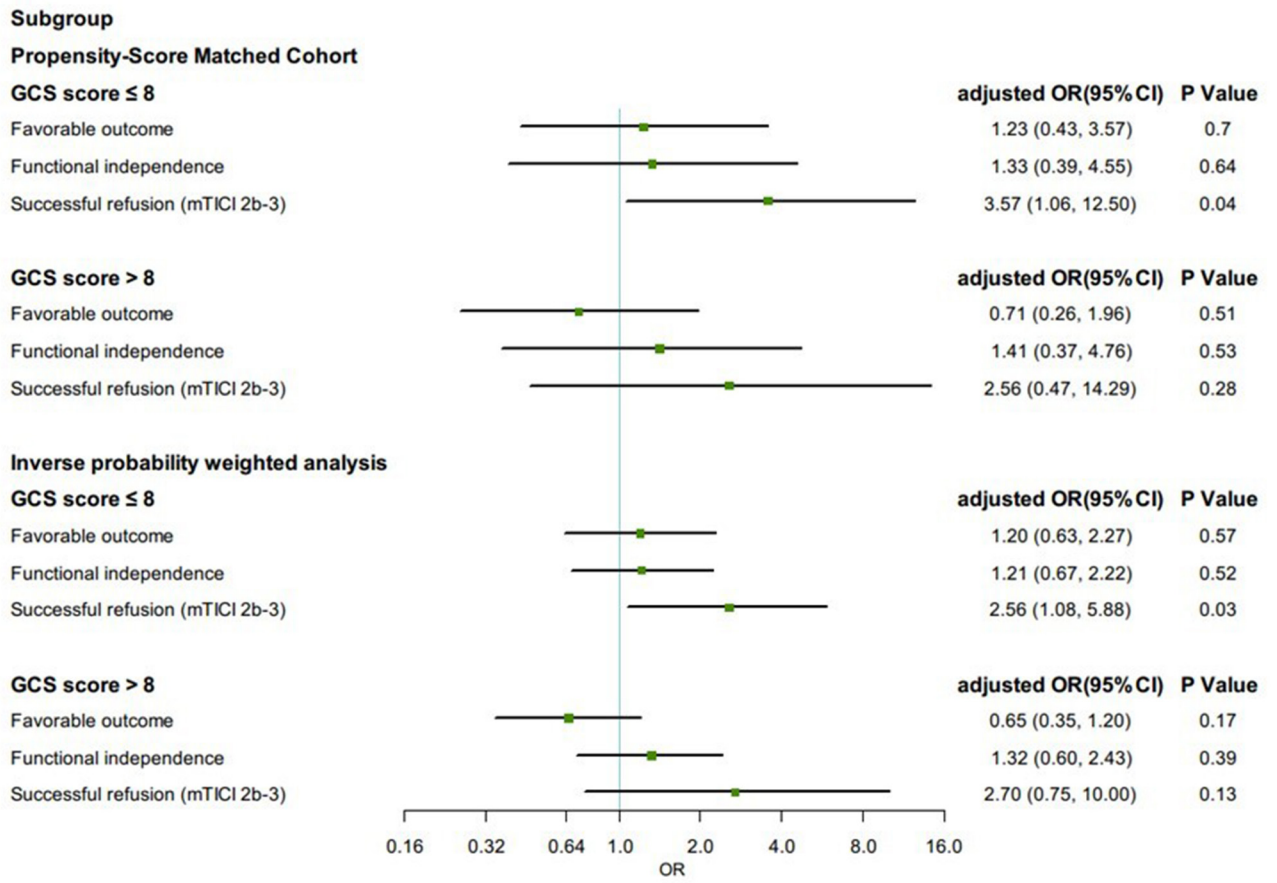

FIGURE 3
Subgroup analysis in clinical and angiographic outcomes according to first-line anesthetic approach in patients treated with thrombectomy in matched and inverse probability of treatment weighting (IPTW) analyses. CI, confidence interval; mTICI, modified thrombolysis in cerebral infarction; OR, odds ratio; sICH, symptomatic intracranial hemorrhage. All regression analyses were adjusted for the following variables: age, sex, atrial fibrillation, smoking history, systolic blood pressure, glucose, site of occlusion, baseline NIH Stroke Scale score, IV thrombolysis, pneumonia, time from estimated occlusion to groin puncture, door to groin puncture, and groin puncture to reperfusion.

(HERMES) collaboration (21), for example, the time interval between randomization and reperfusion was 20 min later in patients who had GA vs. patients who had non-GA during EVT in anterior circulation ischemic stroke. However, in the present study, puncture to reperfusion times were significantly longer in the GA group that did not confer a disadvantage to patients with VBAO compared to non-GA. A recent meta-analysis showed that in spite of the longer onset-to-EVT and onset-to-groin puncture times in VBAO, favorable functional outcome at 90 days in VBAO was comparably no difference just as in anterior circulation large vessel occlusion during EVT (22). This could be supported by the hypothesis that benefit of recanalization is less time-dependent in VBAO than in anterior circulation large vessel occlusion due to the anatomical vascular layout of the brainstem being different from that in usual anterior circulation stroke (23).

Compared with anterior circulation stroke, VBAO has its own characteristics. Patients with VBAO are more likely to have consciousness disorders, or even remain in a deep coma. Therefore, local anesthesia with or without conscious sedation is safe and effective for these patients, especially for operations that can be completed quickly. Most patients with consciousness disorders are prone to restless, accompanied by irregular breathing patterns, hypoxemia, vomiting and aspiration, especially patients with difficult vascular approach and predicted long operation time, so general anesthesia remains widely used for mechanical thrombectomy treatment of acute ischemic stroke. General anesthesia may provide optimal conditions for procedural operations, fewer technical failures and complications occur and higher recanalization rates are achieved, resulting in better clinical outcomes (24). However, no studies have certified that conscious sedation is associated with higher rates of wire perforation, dissection, or intracranial hemorrhage than general anesthesia (20). Additionally, general anesthesia is more frequently associated with hemodynamic instability, such as intraoperative hypotension, which may lead to worse outcomes. Therefore, it is likely that standard circulation management is essential in reducing the negative effects of hemodynamic fluctuations. To our knowledge, the first RCT to compare anesthetic management in patients with VBAO during EVT found that there were not notably different in rates of 90-day favorable outcomes, mortality successful reperfusion, intraoperative hypotension, or perioperative changes in systolic blood pressure between conscious sedation and GA (8). Our study found similar clinical outcomes and safety outcomes between the GA and non-GA groups during

vertebrobasilar stroke endovascular therapy, in line with several RCTs and observational studies (4–6, 8, 25, 26). However, a systematic review and meta-analysis found that non-GA was associated with better outcomes than GA in patients with acute posterior circulation stroke undergoing EVT (27). These findings were inconsistent with ours, which might be explained by the differences in baseline patient characteristics in the meta-analysis, such as stroke severity.

VBAO may lead to bulbar palsy and/or consciousness impairment, which increases periprocedural complications; hence, selection bias was prone to general anesthesia for patients with more severe illness. A lower presenting Glasgow Coma Scale score (≤8) was predictive of poor patient outcome in endovascular treatment for acute posterior large-vessel occlusion (28, 29). According to subgroup analysis, in patients with a GCS score ≤8, a significantly higher rate of successful reperfusion was observed in the GA group. However, the difference in recanalization rate of this size was not sufficient to explain the other outcomes that we observed in our study. Due to the small sample size of the subgroup analysis, the width of 95% confidence intervals was comparatively large. Interestingly, a pilot trial of 43 patients with acute anterior circulation ischemic stroke who underwent EVT found results similar to us, which showed that the rate of successful reperfusion (mTICI score 2b-3) was greater in the patients allocated to receive general anesthesia, and showed no difference in NIHSS scores at 24 h or 7 days or mRS scores at 30 days (30). For patients with poorer clinical presentation and more severe stroke, GA is perhaps more favorable than non-GA in rates of successful reperfusion.

We acknowledge that our study has several limitations. First, it is not clear whether the better functional outcomes in the non-GA group are merely related to non-intubated anesthesia. Second, as a retrospective study, we took advantage of propensity score to adjust for potential confounders between groups. However, the results could have been confounded by variables not accounted for in the propensity model. Third, the anesthesiologist and operator adopted the most appropriate anesthesia strategy for each patient, based on their experience combined with the patient's situation before operation, which lacked a unified agreement. In addition, we did not investigate potentially important procedural factors that could have affected our findings, such as periprocedural blood pressure fluctuations. Finally, we could not avoid the bias caused by multiple imputations that were used to deal with missing data.

Conclusions

Our study suggests that GA appears to be as safe and effective as non-GA during EVT of VBAO. In addition, for patients with GCS score ≤8, we may give priority to general anesthesia. Future prospective studies are warranted, at least to extend our understanding of the effect of the anesthesia strategy and help determine the best anesthetic modality during EVT of VBAO.

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 Affiliated Hospital of University of Science and Technology of China (USTC) in Hefei, China. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

YL, PZ, WS, ZC, and ML contributed to the study concept and design. PX, JW, LX, ZD, DeW, CZ, and TY contributed to acquisition and analysis of data. DL, CL, and DiW contributed to image review and drafting figures of the manuscript. YL, WS, ZC, and ML contributed to drafting the text. 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.2023.1104487/full#supplementary-material

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Analyzing and predicting the risk of death in stroke patients using machine learning

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Background: Stroke is an acute disorder and dysfunction of the focal neurological system that has long been recognized as one of the leading causes of death and severe disability in most regions globally. This study aimed to supplement and exploit multiple comorbidities, laboratory tests and demographic factors to more accurately predict death related to stroke, and furthermore, to make inferences about the heterogeneity of treatment in stroke patients to guide better treatment planning.

Methods: We extracted data from the Medical Information Mart from the Intensive Care (MIMIC)-IV database. We compared the distribution of the demographic factors between the control and death groups. Subsequently, we also developed machine learning (ML) models to predict mortality among stroke patients. Furthermore, we used meta-learner to recognize the heterogeneity effects of warfarin and human albumin. We comprehensively evaluated and interpreted these models using Shapley Additive Explanation (SHAP) analysis.

Results: We included 7,483 patients with MIMIC-IV in this study. Of these, 1,414 (18.9%) patients died during hospitalization or 30 days after discharge. We found that the distributions of age, marital status, insurance type, and BMI differed between the two groups. Our machine learning model achieved the highest level of accuracy to date in predicting mortality in stroke patients. We also observed that patients who were consistent with the model determination had significantly better survival outcomes than the inconsistent population and were better than the overall treatment group.

Conclusion: We used several highly interpretive machine learning models to predict stroke prognosis with the highest accuracy to date and to identify heterogeneous treatment effects of warfarin and human albumin in stroke patients. Our interpretation of the model yielded a number of findings that are consistent with clinical knowledge and warrant further study and verification.

KEYWORDS

stroke, stroke mortality, machine learning, deep learning, treatment heterogeneity

1. Introduction

Stroke is an acute disorder characterized by dysfunction of the focal neurological system, underlying cerebral vascular spontaneous hemorrhage, and inadequate blood supply (1). With its concomitant cardiovascular and cerebrovascular diseases, patients of stroke typically have poor prognosis and outcomes (2, 3). Stroke has been recognized as the second most deadly threat and the second leading contributors to severe disability worldwide (4, 5). The global epidemiology

of stroke has also not been optimistic over the past few decades. The incident cases of stroke were 12.2 million in 2019, among which, 62.4% were of ischemic stroke, while 27.9% were of intracerebral hemorrhage, and 9.7% were subarachnoid hemorrhage cases (6). Meanwhile, the lifetime risk of stroke is approximately 25% from the age of 25 among both men and women (6). In 2019, the deaths caused by stroke amounted to 6.55 million and the disability-adjusted life years (DALYs) of stroke patients also reached 143 million (6).

Clinically, there are many factors that can affect the prognosis of stroke, which in general can be mainly divided into basic information for patients, complications, subtypes of stroke, and the treatments (7–9). Many complications can have an impact on the prognosis of stroke, including atherosclerosis (10), diabetes mellitus (9, 11), atrial fibrillation (12), cerebral palsy (13), and some cancers (14). Some stroke subtypes interact with specific complications and lead to a deterioration in prognosis (15–17). For instance, certain coagulation defects can cause abnormal traumatic injuries with blood-brain barrier disruption and exacerbate risk of hemorrhagic stroke (18, 19). Stroke patients may also face difficult treatment choices, such as the controversy over the use of anticoagulants like warfarin in cases of ischemic stroke complicated by gastrointestinal bleeding (20–22). The influence of a multitude of factors leads to increased complexity in the identification and therapeutic management of stroke patient prognosis.

Several previous studies used machine learning to solve problems related to stroke and other diseases. Cheon et al. used a fully connected neural network (FCNN) to identify factors affecting stroke mortality and had an AUC of 0.8. However, their principal component analysis (PCA) was not clinically interpretable (23). Heo et al. built a model using machine learning to predict long-term outcomes in acute stroke, but they only used six variables from the Analysis of Lausanne (ASTRAL) scores, which did not include comprehensive comorbidities and demographic factors (24). Ambale-Venkatesh et al. combined machine learning with deep phenotyping to improve the accuracy of cardiovascular event predictions (25). Some existing research generally lacks overall consideration of all comorbidities together and sometimes the model is not optimal.

Given the particular complexity and variety of contributing factors to stroke outcomes, which are difficult to predict globally using traditional research methods, such study limitations reduced predictive accuracy and limited to a comprehensive consideration of multiple factors. The aim of our study is to supplement and exploit multiple comorbidities, laboratory tests and demographic factors to more accurately predict death related to stroke, and furthermore, to make inferences about the heterogeneity of treatment in stroke patients to guide better treatment planning.

2. Materials and methods

2.1. Study design

We conducted a retrospective study of the risk factors for death in stroke patients and trained several machine learning (ML) models to predict their mortality during hospitalization and within 30 days after discharge. Patients with stroke were enrolled from the Medical Information Mart from Intensive Care (MIMIC)-IV version 2.0 (26), based on the International Classification of Diseases version 10 (ICD-10), a public dataset maintained by the Beth Israel Deaconess Medical Center. The period for study enrolment was from 2008 to 2019. The study was approved by online certification.

Patients diagnosed with stroke were included according to the ICD-10. We extracted a total of 8,276 patients with stroke, excluding 36 patients admitted to the hospital many times and 757 patients whose records did not contain adequate and relevant information within one month from the outcome (more than 30%). Finally, we extracted and sorted all the information of the remaining 7,483 patients, and a flowchart of patient selection and data collection is shown in Figure 1A.

2.2. Principle variables

The dependent variable was the mortality rate of stroke patients. Participants who died during hospitalization and within 30 days after discharge were considered deaths. The independent variables reflecting sociodemographic status included age, gender, marital status, race, insurance type, height, weight, and body weight index (BMI). Medical variables included the course of the disease, laboratory tests, and complications. Drug-related information included the usage of warfarin and human albumin, which was the most frequently used medicine for stroke patients in this database. We also included the detailed subtypes of stroke and whether the stroke was recurrent as independent variables. We excluded complications without a clear diagnosis to make our study reproducible and explainable. We obtained 145 variables for the ML model.

2.3. Machine learning algorithms and training strategy

We used six ML algorithms, which were Neural Oblivious Decision Ensemble (NODE) (27); CatBoost (28); XGBoost (29); LightGBM (30); fully connected neural network (FCNN); and logistic regression (LR).

NODE, a state-of-the-art Deep Learning (DL) model specialized for tabular data, uses oblivious decision trees (ODTs) (31) as weaker learners and inherits hierarchical representation and attention mechanism from neural networks. Every layer of NODE is densely connected to the original inputs and is trained end-to-end *via* backpropagation. The final prediction of NODE was obtained by averaging the outputs of all ODTs from all layers. We used Quasi-Hyperbolic Adam as an optimization strategy, which was recommended in the original paper (32). An FCNN is a common DL structure that contains several fully connected layers and uses ReLU as a nonlinear activation function. CatBoost is a gradient-boosted decision tree (GBDTs) model released in 2018 that also uses ODTs as weaker learners. The other two GBDTs models are XGBoost and LightGBM. We also used LR for comparison.

All patients were randomly allocated to a testing set of 25% samples unseen in the model development and used to evaluate the final model performance. A training set of 75% of the samples was used for building the model. During the training period, we used 3-folds-cross-validation to tune the model hyperparameters; for each

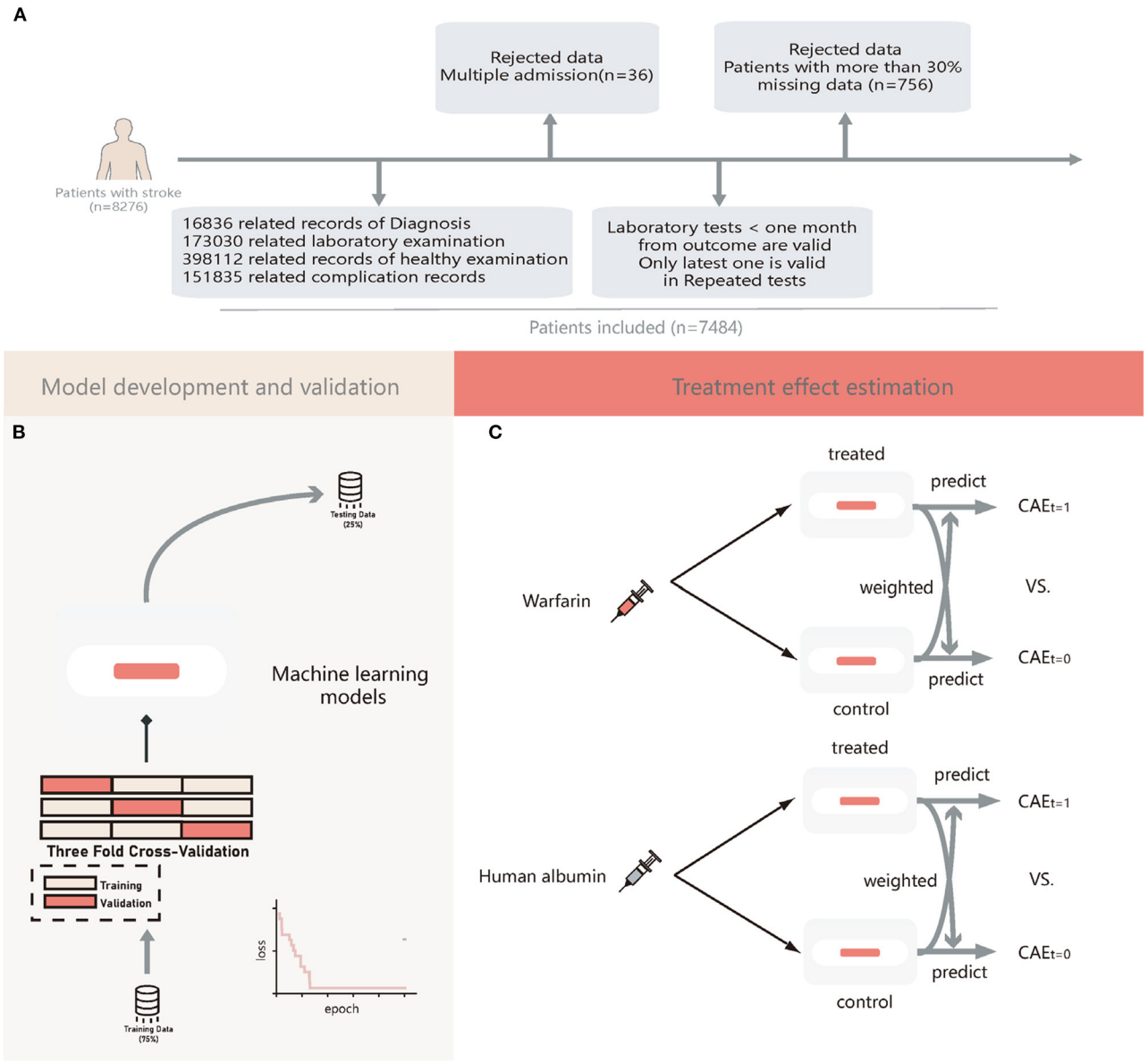

FIGURE 1
The flow chat of patients' enrollment, model development and validation, and treatment effect estimation. **(A)** Flow chart of patients' enrollment; **(B)** Flow chart machine learning models development and validation; **(C)** Flow chart of estimation of treatment heterogeneity effects. CATE, conditional average treatment effect; t = 1, patients treated with warfarin or human albumin; t = 0, patients not treated with warfarin or human albumin.

time, the model trained on two-thirds of the training set and validated on the remaining one-third of the training set. For DL models, the loss of each step was recorded. The training was terminated automatically if it did not decrease in 1,000 iterations. For GBDTs, we used a random search algorithm to obtain the best models. A flowchart of the model development is shown in Figure 1B. We used the median to fill in missing values.

2.4. Estimation of treatment heterogeneity effects

We further estimated the therapeutic effects of human albumin and warfarin in individual patients using Meta-learner (33). It was a three-stage estimation, in which two GBDTs were used in the first stage to estimate the conditional average treatment effects (CATE) for the treatment and control groups separately, followed by estimation of the control group outcome using a GBDT built on the treatment group and estimation of the treatment group outcome using another one built on the control group, and finally estimation of the final CATE was weighted by the estimates obtained in the second stage. This leads to a more causal inference for individual treatment effects (ITE).

ITE was defined as the outcome estimation of a patient receiving human albumin or warfarin minus the outcome estimation for the same patient not receiving human albumin or warfarin. We then divided patients into consistent (Consis.) and inconsistent (In-consis.) groups in the testing set based on the actual treatment

TABLE 1 Comparison of demographic status.

	Control	Dead	*p*-value
Age, median (IQR), y	68.0 (57.0–77.0)	74.0 (64.0–83.0)	<0.001**
BMI, median (IQR)	27.3 (23.8–31.4)	26.3 (22.9–30.3)	<0.001**
Sex			0.5136
Male	3042 (50.1%)	723 (51.1%)	
Female	3027 (49.9%)	691 (48.9%)	
Race			0.505
Asian	212 (3.5%)	60 (4.3%)	
Black	801 (13.2%)	194 (13.7%)	
White	3975 (65.6%)	926 (65.6%)	
Latin	227 (3.7%)	46 (3.3%)	
Other race	844 (13.9%)	185 (13.1%)	
Insurance type			<0.001**
Medicare	2896 (47.7%)	796 (56.3%)	
Other insurance	2906 (47.9%)	563 (39.8%)	
Medicaid	267 (4.4%)	55 (3.9%)	
Marital status			<0.001**
Married	2767 (45.6%)	532 (37.6%)	
Widowed	878 (14.5%)	295 (20.9%)	
Single	1492 (24.6%)	285 (20.2%)	
Divorced	537 (8.8%)	92 (6.5%)	

Other races, including unknown, unable to obtain, and multiple races; other insurance, including no insurance, employer-based insurance plan, and individual health insurance. Statistical analysis: **p < 0.01.

they received and the ITE values. This process was illustrated in Figure 1C.

2.5. Statistical analysis

PostgreSQL was used to extract and store the data from MIMIC-IV. All statistical analyses were performed using R, continuous variables were reported as the median and interquartile range (IQR), and categorical variables were presented as numbers and percentages (%). To compare continuous variables between the two groups, we used the Welch *t*-test and Mann–Whitney *U*-test, as appropriate. The chi-square test and Fisher's exact test were used to compare categorical variables, as appropriate.

3. Results

3.1. Demographic results

A total of 7,483 participants were included in this study, with 6,069 and 1,414 participants in the control and death groups, respectively. The median age was 69.0 years (59.0–79.0 years), and 50.3% of the patients were male. The total mortality rate was 18.9% (95% CI 18.0–19.8%).

A comparison of demographic status is shown in Table 1. The death group was older than the control group (74.0 vs. 68.0, $p < 0.001$). Most of the participants in the control group were married (45.6%, 95% CI 44.3–46.9%), whose proportion was higher than that of the death group (37.6%, 95% CI 35.1–40.2%). The proportion of single and divorced showed the same traction. However, the proportion of widowed individuals showed an opposite trend. The proportion of widowed individuals was 20.9% (95% CI 18.8–23.1%) in the death group and 14.5% (95% CI 13.6–15.4%) in the control group. The body mass index (BMI) of the death group was lower than that of the control group (26.3 vs. 27.3, $p < 0.001$). No statistically significant differences were found in sex or race.

3.2. Model predictive performance

We calculated accuracy (ACC.), the area under the receiver operating characteristic curve (AUC), which is the ability to weigh true positives and false positives, precision score (Prec.), and F-measure (F1), which is a comprehensive indicator reflecting the true positive rate and sensitivity rate. The validation AUC curve during the training period is shown in Figure 2A, which exhibits oscillation owing to 3-fold cross-validation. The predictive performance of each model is presented in Table 2. The performances of GBDTs and NODE were close to acceptable levels. CatBoost has the highest ACC., Prec., and F1 (ACC: 0.8993 [0.8972–0.9014]; Prec.: 0.8155 [0.8072–0.8214]; AUC: 0.9217 [0.9188–0.9238]; F1: 0.6805 [0.6735–0.6855]), XGBoost has the highest F1 (ACC.: 0.8969 [0.8955–0.8987]; Prec.: 0.7783 [0.7689–0.7841]; AUC: 0.9175 [0.9153–0.9194]; F1: 0.6890 [0.6824–0.6939]). However, FCNN performed worse than GBDTs in ACC., AUC, and F1 (ACC.: 0.8726 [0.8699–0.8744]; Prec.: 0.8129 [0.8021–0.8227]; AUC: 0.8796 [0.8763–0.8832]; F1: 0.5328 [0.5231–0.5391]). LR has the lowest Prec. and AUC (ACC.: 0.8753 [0.8729–0.8773]; Prec.: 0.7298 [0.7206–0.7372]; AUC: 0.8591 [0.8555–0.8622]; F1: 0.6003 [0.5923–0.6069]). Additionally, we demonstrated the receiver operating characteristic (ROC) curve in Figure 2B.

3.3. Recognition of the heterogenic treatment effects

We presented the fatality rates (FR) of the treatment group, control group, Consis. group and In-consis. group of warfarin and human albumin in Figures 3A, C, respectively. We also demonstrated their average treatment effects (ATE), which was the Figures 3B, D. In the calculation of the ATE of the factor that whether the patient's actual treatment is in line with ITE (Consis.), treatment was considered as a mediator and was controlled. Meanwhile, subtypes of stroke, recurrent stroke, age, and sex were considered as confounders in the calculation of standardized mortality rate (SMR) and ATE for both Consis. and treatment. In the estimation of ATE, we used augmented inverse probability weighting (AIPW) (34) to correct the OR values. Additionally, we calculated controlled direct effects (CDE) and natural direct effects (NDE) (35), in which, CDE measures whether a specific patient's outcome would have improved if they had been treated (or be in Consis.) when the confounders hold at a predetermined level, while

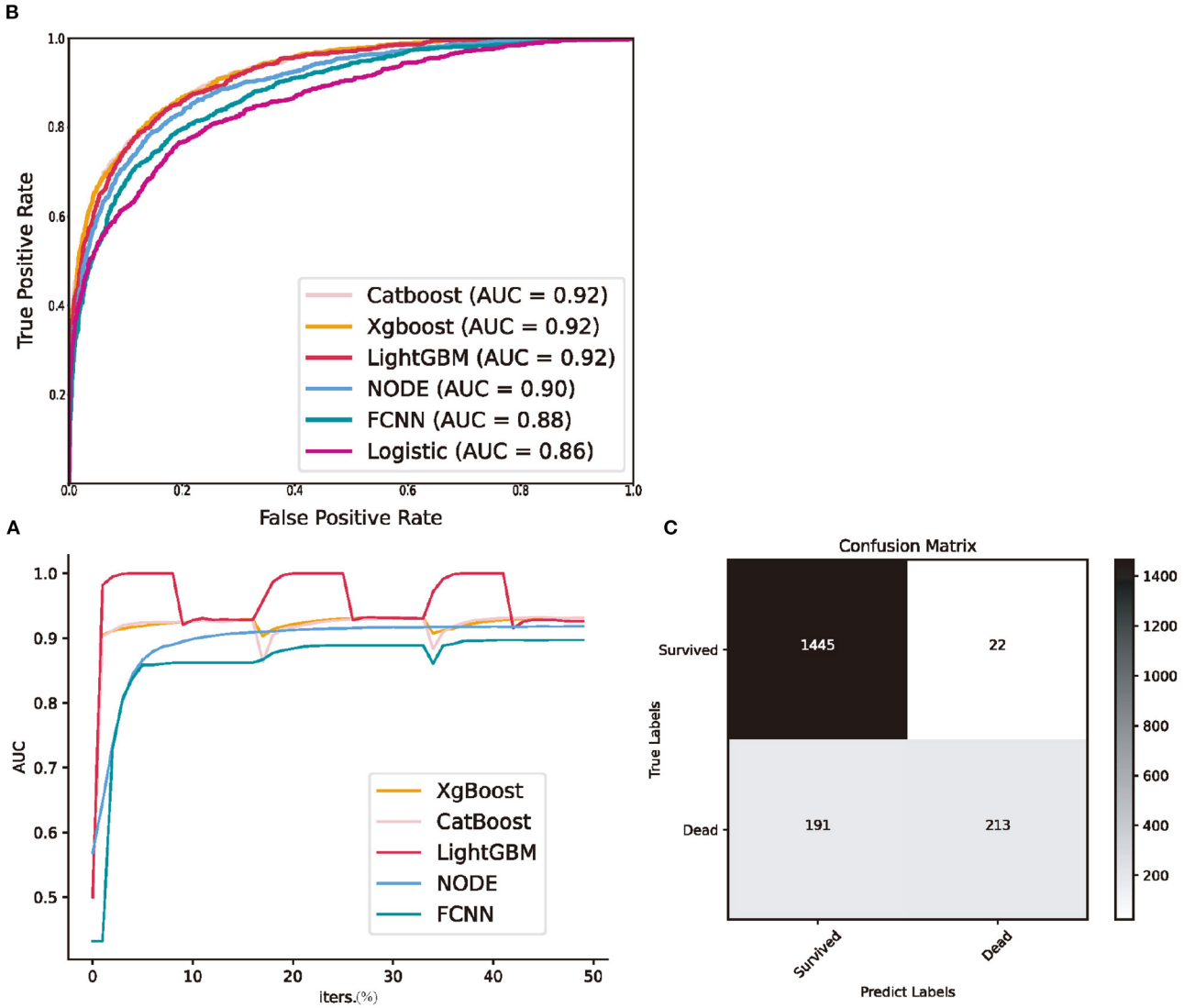

FIGURE 2
Predictive performance of machine learning models in the testing set. **(A)** the validation area under receiver operating characteristic curve during three folds cross validation; **(B)** receiver operating characteristic curve of all machine learning models; **(C)** the confusion matrix of CatBoost. NODE, Neural Oblivious Decision Ensembles; FCNN, fully connected neural network; Logistic, logistic regression; AUC, area under receiver operating characteristic curve; iters., iterations.

NDE holds confounders fixed in the same level under untreated condition. The CDE and NDE were presented as the slope of a linear regression.

In the testing set (1,871 of patients), 1,706 (91.2%) patients have been taken warfarin while the treatment was deemed appropriate for 987 (57.3% of treated with warfarin) patients, and 96 (58.2% of non-treated with warfarin) patients were considered should be on warfarin. 294 (15.7%) patients were in human albumin treatment group; 821 (52.1% of non-treated with human albumin) patients were considered by the model that should be treated.

We observed a lower fatality rate (FR) in the Consis. than in the In-consis. (Consis vs. In-consis: in warfarin, 14.1% vs. 25.6%, $p < 0.0001$; in human albumin, 13.3% vs. 22.4%, $p < 0.0001$). Significant differences remained after correction for confounders (SMR^b of Consis: in warfarin, 0.82, 95% CI: 0.75–0.89; in human albumin, 0.84, 95% CI: 0.76–0.93). The odds ratio (OR), AIPW adjusted OR (adj-OR), CDE, and NDE of Consis. remained significant and lower than treatment, except for CDE of human albumin (Consis.: −0.08, 95% CI: −0.10 to −0.06); human albumin: −0.06). The adj-OR of Consis. in warfarin was 0.54 (0.47–0.62) and that of human albumin was 0.66 (0.57–0.76), indicating a strong protective factor. The CDE and NDE also showed that Consis. had a direct effect on outcome (unaffected by treatment ratio and other confounders).

3.4. Model interpretation

CatBoost exhibits the highest ACC., Prec., and F1. Thus, we conducted a Shapley Additive Explanations (SHAP) analysis to reveal the distribution of the effect of each input acting on CatBoost. Figure 4A shows a SHAP summary plot sorted by the feature importance of the top 20 important features, wherein every point represents a sample, and the horizontal coordinate is the SHAP value of each feature. A higher intensity of red indicates a higher

TABLE 2 Predictive performance of each model.

Model	ACC. [95% CI]	Prec. [95% CI]	AUC [95% CI]	F1 [95% CI]
NODE	0.8857 [0.8833–0.8874]	0.7368 [0.7274–0.7432]	0.9008 [0.8975–0.9029]	0.6528 [0.6455–0.6580]
FCNN	0.8726 [0.8699–0.8744]	0.8129 [0.8021–0.8227]	0.8796 [0.8763–0.8832]	0.5328 [0.5231–0.5391]
XGBoost	0.8969 [0.8955–0.8987]	0.7783 [0.7689–0.7841]	0.9175 [0.9153–0.9194]	**0.6890 [0.6824–0.6939]**
CatBoost	**0.8993 [0.8972–0.9014]**	**0.8155 [0.8072–0.8214]**	**0.9217 [0.9188–0.9238]**	0.6805 [0.6735–0.6855]
LightGBM	0.8955 [0.8931–0.8972]	0.7753 [0.7657–0.7827]	0.9086 [0.9059–0.9106]	0.6804 [0.6730–0.6854]
LR	0.8753 [0.8729–0.8773]	0.7298 [0.7206–0.7372]	0.8591 [0.8555–0.8622]	0.6003 [0.5923–0.6069]

ACC., accuracy; AUC, the area under the receiver operating characteristic curve; Prec., precision score; F1, F-measure. NODE, Neural Oblivious Decision Ensemble; FCNN, fully connected neural network; LR, Logistic Regression Model. Bolded font indicates the best indicator among all models.

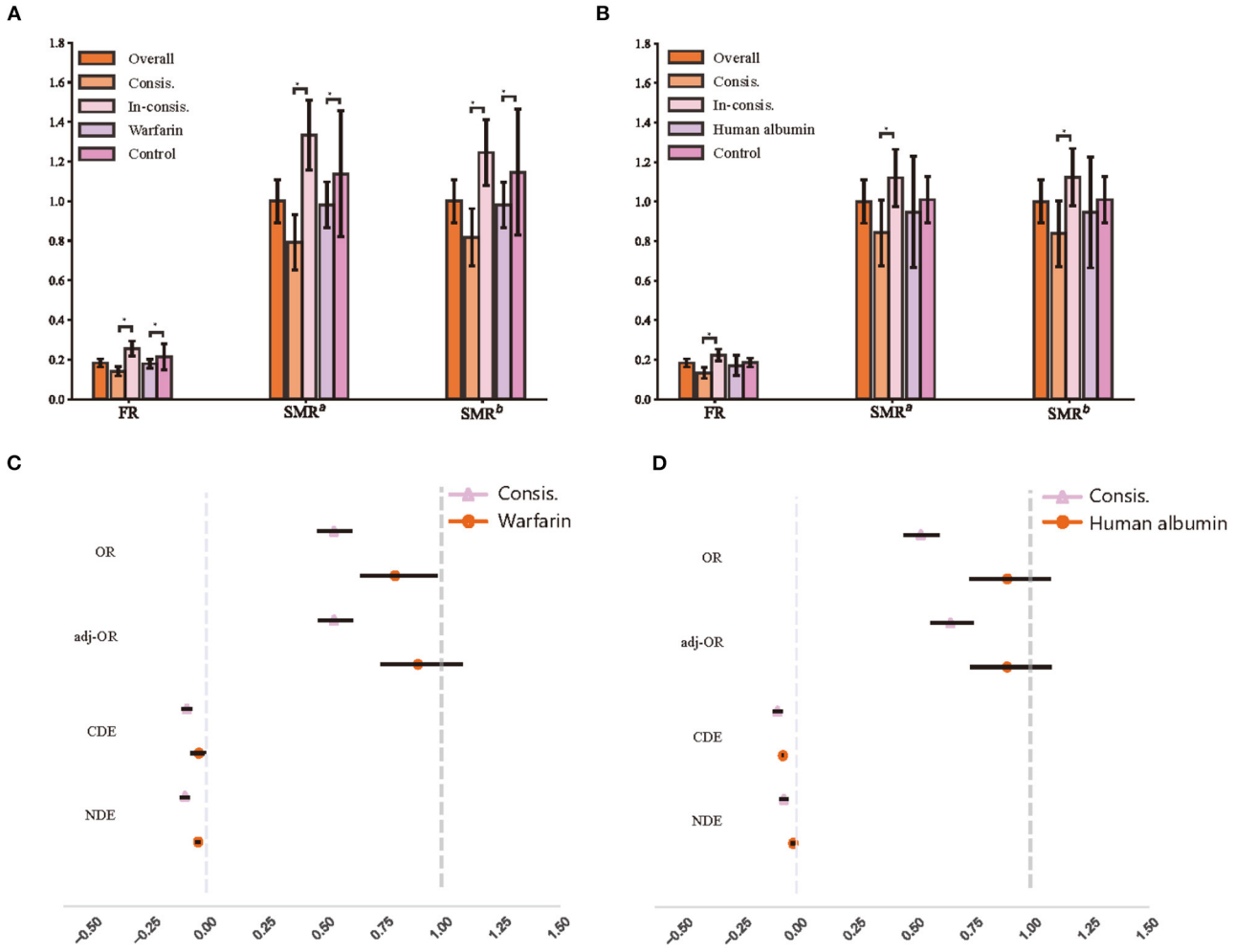

FIGURE 3
The recognition of treatment heterogeneity effects. **(A)** Fatality of different warfarin treatment groups; **(B)** Fatality of different human albumin treatment groups; **(C)** The average treatment effects of warfarin treatment groups; **(D)** The average treatment effects of human albumin treatment groups. FR, fatality rate; SMR, standardized mortality rate; SMR^a, standardized mortality rate that controls age and sex; SMR^b, standardized mortality rate that controls age, sex, and all subtypes of stroke; OR, odds ratio; adj-OR, odds ratio that adjusted with augmented inverse probability weighting; CDE, controlled direct effect; NDE, natural direct effect.

feature value, while a higher intensity of blue indicates a lower feature value.

First, the lower the oxygen saturation (SpO_2), the higher the SHAP value, which means that the patient is more likely to die. The protective factors were red blood cell count (RBC), followed by weight, course of disease, and blood bicarbonate (HCO_3_). The most important risk factor was other disorders of the nervous system (ODNS), followed by white blood cell count (WBC), age, glucose (GLC), and malignant neoplasms of ill-defined, secondary and unspecified sites (MNISUS). We also presented the SHAP plot of Meta-learner of warfarin (Figure 4B) and human albumin (Figure 4C) that indicated which

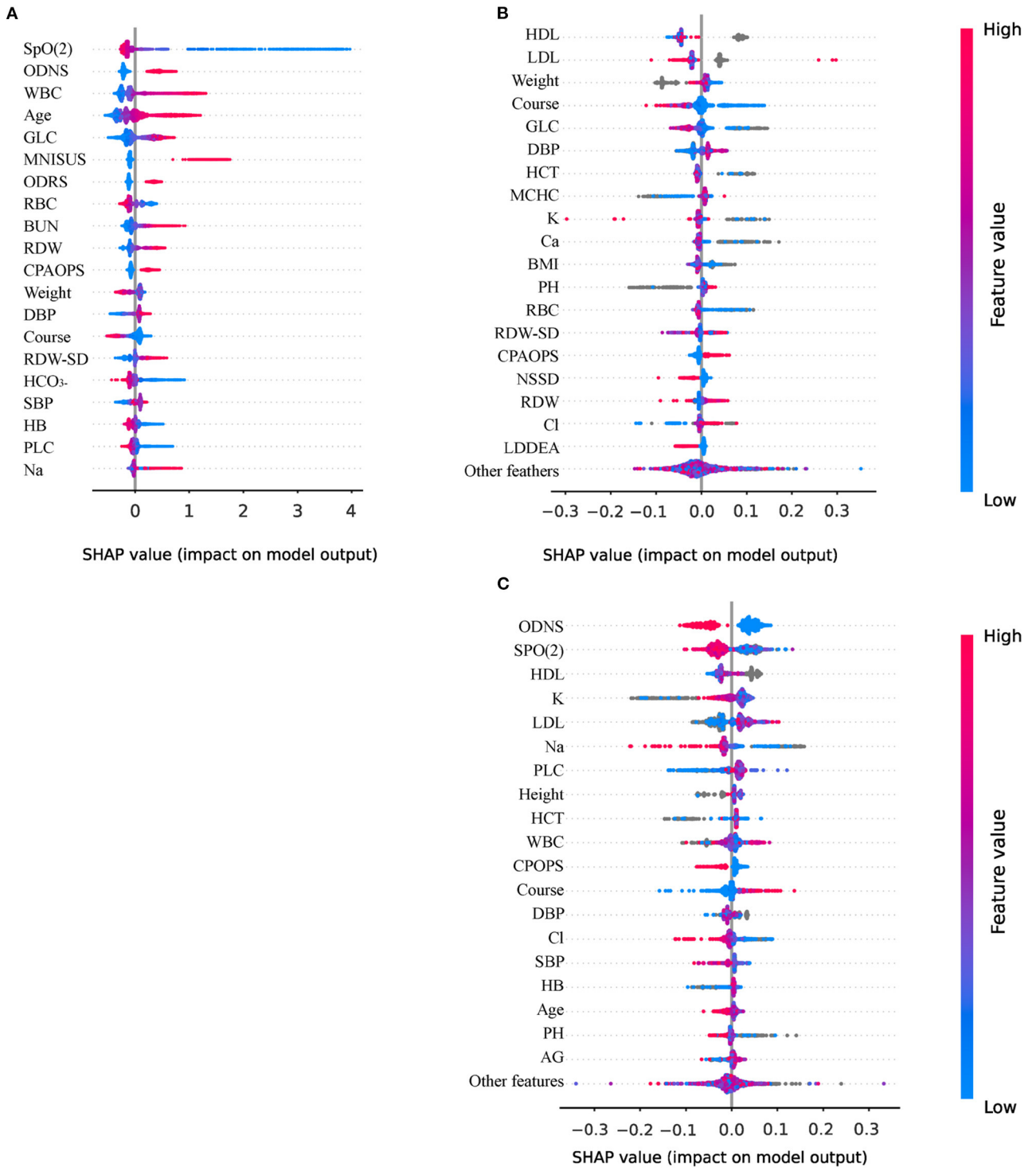

FIGURE 4
Interpretation of models using Shapley Additive Explanations (SHAP) analysis. **(A)** The variables importance of CatBoost; **(B)** The importance of variables to explain warfarin treatment heterogeneity; **(C)** The importance of variables to explain human albumin treatment heterogeneity. ODNS, other disorders of the nervous system; WBC, white blood cell count; GLC, blood glucose; MNISUS, malignant neoplasms of ill-defined, secondary and unspecified sites; ODRS, other diseases of the respiratory system; RBC, red blood cell count; BUN, urea nitrogen; RDW, red blood cell distribution width; CPAOPS, cerebral palsy and other paralytic syndromes; DBP, diastolic blood pressure; RDW-SD, standard deviation of red blood cell distribution width; HCO_{3-}, blood bicarbonate; SBP, Systolic pressure; HB, hemoglobin; PLC, platelet count; Na, blood natrium; HCT, hematocrit; MCHC, mean red blood cell hemoglobin concentration; Ca, serum calcium; K, blood potassium; NSSD, neurotic, stress-related and somatoform disorders; LDDEA, lung diseases due to external agents; Cl, blood chloride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AG, anion gap, PH; phosphoric acid.

variables more significantly affect the inference of heterogeneity of treatment.

4. Discussion

Stroke remains one of the most destructive and prevalent nervous system diseases worldwide, responsible for disability or death in many individuals every year and a significant increase in DALY (36, 37). The importance of our study in the context of stroke mortality is that the features recognized in this study can identify the factors significantly related to mortality and treatment effects using statistical methods and ML models.

We analyzed the distribution of the demographic factors included in the study. Elderly individuals are more likely to have a stroke and a serious clinical outcome (38), which is consistent with our study. We also found that patients with higher BMI were more likely to survive after stroke (control vs. death, 26.3 vs. 27.3, $p < 0.001$), however, this association may depend on age (39). Additionally, we found more patients with other insurance types in the control group than in the death group [control vs. death, 47.9 vs. 39.8%, 95% CI (control), 46.6–49.1%]. Other insurance statuses include no insurance, employer-based insurance plans, and individual health insurance, most of which are commercial insurance, which may be related to a better economic level.

This study showed that the use of the ML method helps predict death after a stroke. To the best of our knowledge, this study achieved the highest AUC (AUC: 0.9217 [0.9188–0.9238]). Previous studies have suggested that FCNN or Deep Neural Network (DNN) is the best model for predicting post-stroke mortality and outperforms other traditional ML models (23, 24). However, in our study, the FCNN performed the worse than GBDTs and NODE (ACC.: 0.8857 [0.8833–0.8874]; AUC: 0.9008 [0.8975–0.9029]). The ML model with the highest performance was CatBoost and the rest of the GBDTs achieved high performance. Tree-based models appear more suitable for structured medical data, regardless of whether the model is implemented with ensemble methods, such as GBDTs, or layer-wise structures, such as NODE. However, GBDTs and DL models have their own advantages. GBDTs can help achieve relatively high accuracy in a very short period. DL models are fully differentiable and scalable, which means that researchers can arbitrarily change their model structure to fit the data better.

We further used meta-learner to identify heterogeneous treatment effects in the stroke population. We observed that 55.6% of the warfarin current use (or not use) and 52.1% of the status quo human albumin treatment (or control) in the testing set were considered inappropriate by model. Even after controlling for treatment factors, demographic factors and subtypes, survival outcomes were still significantly better in those who were consistent with the model judgments than in those who were inconsistent. Extrapolation of treatment effects for the population level does not necessarily hold in individual patients (34) and treatment heterogeneity has been reported to exist in stroke patients (40). However, studies of ITE in stroke patients are scarce (41) and, to our best knowledge, there is no such discussion of warfarin and human albumin, which are common drugs (42–45). Our study shows that ML can be used to help identify individuals with heterogeneous responses to treatment in stroke patients and thus make better treatment plans.

ML is a good predictive tool and usually has high accuracy. However, it has always been regarded as a "black box," indicating poor interpretability. In our study, we conducted SHAP analysis to interpret one of our best models and obtain several risk and protective factors to help better understand the role of various factors in post-stroke mortality. Most of the results were consistent with clinical knowledge (46–49). In addition, we showed the 20 variables considered the most important by the model for the estimation of treatment heterogeneity, mostly laboratory indicators, which are worthy of further investigation. These results can be referenced in subsequent studies as a screening of important variables to narrow the scope.

In summary, we used several highly interpretive machine learning models to predict stroke prognosis with the highest accuracy to date and to identify heterogeneous treatment effects of warfarin and human albumin in stroke patients. Our interpretation of the model yielded a number of findings that are consistent with clinical knowledge and warrant further study and verification.

Our study has some limitations. The data we used included only inpatients from one hospital. These inpatients are already affected by stroke and usually have more serious conditions than the average population of stroke patients (50, 51). This narrow scope may limit the general applicability of our results. Since this is the first study to use machine learning to analyze such a wide range of variables in a population that has complex comorbidity factors, such as simultaneous hemorrhagic stroke and ischemic stroke [839 (11.2%)], we did not perform further analysis and inferences on all the conclusions obtained. In further studies, we will explore in depth the factors affecting survival or treatment effects and group subtypes of stroke to draw even further conclusions.

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 approved by the Massachusetts Institute of Technology (Cambridge, MA) and Beth Israel Deaconess Medical Center (Boston, MA). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

EZ: experimental design, data analysis, model development, and manuscript writing. ZC: data acquisition, data analysis, and manuscript writing. PA: experimental design, data analysis, and manuscript writing. JW: experimental design and manuscript writing. MZ and ZX: data analysis and model development. JL: data analysis and manuscript revise. ZA: experimental design and manuscript revise. 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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Prognostic value of inflammation biomarkers for 30-day mortality in critically ill patients with stroke

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Objective: To explore the values of neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), neutrophil to albumin ratio (NAR), prognostic nutritional index (PNI), systemic immune inflammatory index (SII) and red cell distribution width to albumin ratio (RA) for evaluating the risk of 30-day mortality of ischemic stroke or hemorrhagic stroke patients.

Methods: In this cohort study, the data of 1,601 patients diagnosed with stroke were extracted from the Medical Information Mart for Intensive Care III (MIMIC-III) database. Among them, 908 were hemorrhagic stroke patients and 693 were ischemic stroke patients. Demographic and clinical variables of patients were collected. Univariate and multivariable Cox regression were performed to evaluate the predictive values of NLR, PLR, SII, NAR, RA, and PNI for 30-day mortality in hemorrhagic stroke or ischemic stroke patients. The receiver operator characteristic (ROC) curves were plotted to assess the predictive values of NLR, NAR, and RA for 30-day mortality of hemorrhagic stroke patients.

Results: At the end of follow-up, 226 hemorrhagic stroke patients and 216 ischemic stroke patients died. The elevated NLR level was associated with increased risk of 30-day mortality in hemorrhagic stroke [hazard ratio (HR) = 1.17, 95% confidence interval (CI): 1.06–1.29]. The increased NAR level was associated with elevated risk of 30-day mortality in hemorrhagic stroke (HR = 1.16, 95% CI: 1.02–1.30). The high RA level was linked with increased risk of 30-day mortality (HR = 1.44, 95% CI: 1.23–1.69). No significant correlation was observed in these inflammation biomarkers with the risk of 30-day mortality in ischemic stroke patients. The area under the curves (AUCs) of NLR, RA, and NAR for evaluating the risk of 30-day mortality of hemorrhagic stroke patients were 0.552 (95% CI: 0.503–0.601), 0.644 (95% CI: 0.590–0.699) and 0.541 (95% CI: 0.490–0.592).

Conclusion: NLR, NAR, and RA were potential prognostic biomarkers for predicting 30-day mortality of hemorrhagic stroke patients, which might provide clinicians an easy and cheap way to quickly identify patients with high risk of mortality.

KEYWORDS

inflammation, biomarkers, 30-day mortality, ischemic stroke, hemorrhagic stroke

Introduction

Stroke is a serious disease affecting a quarter of people during their lifetime with high risk of death and disability (1). Stroke has two main subtypes (ischemic stroke and hemorrhagic stroke), and they have distinct clinical and epidemiological characteristics (2). Ischemic stroke and hemorrhagic stroke are accounted for ∼85 and 15% of all stroke cases, respectively (3). Ischemic stroke is caused by the reduction or interruption of blood flow to the brain while hemorrhagic stroke is due to the bleeding in or around the brain (4). Ischemic stroke is the major cause of disability and second cause of deaths globally with a mortality rate of 15% at 90

days (5, 6). As for hemorrhagic strokes, the mortality rate is 25–30% in high-income countries and 30%-48% in low- to middle-income countries (7). Given the prognosis of stroke patients, more reliable biomarkers were essential to help improve the outcomes of these patients.

Numerous studies have demonstrated that neuroinflammatory response plays an essential role in the pathophysiology of ischemic stroke (8, 9). Inflammation associated biomarkers such as monocyte and plateletcrit were reported to be associated with the development of cerebrovascular events including acute ischemic stroke (10, 11). Recently, neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), neutrophil-albumin ratio (NAR), prognostic nutritional index (PNI), systemic immune inflammatory index (SII) and red cell distribution width (RDW) to albumin ratio (RA) have been reported as potential novel biomarkers of baseline inflammatory process and they were identified to be associated with the mortality of stroke (12–17). These studies mainly explored the associations between these inflammation biomarkers and all stroke patients or ischemic stroke patients. They did not compare the differences of these inflammation biomarkers with the mortality of different subtypes of stroke patients. Whether there were differences in the prognostic values of these inflammation biomarkers between ischemic stroke and hemorrhagic stroke was unclear. Which inflammation biomarker was more clearly related to the prognosis of ischemic stroke or hemorrhagic stroke still needs investigation.

In the present study, we hypothesized that ischemic stroke and hemorrhagic stroke might have different prognostic inflammation biomarkers. We planned to explore the prognostic values of NLR, PLR, NAR, PNI, SII, and RA for 30-day mortality of ischemic stroke or hemorrhagic stroke patients based on the data from the Medical Information Mart for Intensive Care III (MIMIC-III) to verify our hypothesis.

Methods

Study population

In the current cohort study, the data of 3,534 patients diagnosed with stroke were extracted from MIMIC-III database. MIMIC-III is a large, free database involving in de-identified health-related data of over 40,000 patients who stayed in intensive care unit (ICU) of the Beth Israel Deaconess Medical Center (Boston, USA) between 2001 and 2012 (18). The data analyzed using the first measurement data within 24 h after admitting to ICU. Patients who aged <18 years, and those who stayed in ICU <24 h were excluded. Those who had no data on SII, NAR, systolic blood pressure (SBP), international normalized ratio (INR), Glasgow coma scale (GCS), or Elixhauser comorbidity index (ECI), and patients with abnormal follow-up time were also excluded. Finally, 1,601 patients were included. Among them, 908 were hemorrhagic stroke patients and 693 were ischemic stroke patients. The project was approved by the Institutional Review Boards of Beth Israel Deaconess Medical Center (Boston, MA) and the Massachusetts Institute of Technology (Cambridge, MA). Requirement for individual patient consent was waived because the project did not impact clinical care and all protected health information was deidentified. As the samples were not from The Eighth Medical Center of PLA General Hospital, and this study was exempt from our Institutional Review Board approval.

Main variables

Main variables analyzed in our study included NLR, PLR, NAP, PNI, SII and RA. NLR (neutrophil to lymphocyte ratio) = neutrophil count/lymphocytes count. PLR (platelet to lymphocyte ratio) = platelet count/lymphocytes count. NAR (neutrophil to albumin ratio) = neutrophil count/albumin. PNI (prognostic nutritional index) = 10 × albumin (g/dL) + 5 × lymphocytes count (10^9/L). SII (systemic immune inflammatory index) = PLT × neutrophil/lymphocyte. RA (RDW to albumin ratio) = RDW/albumin (g/dL).

Potential covariables and definition

Potential covariables analyzed in this study included demographic variables including age (years), gender (female or male), marital status (married, unmarried or unknown), and race [White, or others (Asian, Black, Hispanic or Latino, Unknown)], and clinical variables including respiratory rate (beat/min), SBP (mmHg), diastolic blood pressure (DBP, mmHg), blood oxygen saturation (SpO_2), red blood count (RBC, m/μL), INR, hemoglobin (g/dL), hematocrit (%), creatinine (mg/dL), blood urea nitrogen (BUN, mg/dL), fasting blood-glucose (mg/dL), sodium, potassium, chloride, bicarbonate (mEq/L), ECI score, GCS Score, acute kidney failure (AKI, yes or no), infection diseases and treatments.

Infectious diseases was identided from MIMIC-III database based on the ICD-9 code with the first three digits of 001–009, 010–018, 020–027, 030–042, 045–049, 050–059, 060–066, 070–079, 080–088, 090–099, 100–104, 110–118, 120–129, 130–136, and 137–139. Treatments of ischemic stroke included intravenous tissue plasminogen activator (IV-tPA) (ICD-9 procedure code 9910 and 3604), endovascular treatment (ICD-9 procedure code 3974), and the ICD for stent in the procedure (0045, 0046, 0047, and 0048). The main treatments for hemorrhagic stroke were surgery including craniotomy (ICD-9 procedure code: 0120–0129), and minimally invasive surgery (ICD-9 procedure code 0221, 0222, 0139, 0101, 0102, and 0109).

Outcome variable

The 30-day mortality of patients was regarded as outcome in our study. The median follow-up was 30 (21.38, 30.00) days. The follow-up was ended when patients died within 30 days. The outcome was obtained through in-hospital observations or through the Social Security Number of patients. At the end of follow-up, 226 hemorrhagic stroke patients and 216 ischemic stroke patients died.

Statistical analysis

Normally distributed measurement data were described as mean and standard deviation (Mean ± SD), while non-normally distributed measurement data were shown as median and quartile spacing [M (Q_1, Q_3)]. Mann-whitney U rank-sum test was applied for comparison between groups. Enumeration data were expressed as n (%), and χ^2 test was used for comparisons between groups. Univariate cox models were established for 30-day mortality

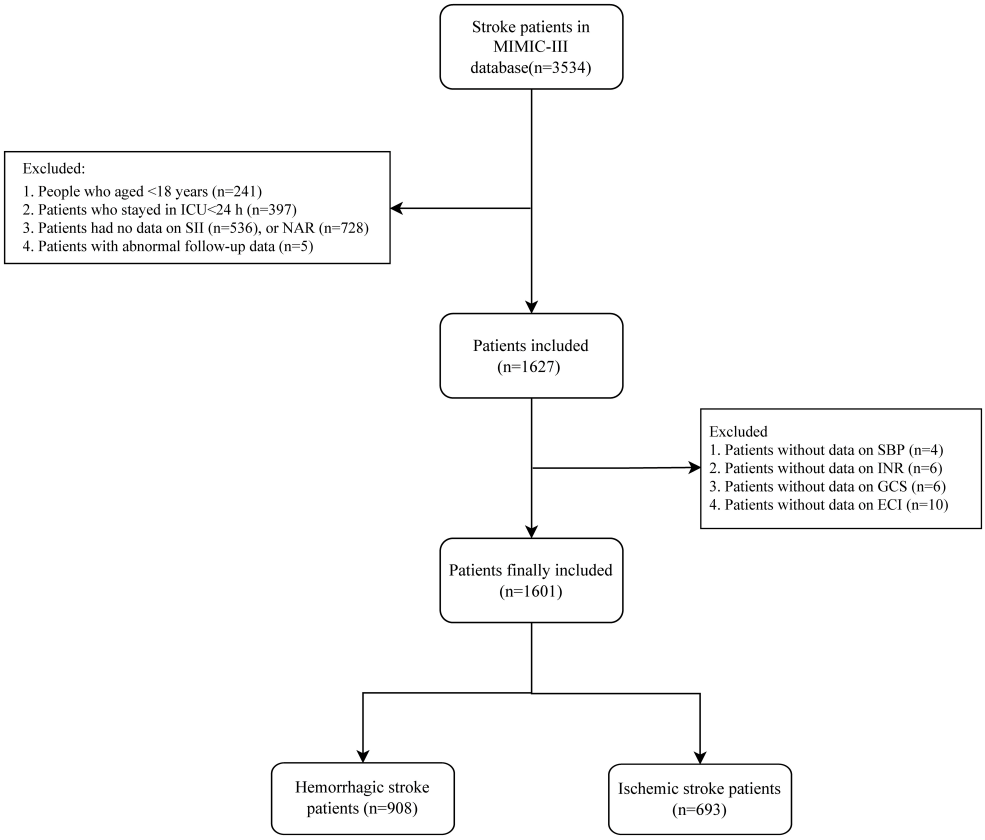

FIGURE 1
The screen process of the participants in the current study.

and hazards ratio (HR) and 95% confidence interval (CI) were standardized with $P < 0.05$ as potential covariables. Univariate and multivariable cox regression were performed to evaluate the prognostic values of NLR, PLR, SII, NAR, RA, and PNI for 30-day mortality of hemorrhagic stroke or ischemic stroke patients. To evaluate the associations between NLR, PLR, SII, NAR, RA, or PNI and 30-day mortality in hemorrhagic stroke patients, confounding factors including age, marital status, respiratory rate, hemoglobin, hematocrit, BUN, fasting blood-glucose, chloride, ECI and AKI were adjusted in the multivariable cox regression model. To assess the associations between NLR, PLR, SII, NAR, RA, or PNI and 30-day mortality in ischemic stroke patients, age, marital status, race, creatinine, BUN, bicarbonate, potassium, ECI, GCS, and AKI were adjusted in the multivariable cox regression model. The receiver operator characteristic (ROC) curves were plotted to evaluate the diagnostic values of NLR, NAR, and RA for 30-day mortality of hemorrhagic stroke. R Studio Version 4.0.3 (2020-10-10) and SAS 9.4 (SAS Institute Inc., Cary, USA) were applied for data analysis.

Results

The baseline characteristics of patients with hemorrhagic stroke or ischemic stroke

In total, 3,534 stroke patients were found in MIMIC-III database, among them, 241 people who aged <18 years and 397 patients who stayed in ICU<24 h were excluded. Five hundred and thirty-six patients had no data on SII and 728 patients had no data on NAR, and they were excluded. Five patients with abnormal follow-up data (the day admitted to ICU was after the death day) were excluded. Patients without data on SBP ($n = 4$), INR ($n = 6$), GCS ($n = 6$) and ECI ($n = 10$) were not included. Finally, 1,601 stroke patients were involved in with 908 hemorrhagic stroke patients and 693 ischemic stroke patients. The screen process was shown in Figure 1.

As observed in Table 1, the median NLR (8.39 vs. 7.06) and RA (4.58 vs. 3.97) in the death group were higher than the survival group in hemorrhagic stroke patients. The median PNI in the death group was lower than the survival group in hemorrhagic stroke patients (39.90 vs. 43.17). The median NLR (8.17 vs. 6.82), SII (1.90 vs. 1.58), NAR (2.93 vs. 2.51) and RA (4.52 vs. 4.26) in the death group were higher than the survival group in patients with ischemic stroke. The median PNI in the death group was lower than the survival group in patients with ischemic stroke (38.64 vs. 41.11).

Potential covariables associated with 30-day mortality in hemorrhagic or ischemic stroke patients

Potential covariables with statistical difference in Table 1 was involved in univariate cox analysis. The results depicted that age (HR = 1.01, 95% CI: 1.00–1.01), marital status, respiratory rate (HR

TABLE 1 Baseline characteristics between patients survived and died within 30 days with ischemic stroke or hemorrhagic stroke.

Variables	Hemorrhagic stroke		P	Ischemic stroke		P
	Survival group ($n = 682$)	Death group ($n = 226$)		Survival ($n = 477$)	Death group ($n = 216$)	
Age, years M (Q_1, Q_3)	62.79 (51.52, 74.13)	74.44 (59.17, 81.98)	<0.001	68.02 (55.69, 78.13)	77.26 (66.44, 84.51)	<0.001
Gender, *n* (%)			0.733			0.402
Female	314 (46.04)	107 (47.35)		242 (50.73)	117 (54.17)	
Male	368 (53.96)	119 (52.65)		235 (49.27)	99 (45.83)	
Marital status, n (%)			0.001			0.024
Married	351 (51.47)	106 (46.90)		227 (47.59)	90 (41.67)	
Unmarried	289 (42.38)	89 (39.38)		222 (46.54)	101 (46.76)	
Unknown	42 (6.16)	31 (13.72)		28 (5.87)	25 (11.57)	
Race, *n* (%)			0.179			0.037
White	482 (70.67)	149 (65.93)		325 (68.13)	164 (75.93)	
Others*	200 (29.33)	77 (34.07)		152 (31.87)	52 (24.07)	
Respiratory rate, Mean ± SD	17.65 ± 4.49	18.56 ± 5.76	0.031	18.00 (14.00, 22.00)	19.00 (16.00, 24.00)	0.025
SBP, mmHg, Mean ± SD	140.26 ± 25.94	138.92 ± 28.27	0.512	136.16 ± 27.82	135.00 ± 31.68	0.646
DBP, mmHg, Mean ± SD	72.17 ± 17.12	70.16 ± 19.44	0.165	69.39 ± 18.28	69.40 ± 20.70	0.994
SpO_2, Mean ± SD	97.93 ± 4.92	97.63 ± 5.47	0.476	97.50 ± 4.80	97.13 ± 4.00	0.286
RBC, m/ul, Mean ± SD	4.33 ± 0.64	4.12 ± 0.82	<0.001	4.07 ± 0.75	3.99 ± 0.72	0.214
INR, M (Q_1, Q_3)	1.10 (1.00, 1.20)	1.20 (1.10, 1.60)	<0.001	1.20 (1.10, 1.30)	1.20 (1.10, 1.40)	<0.001
Hemoglobin, g/dL, Mean ± SD	13.13 ± 1.89	12.54 ± 2.31	<0.001	12.22 ± 2.30	12.01 ± 2.16	0.258
Hematocrit, percent, Mean ± SD	38.38 ± 5.30	36.95 ± 6.52	0.003	36.22 ± 6.35	35.83 ± 5.98	0.451
Creatinine, mg/dl, M (Q_1, Q_3)	0.90 (0.70, 1.10)	1.00 (0.80, 1.30)	<0.001	1.00 (0.80, 1.40)	1.20 (0.90, 1.70)	<0.001
BUN, mg/dl, M (Q_1, Q_3)	16.00 (13.00, 22.00)	20.00 (15.00, 28.00)	<0.001	19.00 (14.00, 29.00)	25.00 (16.00, 41.00)	<0.001
Fasting blood-glucose, mg/dl, M (Q_1, Q_3)	138.00 (116.00, 169.00)	154.50 (126.00, 213.00)	<0.001	127.00 (108.00, 167.00)	136.00 (111.00, 170.50)	0.044
Bicarbonate, mEq/L, Mean ± SD	24.48 ± 3.40	24.05 ± 4.04	0.152	24.38 ± 4.36	23.35 ± 4.64	0.005
Sodium, Mean ± SD	138.96 ± 4.14	138.23 ± 5.05	0.049	139.25 ± 4.60	138.60 ± 4.74	0.085
Potassium, Mean ± SD	4.07 ± 0.70	4.13 ± 0.85	0.343	4.19 ± 0.80	4.40 ± 0.92	0.005
Chloride, Mean ± SD	103.13 ± 4.71	101.93 ± 5.84	0.005	103.79 ± 5.90	102.93 ± 5.82	0.075
Bicarbonate, mEq/L, Mean ± SD	24.48 ± 3.40	24.05 ± 4.04	0.152	24.38 ± 4.36	23.35 ± 4.64	0.005

(Continued)

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TABLE 1 (Continued)

Variables	Hemorrhagic stroke		P	Ischemic stroke		P
	Survival group ($n = 682$)	Death group ($n = 226$)		Survival ($n = 477$)	Death group ($n = 216$)	
Infection diseases, *n* (%)			0.106			0.446
No	472 (98.95)	210 (97.22)		555 (81.38)	189 (83.63)	
Yes	5 (1.05)	6 (2.78)		127 (18.62)	37 (16.37)	
IV-tPA, *n* (%)			0.130	–	–	–
No	411 (86.16)	195 (90.28)		—-	–	
Yes	66 (13.84)	21 (9.72)		–	–	
Endovascular treatment, *n* (%)			0.586	–	–	–
No	455 (95.39)	208 (96.30)		–	–	
Yes	22 (4.61)	8 (3.70)		–	–	
Craniotomy, *n* (%)	–	–	–			0.137
No	–	–		644 (94.43)	219 (96.90)	
Yes	–	–		38 (5.57)	7 (3.10)	
Minimally invasive surgery, *n* (%)	–	–	–			
No	–	–		633 (92.82)	208 (92.04)	
Yes	–			49 (7.18)	18 (7.96)	
ECI, M (Q_1, Q_3)	6.00 (0.00, 13.00)	9.00 (0.00, 16.00)	0.019	8.00 (4.00, 16.00)	12.00 (6.00, 18.50)	<0.001
GCS score, M (Q_1, Q_3)	14.00 (11.00, 15.00)	14.00 (7.00, 15.00)	0.304	14.00 (11.00, 15.00)	14.00 (9.00, 15.00)	0.032
AKI, *n* (%)			<0.001			<0.001
No	353 (51.76)	80 (35.40)		191 (40.04)	51 (23.61)	
Yes	329 (48.24)	146 (64.60)		286 (59.96)	165 (76.39)	
NLR, M (Q_1, Q_3)	7.06 (4.01, 11.53)	8.39 (4.41, 13.17)	0.020	6.82 (3.57, 11.54)	8.17 (4.80, 14.31)	0.002
PLR, M (Q_1, Q_3)	184.71 (124.64, 268.00)	188.28 (104.73, 316.82)	0.850	182.60 (117.94, 306.12)	211.89 (134.90, 309.61)	0.295
SII, M (Q_1, Q_3)	1,712.54 (888.13, 2,750.82)	1,701.81 (676.02, 2,992.99)	0.861	1,577.03 (814.29, 2,812.50)	1,900.55 (891.06, 3,461.58)	0.028
NAR, M (Q_1, Q_3)	2.45 (1.66, 3.33)	2.62 (1.72, 4.03)	0.062	2.51 (1.58, 3.77)	2.93 (1.94, 4.18)	<0.001
PNI, M (Q_1, Q_3)	43.17 (38.39, 48.33)	39.90 (34.10, 46.40)	<0.001	41.11 (34.94, 46.45)	38.64 (33.21, 43.19)	<0.001
RA, Mean ± SD	3.97 ± 0.87	4.58 ± 1.31	<0.001	4.26 (3.66, 5.22)	4.52 (3.90, 5.42)	0.003
Lymphocytes, M (Q_1, Q_3)	11.80 (7.60, 18.40)	10.00 (6.00, 16.80)	0.003	12.00 (7.20, 19.80)	10.00 (6.00, 15.80)	0.002
Neutrophil,%, Mean ± SD	79.02 ± 13.12	78.97 ± 16.04	0.964	77.07 ± 14.47	79.40 ± 13.63	0.046

(Continued)

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TABLE 1 (Continued)

Variables	Hemorrhagic stroke			Ischemic stroke		
	Survival group ($n = 682$)	Death group ($n = 226$)	*P*	Survival ($n = 477$)	Death group ($n = 216$)	*P*
RDW, percent, Mean ± SD	13.96 ± 1.46	14.85 ± 2.14	<0.001	14.69 ± 2.05	14.89 ± 1.79	0.189
PLT, K/uL, M (Q_1, Q_3)	241.00 (184.00, 295.00)	215.00 (142.00, 287.00)	<0.001	241.00 (175.00, 309.00)	236.00 (162.50, 300.50)	0.420
Albumin,%, Mean ± SD	3.62 ± 0.56	3.40 ± 0.65	<0.001	3.34 ± 0.67	3.19 ± 0.63	0.007

SBP, systolic blood pressure; DBP, diastolic blood pressure; SpO_2, blood oxygen saturation; RBC, red blood count; INR, international normalized ratio; BUN, blood urea nitrogen; GCS, Glasgow coma scale; IV-tPA, intravenous tissue plasminogen activator; ECI, Elixhauser comorbidity index; AKI, acute kidney failure; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, systemic immune inflammatory index; NAR, neutrophil to albumin ratio; PNI, prognostic nutritional index; RA, red cell distribution width to albumin ratio.

Others*: Asian, Black, Hispanic or Latino, Unknown.

= 1.03, 95% CI: 1.00–1.06), RBC (HR = 0.60, 95% CI: 0.38–0.95), hemoglobin (HR = 0.74, 95% CI: 0.58–0.95), hematocrit (HR = 0.96, 95% CI: 0.94–0.98), BUN (HR = 1.01, 95% CI: 1.01–1.01), fasting blood–glucose (HR = 1.00, 95% CI: 1.00–1.01), chloride (HR = 0.96, 95% CI: 0.94–0.99), ECI (HR = 1.02, 95% CI: 1.00–1.03) and AKI (HR = 1.74, 95% CI: 1.32–2.29) were covariables that might be associated with 30–day mortality in hemorrhagic stroke patients (Table 2). Age (HR = 1.00, 95% CI: 1.00–1.01), marital status, race (HR = 1.39, 95% CI: 1.02–1.91), creatinine (HR = 1.08, 95% CI: 1.00–1.16), BUN (HR = 1.01, 95% CI: 1.00–1.02), bicarbonate (HR = 0.96, 95% CI: 0.93–0.99), potassium (HR = 1.26, 95% CI: 1.09–1.45), ECI (HR = 1.03, 95% CI: 1.01–1.04), GCS (HR = 0.93, 95% CI: 0.90–0.97) and AKI (HR = 1.95, 95% CI: 1.42–2.67) were covariables that might be associated with 30-day mortality in ischemic stroke patients (Table 3).

Associations between NLR, PLR, SII, NAR, RA or PNI and 30-day mortality in hemorrhagic stroke or ischemic stroke patients

As exhibited in Figure 2, univariate analysis revealed that NLR (HR = 1.27, 95% CI: 1.16–1.39), SII (HR = 1.16, 95% CI: 1.07–1.25), NAR (HR = 0.45, 95% CI: 0.32–0.64), RA (HR = 1.23, 95% CI: 1.10–1.39) or PNI (HR = 1.66, 95% CI: 1.46–1.89) might have associations with 30-day mortality in hemorrhagic stroke patients. After adjusting for confounders including age, marital status, respiratory rate, hemoglobin, hematocrit, BUN, fasting blood-glucose, chloride, ECI and AKI, the elevated NLR level was associated with increased risk of 30-day mortality in hemorrhagic stroke (HR = 1.17, 95% CI: 1.06–1.29). The high level of NAR was associated with elevated risk of 30-day mortality in hemorrhagic stroke (HR = 1.16, 95% CI: 1.02–1.30). The increased level of RA was linked with elevated risk of 30-day mortality (HR = 1.44, 95% CI: 1.23–1.69). The higher level of NLR (HR = 1.13, 95% CI: 1.03–1.25), and NAR (HR = 1.18, 95% CI: 1.07–1.32) might correlate with increased risk of 30-day mortality in ischemic stroke patients. No significant correlation was observed in these inflammation biomarkers with the risk of 30-day mortality in ischemic stroke patients after adjusting for age, marital status, race, creatinine, BUN, bicarbonate, potassium, ECI, GCS and AKI (all $P > 0.05$).

The predictive values of NLR, NAR, or RA for 30-day mortality in hemorrhagic stroke patients

The C-indexes of NLR, NAR, and RA for evaluating the 30-day mortality in hemorrhagic stroke patients were 0.54 (95% CI: 0.50–0.58), 0.53 (95% CI: 0.49–0.57), and 0.61 (95% CI: 0.57–0.65), respectively (Table 4). The AUCs were shown in Figure 3. The AUC values of NLR, NAR and RA for evaluating the risk of 30-day mortality for hemorrhagic stroke patients were 0.552 (95% CI: 0.503–0.601), 0.541 (95% CI: 0.490–0.592) and 0.644 (95% CI: 0.590–0.699). Delong test revealed that the AUCs of NLR and NAR were statistically lower than the AUC of RA($P < 0.001$).

TABLE 2 Potential covariables associated with 30-day mortality in hemorrhagic stroke patients.

Variables	HR (95% CI)	*P*
Age	1.01 (1.00-1.01)	<0.001
Marital status		
Married	Ref	
Unknown	2.10 (1.41–3.13)	<0.001
Unmarried	1.01 (0.76–1.33)	0.961
Respiratory rate	1.03 (1.00–1.06)	0.030
RBC	0.60 (0.38–0.95)	0.028
INR	1.04 (1.00–1.09)	0.075
Hemoglobin	0.74 (0.58–0.95)	0.016
Hematocrit	0.96 (0.94–0.98)	<0.001
Creatinine	1.02 (0.98–1.07)	0.293
BUN	1.01 (1.01–1.01)	<0.001
Fasting blood-glucose	1.00 (1.00–1.01)	<0.001
Sodium	0.97 (0.94–1.00)	0.068
Chloride	0.96 (0.94–0.99)	0.002
ECI	1.02 (1.00–1.03)	0.016
AKI	1.74 (1.32–2.29)	<0.001
Infectious disease		
No	Ref	
Yes	0.86 (0.61–1.23)	0.41
Craniotomy		
No	Ref	
Yes	0.58 (0.27–1.23)	0.153
Minimally invasive surgery		
No	Ref	
Yes	1.12 (0.69–1.81)	0.649

HR, hazard ratio; CI, confidence interval; RBC, red blood count; INR, international normalized ratio; BUN, blood urea nitrogen; ECI, Elixhauser comorbidity index; AKI, acute kidney failure; IV-tPA, intravenous tissue plasminogen activator.

TABLE 3 Potential covariables associated with 30-day mortality in ischemic stroke patients.

Variables	HR (95% CI)	*P*
Age	1.00 (1.00-1.01)	<0.001
Marital status		
Married	Ref	
Unknown	1.86 (1.20–2.91)	0.006
Unmarried	1.12 (0.84–1.49)	0.437
Race	1.39 (1.02–1.91)	0.037
Respiratory rate	1.01 (1.00–1.03)	0.076
INR	1.03 (0.94–1.13)	0.480
Creatinine	1.08 (1.00–1.16)	0.036
BUN	1.01 (1.00–1.02)	<0.001
Fasting blood-glucose	1.00 (1.00–1.00)	0.078
Bicarbonate	0.96 (0.93–0.99)	0.004
Potassium	1.26 (1.09–1.45)	0.001
ECI	1.03 (1.01–1.04)	<0.001
GCS	0.93 (0.90–0.97)	<0.001
AKI	1.95 (1.42–2.67)	<0.001
Infectious disease		
No	Ref	
Yes	2.16 (0.96–4.86)	0.063
IV-tPA		
No	Ref	
Yes	0.74 (0.47–1.15)	0.181
Endovascular treatment		
No	Ref	
Yes	0.86 (0.42–1.73)	0.664

HR, hazard ratio; CI, confidence interval, INR, international normalized ratio, BUN, blood urea nitrogen, ECI, Elixhauser comorbidity index, GCS, Glasgow coma scale, AKI, acute kidney failure, IV-tPA, intravenous tissue plasminogen activator.

Discussion

In this study, the prognostic values of NLR, PLR, NAR, PNI, SII, and RA for 30-day mortality of ischemic stroke or hemorrhagic stroke patients were investigated based on the data form MIMIC-III database. The results unveiled that high levels of NLR, NAR and RA were linked with increased risk of 30-day mortality in hemorrhagic stroke patients. The AUC values of NLR, NAR, and RA for diagnosing the risk of 30-day mortality in hemorrhagic stroke were 0.552, 0.541, and 0.644. respectively. The findings suggested the values of monitoring the levels of inflammation biomarkers for timely identifying hemorrhagic stroke patients with high risk of mortality within 30 days and provide appropriate interventions to improve their outcomes.

Inflammation is one of the most important pathophysiological mechanisms of stroke and the inflammatory response is activated after stroke, which serves a vital part in secondary brain injury in patients (19). Recently, increasing studies have reported the essential role of immunity in predicting the prognosis and treating patients with acute stroke (20). Immunity is a complex process, and the activation and immunosuppression of different inflammatory cells are induced during the process (21). Neutrophils and lymphocytes are two important inflammatory cells, which were reported to have different roles of in the prognosis after stroke (22, 23). NLR is the ratio of neutrophil to lymphocyte, which can be calculated both from the absolute number of neutrophils and lymphocytes, and from their relative number (24). Previously, a high NLR level was found to associate with poor functional outcomes and increased mortality in patients with spontaneous intracerebral hemorrhage (ICH) (25, 26). These studies provided support to the findings of our study, which depicted that increased NLR was linked with higher risk of 30-day mortality of hemorrhagic stroke patients. NAR is the ratio of neutrophil to albumin, which has become a novel index reflecting systemic inflammation and predicting outcomes

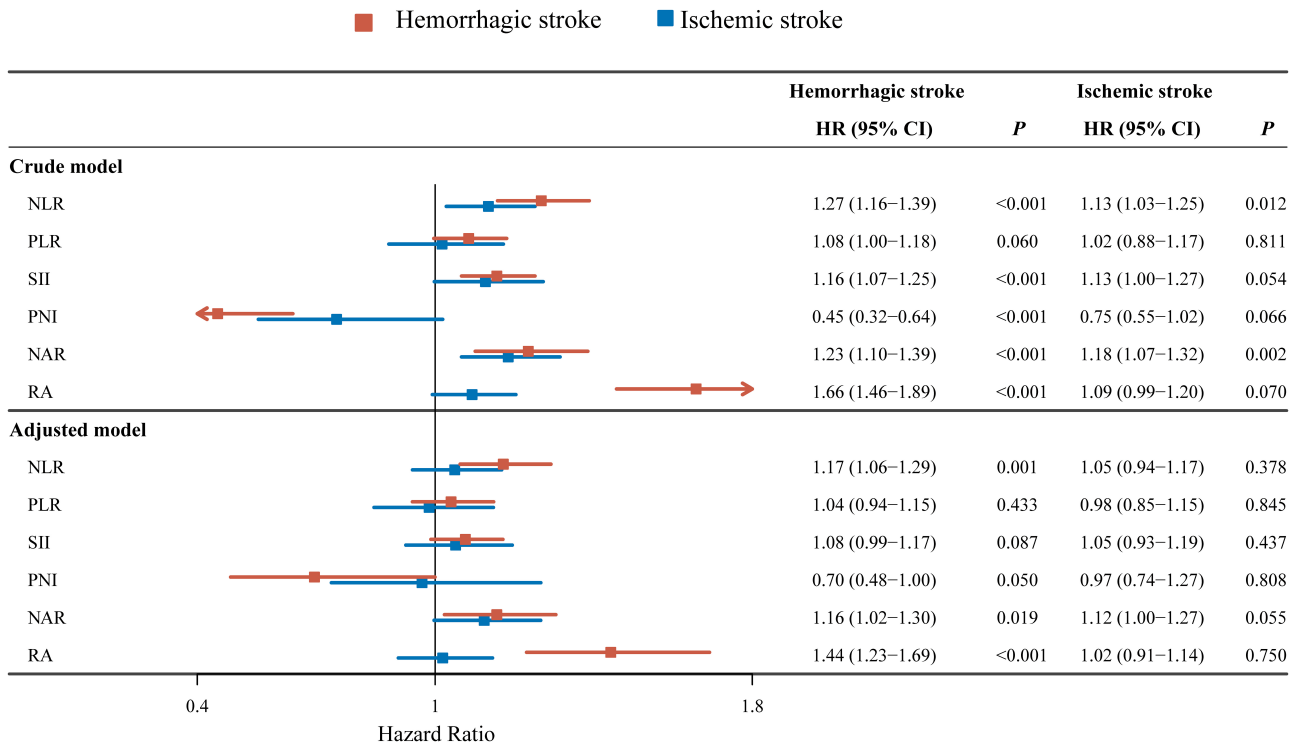

FIGURE 2
Forest plot showing the associations between NLR, PLR, SII, NAR, RA or PNI and 30-day mortality in hemorrhagic stroke or ischemic stroke patients.

TABLE 4 The C-index of NLR, NAR, and RA for evaluating the risk of 30-day mortality of hemorrhagic stroke patients.

	C-index (95% CI)
NLR	0.54 (0.50–0.58)
RA	0.61 (0.57–0.65)
NAR	0.53 (0.49–0.57)

CI, confidence interval; RA, red cell distribution width to albumin ratio; NAR, neutrophil to albumin ratio; NLR, neutrophil to lymphocyte ratio.

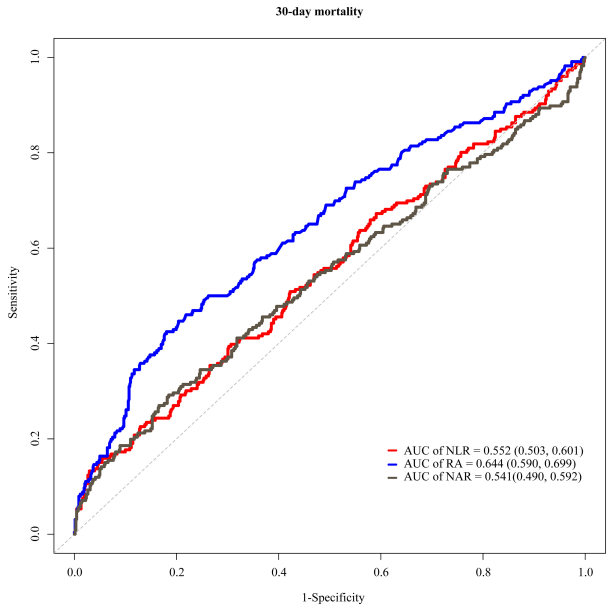

FIGURE 3
ROC curves presenting the values of NLR, NAR and RA for evaluating the risk of 30-day mortality in hemorrhagic stroke.

of patients in diseases (27). Albumin is an abundant protein in human blood plasma which has osmoregulation, anti-oxidation and anti-inflammation functions (28). A low albumin level was associated with increased mortality risk in hospitalized patients (29). Li et al. found that low serum albumin levels were associated with increased risk of total stroke, ischemic stroke, and ICH (30). In our study, a high level of NAR was correlated to increased risk of 30-day mortality of hemorrhagic stroke patients. RA is another inflammation biomarker derived from the ratio of RDW to albumin, which was reported to be associated with mortality of stroke (17). RDW is a hematologic parameter showing the divergence of red blood cell volume (31). In previous studies, RDW was identified to closely associate with prognosis of cardiovascular events (32, 33). Some other studies revealed that RA might be correlated with hemorrhagic transformation in acute ischemic stroke patients (34). Herein, elevated RA level was associated with higher risk of 30-day mortality of hemorrhagic stroke patients.

The mechanisms underlying the association between NLR, NAR, and RA with 30-day mortality in hemorrhagic stroke patients might be the follows. In hemorrhagic stroke patients, the increased number of neutrophils and decreased number of lymphocytes could induce a cytokine-chemokine storm and, ultimately, lead to more complications (35). Increased neutrophils

can release chemical mediators related to increased tissue damage and poor neurological prognosis in stroke patients (36). Lymphocytes were reported to play a brain protective role and the decrease of lymphocytes may lead to deterioration of nerve function (37). Albumin was found to exert an anticoagulant role and inhibitory effect on platelet function by binding antithrombin (38–40), which might aggravate the development of hemorrhagic stroke. In our study, we found that NLR, NAR and RA had potential prognostic values for 30-day mortality in hemorrhagic stroke patients. Previously, ICH score was reported to be a reliable clinical grading scale that allows risk stratification for patients with ICH (41). ICH score includes a basic neurological examination (GCS), a baseline patient characteristic (age), and initial neuroimaging (ICH volume, IVH, infratentorial/supratentorial origin), and compared with ICH score, NLR, NAR and RA are easily available and inexpensive markers that can be routinely detected in clinic. Application of these prognostic biomarkers may help clinicians enhance risk stratification, design individual treatments, and determine follow-up schedules for hemorrhagic stroke patients, which might further improve the outcomes of those patients.

There was evidence indicating that NLR, PLR, or NAR might associate with 30-day mortality of ischemic stroke patients in previous studies (42–44). The mechanisms underlying the findings might be related to the different roles of neutrophils and lymphocytes in the pathophysiologic development of atherosclerosis (45). Neutrophils are found to accumulate in cerebral vessels shortly after stroke and may result in infarctions extension and inhibit microvascular perfusion (46). PNI reflects nutritional status of patients, and previous studies revealed that malnutrition was associated with increased mortality in older Chinese adults with ischemic stroke (47). In our study, no significant association between NLR, PLR, NAR, PNI, SII or RA with 30-day mortality was found in ischemic stroke patients, this might because some other variables related to 30-day mortality of ischemic stroke patients were not included. The association between NAR and 30-day mortality of ischemic stroke patients showed a *P*-value of 0.055, this suggested that there might be association between NAR and 30-day mortality of ischemic stroke patients.

The strength in our study was that we focused on the prognostic values of LR, PLR, NAR, PNI, SII, and RA for 30-day mortality of different subtypes of stroke including ischemic stroke or hemorrhagic stroke. The finding might help identify potential reliable biomarkers in predicting those with high risk of 30-day mortality of different subtypes of stroke. There were several limitations in the current study. Firstly, this was a retrospective study from single-center, recall bias might exist. Secondly, due to the limitation of the database, some variables including the site or size of the hemorrhage or ischaemic stroke were unavailable, which might affect the results of our study. Thirdly, we analyzed the baseline data of inflammation biomarkers in ICU, and in the future, dynamic changes of the inflammation biomarkers during ICU stay will be analyzed to verify the results of our study. We will also conduct a study based on the samples from our hospital, and more important variables will be included.

Conclusion

This study evaluated the predicitive values of NLR, PLR, NAR, PNI, SII, and RA for 30-day mortality of ischemic stroke or hemorrhagic stroke patients. We found that NLR, NAR and RA were potential prognostic biomarkers for predicting 30-day mortality in hemorrhagic stroke patients, which might help clinicians enhance risk stratification, design individual treatments, and determine follow-up schedules for hemorrhagic stroke patients.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found in MIMIC-III database.

Ethics statement

Requirement for individual patient consent was waived because the project did not impact clinical care and all protected health information was deidentified. As the samples were not from The Eighth Medical Center of PLA General Hospital, and this study was exempt from our Institutional Review Board approval.

Author contributions

JZ and JF collected and analyzed the clinical data, reviewed the literature, and drafted the article. CL and FQ designed the study, supervised the initial drafting, and critically revised the article. All authors contributed to the article and approved the submitted version.

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

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Time course of recovery of different motor functions following a reproducible cortical infarction in non-human primates

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A major challenge in human stroke research is interpatient variability in the extent of sensorimotor deficits and determining the time course of recovery following stroke. Although the relationship between the extent of the lesion and the degree of sensorimotor deficits is well established, the factors determining the speed of recovery remain uncertain. To test these experimentally, we created a cortical lesion over the motor cortex using a reproducible approach in four common marmosets, and characterized the time course of recovery by systematically applying several behavioral tests before and up to 8 weeks after creation of the lesion. Evaluation of in-cage behavior and reach-to-grasp movement revealed consistent motor impairments across the animals. In particular, performance in reaching and grasping movements continued to deteriorate until 4 weeks after creation of the lesion. We also found consistent time courses of recovery across animals for in-cage and grasping movements. For example, in all animals, the score for in-cage behaviors showed full recovery at 3 weeks after creation of the lesion, and the performance of grasping movement partially recovered from 4 to 8 weeks. In addition, we observed longer time courses of recovery for reaching movement, which may rely more on cortically initiated control in this species. These results suggest that different recovery speeds for each movement could be influenced by what extent the cortical control is required to properly execute each movement.

KEYWORDS

non-human primate (NHP), stroke, common marmoset, photothrombosis, visually-guided reaching

Introduction

The most common deficit after stroke is motor impairment (1), and ∼60% of stroke patients do not completely recover their upper limb function, such as target-reaching and hand-grasping (2). The relationship between the extent of the lesion and the degree of deficits is well established (3, 4); however, factors that determine the speed of recovery remain uncertain. A lack of an optimal animal model of stroke for reproducing upper limb motor deficits in terms of both the extent and recovery process is a major limitation that has hindered the development of an effective therapeutic intervention.

Although several stroke models using rodents have been established (5), non-human primate (NHP) models remain indispensable (6–9) because NHPs provide an advantage over rodents when reproducing the reaching and grasping movements of a human stroke patient. First, the musculature and functionality of the hand differ between rodents and primates [for review see (10)]. For example, the intrinsic hand muscles have vast differences in anatomy between

the two animals. Thus, finger individualization is less frequently measured in rodents than in primates (11, 12). Second, the cortical visual pathway for visually guided behaviors is developed in primates (13). For example, neurons in the parietofrontal cortex are activated during visually guided reaching (14–16), and lesions in this pathway cause deficits in reaching performance (17–19). In contrast, rodents primarily use olfaction to identify the location of a target, and thus, reaching toward a target is guided more by olfaction than by vision (20–22). In addition, recent studies demonstrated the advantage of NHPs over rodents in terms of cellular divergency in the central nervous system (CNS), with an important implication in the context of inflammation (23, 24).

Therefore, the use of existing NHP models (8, 25) is advantageous to using the rodent model for stroke research. However, NHP models show significant inter-individual variability in the extent and recovery time course of outcome measures, which is largely due to the technical complexity of applying an infarction and the limited availability of animals to refine such techniques. For example, in an anterior choroidal artery occlusion model, only 60% of animals showed neurological impairment (26). In an internal capsular infarct model, the duration of recovery varied among animals (27, 28). Such inter-animal variability can be compensated for by increasing the number of animals in the case of rodent models, whereas this is more challenging for NHP models. Consequently, NHP stroke models have been less popular for use in stroke research to date (29, 30).

In this study, we aimed to overcome this problem by using a photothrombotic approach (31–38), which involves the intravenous administration of photosensitive dye, followed by irradiation of the cerebral cortex with green light (31). The irradiation triggers the formation of a blood clot that occludes the vessels (5, 39, 40). Because the area of infarction can be controlled by irradiation light, this method has the advantages of high reproducibility and low mortality (7, 8, 39–41).

The purpose of this study was to create a cortical infarction using photothrombosis over the motor cortex of NHPs to establish a reproducible deficit in the reaching and grasping task. We then characterized the time courses of recovery of the reaching and grasping functions.

Materials and methods

Animals

Four adult common marmosets (*Callithrix jacchus*, aged 3–6 years, three males and one female, weighing 300–550 g) were used in the present study (Table 1). All interventions and animal care procedures were performed in accordance with the institutional guideline for animal experiments and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments were approved by the experimental animal committee of the National Institute of Neuroscience.

Surgical procedure

We created an infarction over the unilateral motor cortex using Rose Bengal, which is a light-sensitive dye, according to a previous study (36). A 3 mm diameter liquid light guide connected to the light source (Spectra X light engine, Lumencor, Beaverton, OR, USA) was placed 16 mm above the motor cortex, which was identified with the aid of a stereotaxic atlas (42). After intravenous injection of Rose Bengal (20 mg/kg), green light (542.5–557.5 nm) was irradiated for 5 min at a light intensity of 48 mW. All surgeries were performed under anesthesia induced by intramuscular induction of ketamine hydrochloride (20 mg/kg) and maintained by inhalation of isoflurane (2%−3%). Atropine sulfate, antibiotics, analgesics, and dexamethasone were used to prevent postsurgical infection, pain, and edema. Mannitol (1.0–1.5 mL/h) was infused if necessary to reduce intracranial pressure during surgery. Antibiotics and analgesics were injected twice (morning and afternoon) daily for 5 days following creation of the lesion.

To expose the green light, we performed a craniotomy. The skull was opened between interaural, 5–15 mm anteroposterior (AP) and 2–12 mm mediolateral (ML) from the midline. A probe for light exposure was then placed on the opened skull at the center, 8 mm AP and 4 mm ML. To limit irradiation of the light, a perforated aluminum cover (3 mm AP × 8 mm ML) was placed onto the brain. We have already confirmed that craniotomy itself does not alter the spontaneous in-cage behavior after surgery (26).

Behavioral assessments

Marmoset neurologic score (MNS)

To evaluate the natural recovery process of sensorimotor functions, neurological status was evaluated using a neurological

TABLE 1 Marmosets used in the study.

Subject	Sex	Age (years)	Lesion volume [mm^3]
Monkey K	male	3.4	43.5
Monkey M	female	5.2	38.5
Monkey P	male	6.0	41.1
Monkey U	male	5.0	45.4

TABLE 2 Modified marmoset neurologic score.

General evaluation	Hemilateral evaluation
Stays in back of the cage	Body tilting
Stays still for 1 min	Head tilting
Cannot stand in the perch	Hand waving
Circling behavior	Repeated touching before grasp cage bars
Palpebral ptosis	Hand crossing the chest
No jumping from cage wall	Hand slipping from the cage bars
No rearing without hand support	Hand dangling from the cage bars
	Hand neglect during feeding
	Foot slipping
	Foot dangling
	Dropping crumbs

Total score: 18 points (general: 7 points; hemilateral: 11 points).

score described previously (26). First, we recorded the spontaneous natural behavior of the marmosets in the home cage *via* a video camera placed in front of their cages. Two experienced experimenters then carefully inspected the recorded video and judged the absence (score = 1) or presence (score = 0) of 18 abnormal behavioral signs in their home cage (Table 2). We omitted several test items from the original test (26) that required retrieval of the marmoset from the home cage (e.g., the "stick" and "limb stimuli" tests). The maximum score was 18, and a lower score indicated greater motor impairment. The tests were performed before and 1, 2, 7, 14, 21, and 28 days after creation of the lesion.

Pellet-reaching task

To evaluate the influence of cerebral ischemia on forelimb sensorimotor function, we trained three marmosets (Monkeys K, M, and P) to perform a pellet-reaching task. A clear acrylic food table was attached to the cage, and the monkeys were forced to use their impaired limb (Figure 1A). The table was 180 mm in length and 60 mm in width and was attached 80 mm above the floor of the cage. A transparent wall (180 mm in length and 65 mm in depth) was attached to the front edge of the table in front of the marmoset. A small opening (a 25 mm square) was located 10 mm from the bottom of the transparent wall, which forced the monkeys to use their affected hand. A sweet treat (4–8 mm in diameter) was placed in a small well on the table (8 mm in diameter, 1 mm in depth, and 20 mm from the opening). Two marmosets performed the task with the right hand (Monkeys M and P) and one marmoset performed the task with the left hand (Monkey K). We defined the success rate as a percentage of the ratio between the number of successful retrievals with the affected hand and the number of reaches with the affected hand. All marmosets were trained for 20 min for 5 days per week for up to 7 weeks until their baseline performance plateaued (>80% success rate). The tests were performed before and 1, 2, 4, and 8 weeks after creation of the lesion. In a single test, the marmosets attempted 20–40 food pellet retrievals using the affected hand over 10 min. If the animals did not use the affected hand within 5 min, the success rate was recorded as zero.

To evaluate hand kinematics, recordings using a high-speed camera were acquired during the task before and 4 and 8 weeks after creation of the lesion. Two-dimensional positions of the affected hand were recorded using a high-speed camera (EX−100F; CASIO COMPUTER, Tokyo, Japan). The camera was operated at 240 frames/s at a 640 × 480-pixel resolution. The camera was placed 230 mm from the table horizontally to ensure that the animal's hand was tracked throughout reaching and retrieving movements.

Most tests were performed in the home cage. However, some sessions were performed in the breeding room. In such sessions, we used a cage of identical size as that of the home cage.

Processing of video recordings

To quantify the movement trajectories during pellet-reaching, we measured the two-dimensional positions of the hand and pellets using DeepLabCut (version 2.2b8) (43, 44). First, we annotated the two-dimensional positions of the distal interphalangeal (DIP), proximal interphalangeal (PIP), and metacarpophalangeal (MP) joints of the index finger, interphalangeal (IP) joint of the thumb, and the food pellet (Figure 1B). Next, we trained two deep neural networks (i.e., a right-hand network and a left-hand network) based on transfer learning of the pre-trained network (ResNet50). In the right-hand network, we labeled a total of 1,500 images that were randomly selected from 17 trials (two different targets at three different time points, one or two trials each) in two animals (Monkeys M and P). In the left-hand network, we labeled a total of 1,199 images that were randomly selected from 12 trials (two different targets at three different time points, two trials each) in one animal (Monkey K). The ratios of the training data to the annotated data were 0.95, and the training iterations were 1,030,000.

Once the networks were trained, we performed separate validation procedures for the two networks. The train and test errors were as follows: the right-hand network was 2.51 pixels and 2.12 pixels, respectively, and the left-hand network was 2.09 pixels and 1.95 pixels, respectively. The model provided likelihood estimates for each tracking result at each time point. We regarded the tracking result with a likelihood of <0.7 as an occlusion. We removed results with a likelihood between 0.7 and 0.95 and performed linear interpolation. The ratio of the removed frames to total frames was <1%.

We then converted the tracking results into actual two-dimensional coordinates using the four landmarks on the table for which the two-dimensional coordinates were determined previously. The converted data were low-pass filtered at 30 Hz in each coordinate axis, and the hand position, movement speed, grip aperture, and finger-joint angle were calculated using the filtered data. Specifically, the hand position was calculated from the two-dimensional coordinates of the index finger MP joint, and movement speed was calculated by the differential of the Y-coordinate positions of the index finger MP joint. Grip aperture was calculated according to the Euclidean distance between the two-dimensional coordinates of the IP joint of the thumb and the DIP joint of the index finger. Using the two-dimensional coordinates of the MP, PIP, and DIP joints, we calculated the horizontally projected angle of the index finger PIP joint.

Kinematic analysis

Using hand position, movement speed, finger-joint angle, and likelihood estimates for each data point, we defined the movement phases (Figures 1C, D). We defined eight discrete movement task epochs from the processed video recordings. The "reaching start" was defined as the time at which the MP joint passed the opening when the likelihood estimate of the MP joint exceeded 0.95. The "initial movement" phase was defined as the period from the "reaching start" to the first local minimum in movement speed. The "endpoint" was defined as the time at which the Y-axis movement speed dropped below the speed threshold (0 mm/s) for at least 25 ms. The definitions of "initial movement" and "endpoint" were adopted from the visually guided reaching task in human stroke patients (45). The "maximum grip aperture" was defined as the time at which the grip aperture between the thumb and index finger was the largest. The "reaching end" was defined as the time at which the hand touched the food pellet when the distance between the MP joint and the food pellet was the smallest. The "grasping start" was defined as the time at which the index finger started to flex when the angular velocity of the PIP joint angle first exceeded 5% of the peak angular velocity. Because wrist supination occurred as soon as the food pellet was grasped, "grasping end" was defined as the time at which the index finger DIP joint

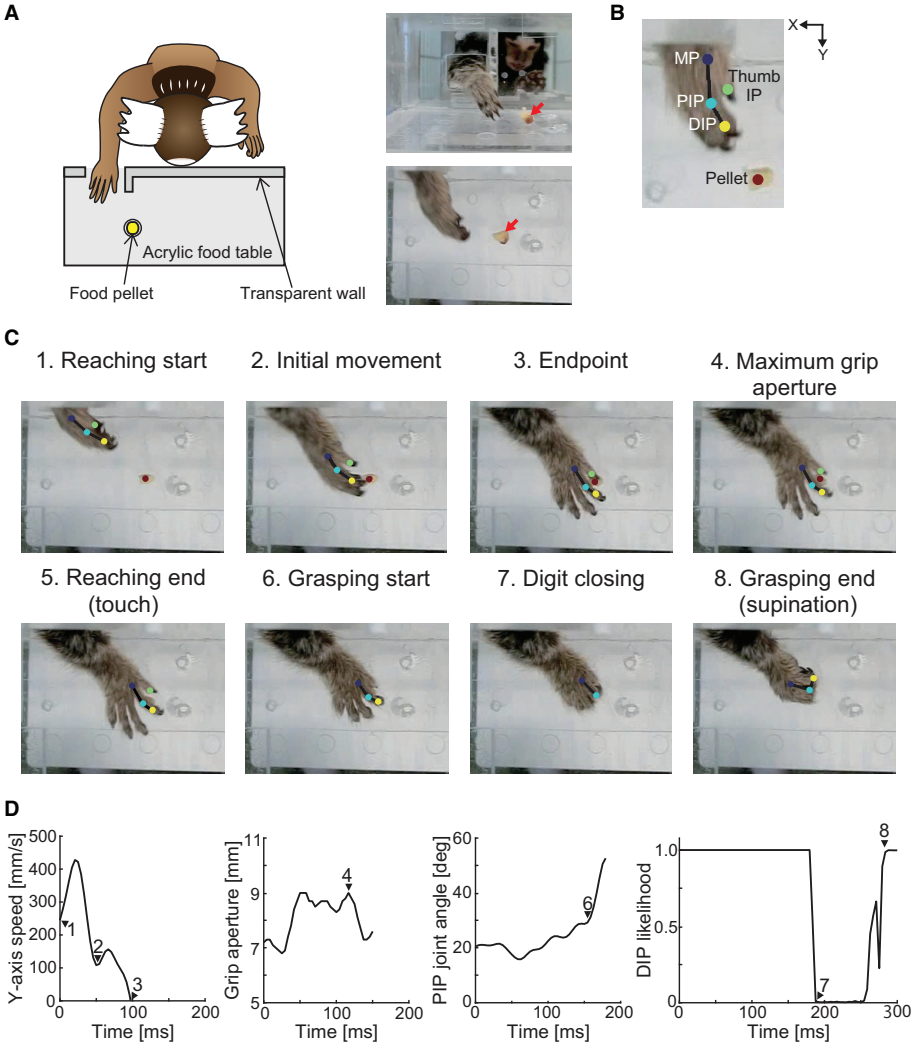

FIGURE 1
Schematic of the experimental setup and definition of the movement phase. **(A)** Experimental setup. Red arrows in the right images indicate the position of the food pellet. **(B)** Positions of the tracking using DeepLabCut. Two-dimensional positions of the distal interphalangeal (DIP), proximal interphalangeal (PIP), and metacarpophalangeal (MP) joints of the index finger, interphalangeal (IP) joint of the thumb, and food pellet were tracked. **(C)** Definition of the movement phases. The reaching movement phase was defined as the time at which the MP joint of the index finger passed the opening ("reaching start") to the time when the hand touched the food pellet ("reaching end"). The grasping movement phase was defined as the time at which the index finger started to flex ("grasping start") to the time when the wrist began to supinate ("grasping end"). **(D)** Y-axis movement speed, grip aperture, PIP joint angle, and likelihood estimates of the index finger DIP joint position for each data point using DeepLabCut. Each number corresponds to those in **(C)**.

could be seen from above after the digits closed, when the likelihood estimate of the index finger DIP joint exceeded 0.95.

The data were visually inspected, and data were discarded when the marmoset failed to perform a successful reaching movement, or when the animal was unable to touch, displaced, or dropped the pellet. The ratios of reaching failures to grasping failures at each time point are shown in Table 3. Data in which the grasping movement took longer than 1 s were also excluded from the statistical analysis. Eventually, we analyzed five reaching and grasping movements for each time point.

To evaluate reaching performance in detail, we used two further movement parameters that represent the initial motor response and feedback corrections of the visually guided reaching task, which are used in human stroke patients (45). An initial movement direction error that represents the initial motor response was defined as the angular deviation between a straight line from the MP joint position at the "reaching start" to the target position and a vector from the MP joint position at the "reaching start" to the "initial movement." The number of speed maxima that represents the feedback corrections was defined as the number of Y-axis movement speed maxima between the "reaching start" and the "endpoint." We also evaluated the grasping performance in detail to measure the grasping time and the maximum grip aperture. Grasping time was defined as the total time from "grasping start" to "grasping end." Maximum grip aperture is a clinically relevant outcome measure of functional impairment in human patients (46), and has been demonstrated to be altered in marmosets after lesion of the cortical visual pathway (13, 19). Following the methods of previous studies, we defined the maximum grip aperture as the maximum value of the grip aperture between the thumb and index finger before "grasping start" (13, 19).

TABLE 3 Details of the pellet-reaching task.

Subject	Time point	Success ratio (%)	Failure ratio (%)		
			Reaching failure	Grasping failure	Pull-back failure
Monkey K	Pre	90	0	10	0
	4 weeks	36	23	23	18
	8 weeks	39	13	17	31
Monkey M	Pre	95	0	5	0
	4 weeks	45	37	13	5
	8 weeks	96	0	4	0
Monkey P	Pre	81	0	5	14
	4 weeks	31	44	10	15
	8 weeks	20	15	50	15

Histology

Immunohistochemistry

After the marmosets had performed all the experiments, including the pre- and post-lesion sessions, they were deeply anesthetized and transcardially perfused with 4% paraformaldehyde in phosphate buffer (pH 7.4). The fixed brains were removed from the skull, postfixed in the same fresh fixative overnight at 4°C, and placed into 0.1 M phosphate buffer (pH 7.4) containing 30% sucrose. The brains were then cut along the coronal plane into 50 μm thickness slices using a freezing microtome. One section out of six was immediately mounted for thionin staining. For immunohistochemistry, adjacent sections were incubated with a mouse monoclonal antibody for glial fibrillary acidic protein (1:1,500 dilution; Sigma-Aldrich, St. Louis, MO, USA) or a rabbit polyclonal antibody for Iba-1 (1:4,000 dilution; WAKO Pure Chemical Industries, Osaka, Japan). Secondary biotinylated anti-mouse (1:200 dilution; Vector Laboratories, Burlingame, CA, USA) or biotinylated anti-rabbit (1:200 dilution; Vector Laboratories) antibodies were also used. Immunoreactive signals were visualized using the ABC Staining Kit (Vector Laboratories) with 3,3'-diaminobenzidine. All stained images were acquired using an inverted microscope (BZ-X700, Keyence, Osaka, Japan).

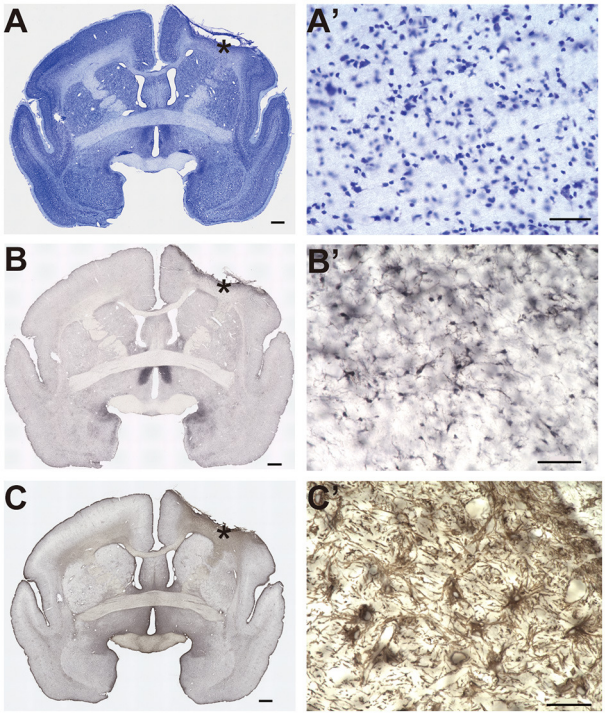

FIGURE 2
Histological identification of the lesion area. **(A–C)** Representative image of Nissl staining **(A)**, Iba-1 immunostaining **(B)**, and glial fibrillary acidic protein immunostaining **(C)**. Asterisks indicate the locations of the higher magnification view shown in **(A'–C')**. Scale bar, 1 mm for **(A–C)**, and 50 μm for **(A'–C')**.

Identification of lesion area

To identify the lesioned cortical area induced by photothrombosis, we detected the area showing an inflammatory response (Figures 2B, 3). First, we selected sections showing an inflammatory response based on Iba-1 immunochemistry with a 300 μm space between serial sections. We then manually traced the cortical area labeled with the Iba-1 antibody. Because several cortical structures were lost under the irradiation area, we estimated the area of the lost cortical structure by tracing the interhemispheric difference between the contralesional hemisphere and the ipsilesional hemisphere in each section. From these tracings, we calculated the total volume of the lesioned areas using ImageJ (National Institutes of Health, MD, USA) using the following formula:

$$volume = d \sum (S_{contra} - S_{ipsi} + S_{Iba-1}) \quad (1),$$

where d is the distance between sections (300 μm), and S_{contra}, S_{ipsi}, and S_{Iba-1} are the traced areas of the contralesional hemisphere, ipsilesional hemisphere, and Iba-1-positive cortical structure, respectively.

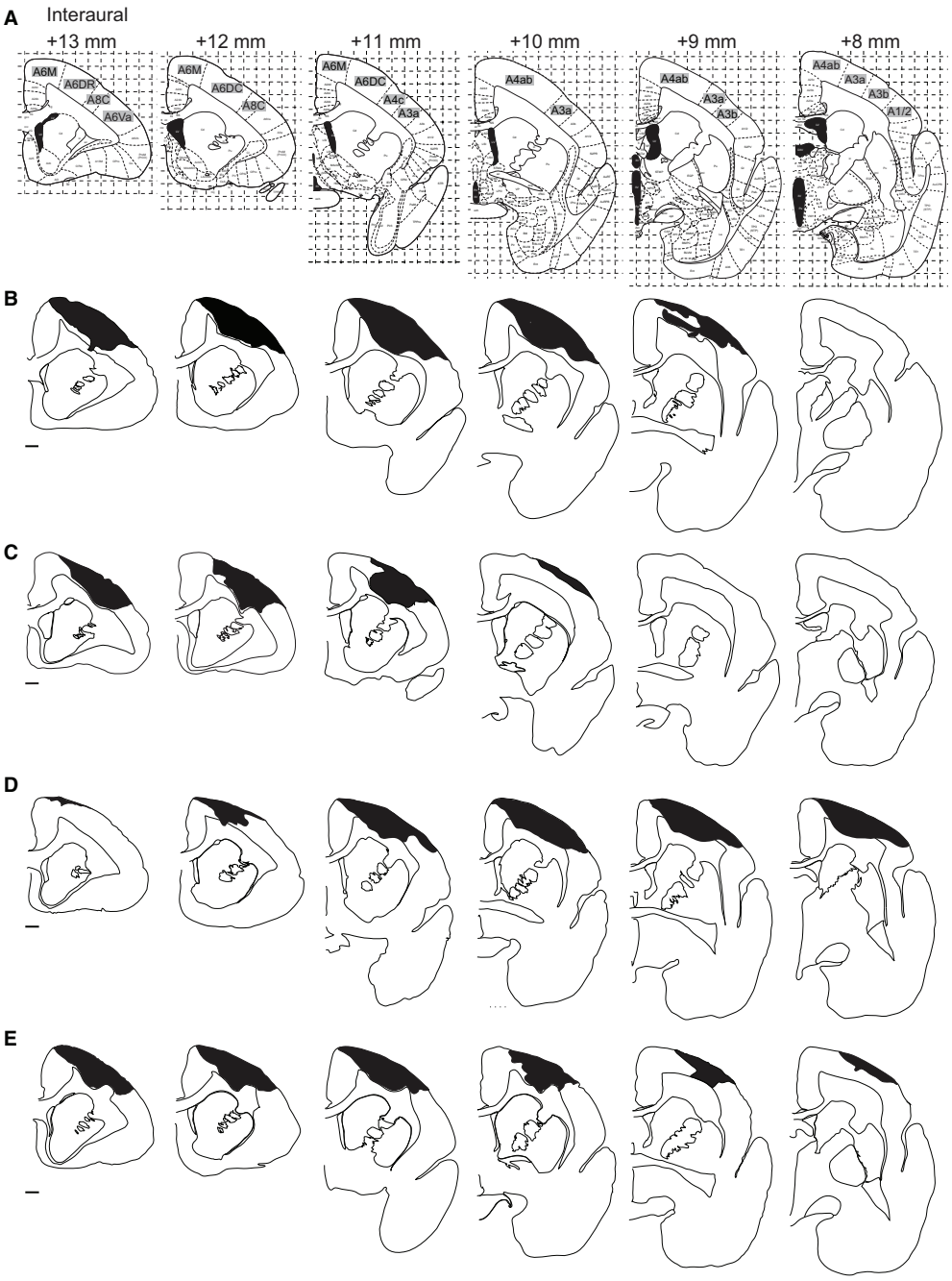

FIGURE 3
Distribution of the lesion area. **(A)** The corresponding coronal sections of the marmoset brain atlas (42). **(B–E)** Serial coronal sections of the lesion area in Monkey K **(B)**, Monkey M **(C)**, Monkey P **(D)**, and Monkey U **(E)**. Black hatched areas indicate the lesion area identified by Iba-1 immunostaining. Only the hemispheres ipsilateral to the lesion site are shown at intervals of 1 mm. Scale bar, 1 mm.

Experimental design and statistical analysis

To assess the differences in the time course of recovery between subjects, a two-way analysis of variance with aligned rank transform [(ART-ANOVA); (47, 48)] was performed for each index, with "time point" ("Pre," "4 weeks," and "8 weeks") and "subject" (Monkeys M, K, and P) as between-subject factors. *Post-hoc* analyses were performed using Holm-Bonferroni correction for multiple comparisons. The level of significance was set at $\alpha = 0.05$. All data analyses and statistical tests were performed using MATLAB 2018b (MathWorks, Natick, MA, USA).

Results

Area and extent of the lesion

An example of the extent of the lesioned cortical area is shown in Figures 2A–C (Monkey P). In this subject, we found dense cell

infiltration under the irradiation area (Figure 2A'). Clear microglia accumulation within the same area indicated an inflammatory response (Figure 2B'). Furthermore, an aggregation of reactive astrocytes was observed within the same area (Figure 2C'). Taken together, these results suggested that the extent of the lesion encompassed the infarction under the irradiation area.

We then compared the area and extent of the lesion among the four animals. The area immunostained by Iba-1 is shown in black hatched area in Figure 3. We found that Brodmann's areas 6M, 6DR, 6DC, 4c, and 4ab were the locus of damage with the highest probability, according to the marmoset brain atlas [(42); Figure 3A]. Area 4 corresponds to the primary motor cortex (M1), area 4b corresponds to the forelimb movement representation in the M1 (42, 49–51), and areas 6M, 6DR, and 6DC correspond to the supplementary motor area and rostral and caudal area of the dorsal premotor cortex (PMd), respectively (42, 52). Therefore, we concluded that the lesioned area was mainly localized to the motor-related cortical areas. In addition, two animals (Monkeys P and U) showed ischemic damage in the primary somatosensory cortex [areas 3a and 3b in Monkeys P and U (Figures 3D, E) and area 1/2 in Monkey P (Figure 3D)]. The average lesion volume among animals was 42.1 $\pm$ 3.0 mm^3 (Table 1).

Time course of behavioral recovery

Figure 4A shows the time course of the changes in the MNS score. Before the lesion was created, all animals scored the maximum score (18 points). One day after creation of the lesion, all marmosets showed an expected decrease in the MNS score (median = 7). The score recovered rapidly over the subsequent weeks in all animals. Specifically, from 2 days to 1 week after creation of the lesion, the median score increased from 12 to 16 points. At 3 weeks after creation of the lesion, animals fully recovered and scored the maximum score (18 points).

This systematic recovery time course in all monkeys as measured by the MNS score (Figure 4A) was also supported by the descriptive observations of the daily behaviors of the marmosets in their cages. One day after creation of the lesion, the animals often stayed at the back of the cage, their body and head were tilted, and they frequently let their hand and feet slip or dangle from the cage bars. They also held the cage bars close to their chest using their intact hand. At 1 to 2 weeks after creation of the lesion, several abnormal behaviors continued to be exhibited, such as dangling their hands from the cage bars. However, these abnormal behaviors were not observed from 3 weeks after creation of the lesion.

Figure 4B shows the weekly change in the success rate of the pellet-reaching task. Before the lesion, success rates were >80% in all animals. After creation of the lesion, their performance was completely impaired for 2 weeks (i.e., 0% success rate), which suggested that the lesion drastically affected the function of the contralesional limb. At 2 to 4 weeks after creation of the lesion, the animals started to use their impaired limb to reach and retrieve pellets. However, in contrast to the MNS score (Figure 4A), success rates remained lower (31–45%) than those before creation of the lesion, and recovery varied among animals. For example, one marmoset (Monkey M) showed an improvement in success rate, whereas the other two marmosets (Monkeys K and P) showed little improvement. This heterogeneous recovery among animals is further described in Table 3. At 4 weeks after creation of the lesion, the predominant reason for failed trials was "reaching failure," where 23–44% (Table 3) of failures were caused by this error. Reaching failures are primarily due to reaching for the pellet in an inappropriate direction, which resulted in their hand not reaching the pellet, based on our visual observations. Another reason for failed trials at this time point was "grasping failure" (10–23% of all failures; Table 3), in which animals exhibited clumsy digit movements, which resulted in the pellet being displaced or dropped.

At 8 weeks after creation of the lesion, we found mixed results in the three monkeys. In Monkeys K and P, the major sources of failures were both "grasping failures" and "pull-back failures" (Table 3 for Monkeys K and P). In pull-back failures, animals were able to touch the pellet but could not bring the pellet to their mouth. In contrast, we found almost complete recovery in Monkey M. Our analysis of the success ratio for the time course of recovery suggests that controlled cortical lesions can produce a reproducible time course of recovery of motor deficits for at least 4 weeks after creation of the lesion.

A comparison between Figures 4A, B illustrates the unique recovery profiles of pellet-reaching and demonstrates a clear contrast to the earlier recovery of the MNS scores. Although the MNS scores recovered completely and reached a plateau at 3–4 weeks after creation of the lesion (Figure 4A) in a highly similar fashion across all animals, the success rate of pellet-reaching did not recover to the pre-lesion rate at 4 ($n = 3$) or 8 weeks ($n = 2$) after creation of the lesion. These results may reflect the superior resolution of the reaching and grasping test for evaluating the recovery of function represented by the lesioned cortical area.

Reaching kinematics

Figure 5A shows the hand trajectories during the reaching movement of one representative marmoset (Monkey M). Before creation of the lesion ("Pre"), the animal showed a relatively straight trajectory of the hand toward the target from the beginning to the initial movement phase (Figure 5Aa). This straight trajectory was sustained until the endpoint was reached (Figure 5Ab). The animal then showed small corrective movements before touching the pellet (Figure 5Ac). In contrast, at 4 weeks after creation of the lesion ("4 weeks"), the reaching movement occurred in the incorrect direction at the beginning (Figure 5Ad), which was not corrected even by the end of the reaching movement (Figure 5Ae). The direction was eventually corrected before the hand touched the pellet (Figure 5Af). At 8 weeks after creation of the lesion ("8 weeks"), the misdirection in the initial movement recovered partially but a deviation from the pellet persisted (Figure 5Ag). The hand trajectory showed meandering trajectories (Figure 5Ah, i), which suggested that the animal was correcting the direction of movement during the reaching period. These observations (the trajectory superimposed over the pictures in Figure 5A) are quantitatively represented in the X-Y coordinate and summarized in Figure 5B, illustrating that the hand trajectory significantly drifted along the Y-axis in the overshoot direction at "4 weeks" in contrast to the relatively straight path at "Pre."

We then quantified these observations by measuring the initial phase of the reaching movement (initial direction error) and the

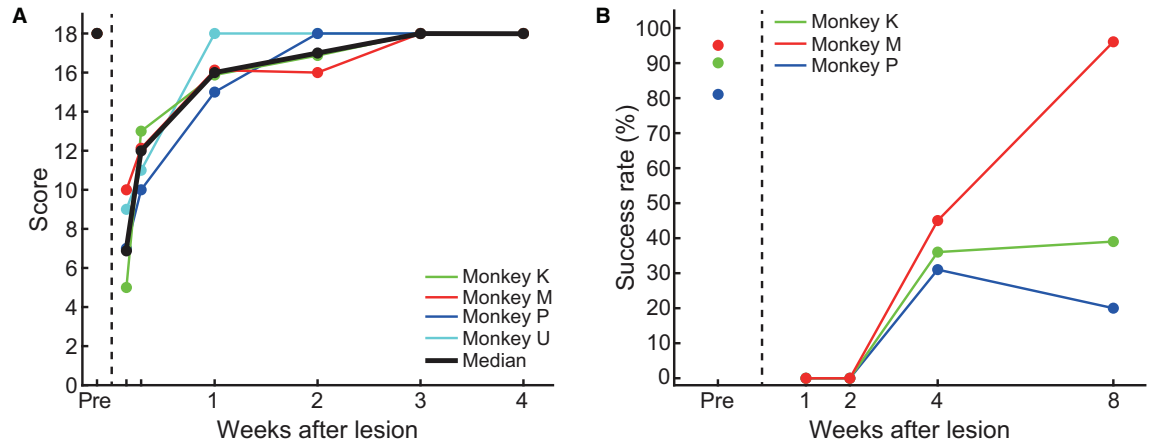

FIGURE 4
Time course of behavioral recovery. **(A)** Transitional changes in the marmoset neurological score. The black line represents the median value at each time point. **(B)** Transitional changes in the success rate of the pellet-reaching task.

corrective feedback control (number of speed maxima), similarly to the reports measuring sensorimotor impairments in human stroke patients (45, 53). We found significant changes at various measurement points in both the initial direction error (two-way ART-ANOVA, "time point" × "subject" interaction: $F_{(4,36)} = 19.6$, $p < 0.0001$) and the number of speed maxima (two-way ART-ANOVA, "time point" × "subject" interaction: $F_{(4,36)} = 9.0$, $p < 0.0001$), as shown in Figures 5C, D. Compared with "Pre", all animals showed significantly larger initial movement direction errors at "4 weeks" (Figure 5C; Monkey K: $p = 0.0002$, Monkey M: $p < 0.0001$, and Monkey P: $p = 0.0001$) and "8 weeks" (Figure 5C; Monkey K: $p < 0.0001$, Monkey M: $p = 0.0082$, Monkey P: $p = 0.0001$). Similarly, the results of the number of speed maxima showed significantly more corrective movements at "8 weeks" than at "Pre" (Figure 5D; Monkey K: $p < 0.0001$, Monkey M: $p < 0.05$, Monkey P: $p < 0.05$). Only one marmoset (Monkey K) made significantly more corrective movements at "4 weeks" than at "Pre" (Figure 5D; $p < 0.0001$). These results indicated that the impairment in reaching trajectory is characterized by an increase in initial movement direction errors and greater corrective feedback control, which were both comparable to observations in human stroke patients (45, 53). Neither parameter recovered to presurgical levels, even 8 weeks after creation of the lesion.

Grasping function

We observed that the impaired feed-forward and feedback control for the reaching movement did not recover, even 8 weeks after creation of the lesion. To test whether the grasping movement after reaching the target follows the same time course of recovery, we analyzed the grasping kinematics and their changes (Figure 6). Examples of typical grasping movements in one marmoset are shown in Figure 6A (Monkey M). A smooth continuous motion of finger extension (Figure 6Aa), finger flexion (Figure 6Ab) and wrist supination (Figure 6Ac) was observed at "Pre". However, at "4 weeks," we noticed a larger grip aperture and clumsiness in the hand-closing movement, although the animal was able to open and close the affected hand (Figure 6Ad, e, f). At "8 weeks," the grasping movement recovered, and the smooth hand-closing movement was restored (Figure 6Ag, h, i). To characterize these observations, we analyzed the total grasping time from the start to the end of the grasping motion and found a significant interaction between "time point" and "subject" (two-way ART-ANOVA, $F_{(4,36)} = 4.06$, $p = 0.0081$). However, all animals showed significantly longer grasping times at "4 weeks" (Figure 6B; Monkey K: $p < 0.0001$, Monkey M: $p < 0.0001$, Monkey P: $p = 0.002$) and "8 weeks" (Figure 7B; Monkey K: $p = 0.0142$, Monkey M: $p = 0.0016$, Monkey P: $p = 0.0003$) than at "Pre." In addition, a similar homogeneous time course of recovery was also observed in the maximum grip aperture (two-way ART-ANOVA, "Time point" × "Subject" interaction: $F_{(4,36)} = 1.95$, $p = 0.1236$, "Time point" main effect: $F_{(2,36)} = 37.1$, $p < 0.0001$). Maximum grip aperture was significantly larger at "4 weeks" (Figure 6C; $p < 0.0001$) and "8 weeks" (Figure 6C; $p = 0.0230$) than at "Pre", and significantly decreased from "4 weeks" to "8 weeks" (Figure 6C; $p < 0.0001$). These findings indicated that the time course of the recovery of the grasping movement differed from that of the reaching movement. Although the animals did not recover their reaching movement, all animals partially recovered their grasping movement 8 weeks after creation of the lesion.

Control experiment

To test whether the photochemically induced cerebral infarction only affected the reach and grasping functions of the contralesional limb, we performed the same behavioral assessments on the ipsilesional limb. Two measurements were performed before and 2 weeks after creation of the lesion in each monkey and compared. The change in the success rate of the pellet-reaching task is provided in Figure 7A. We found no difference in the success rate between pre- and post-surgery.

Figures 7B–E shows the results of the comparisons of the reaching and grasping function indices of initial movement direction error,

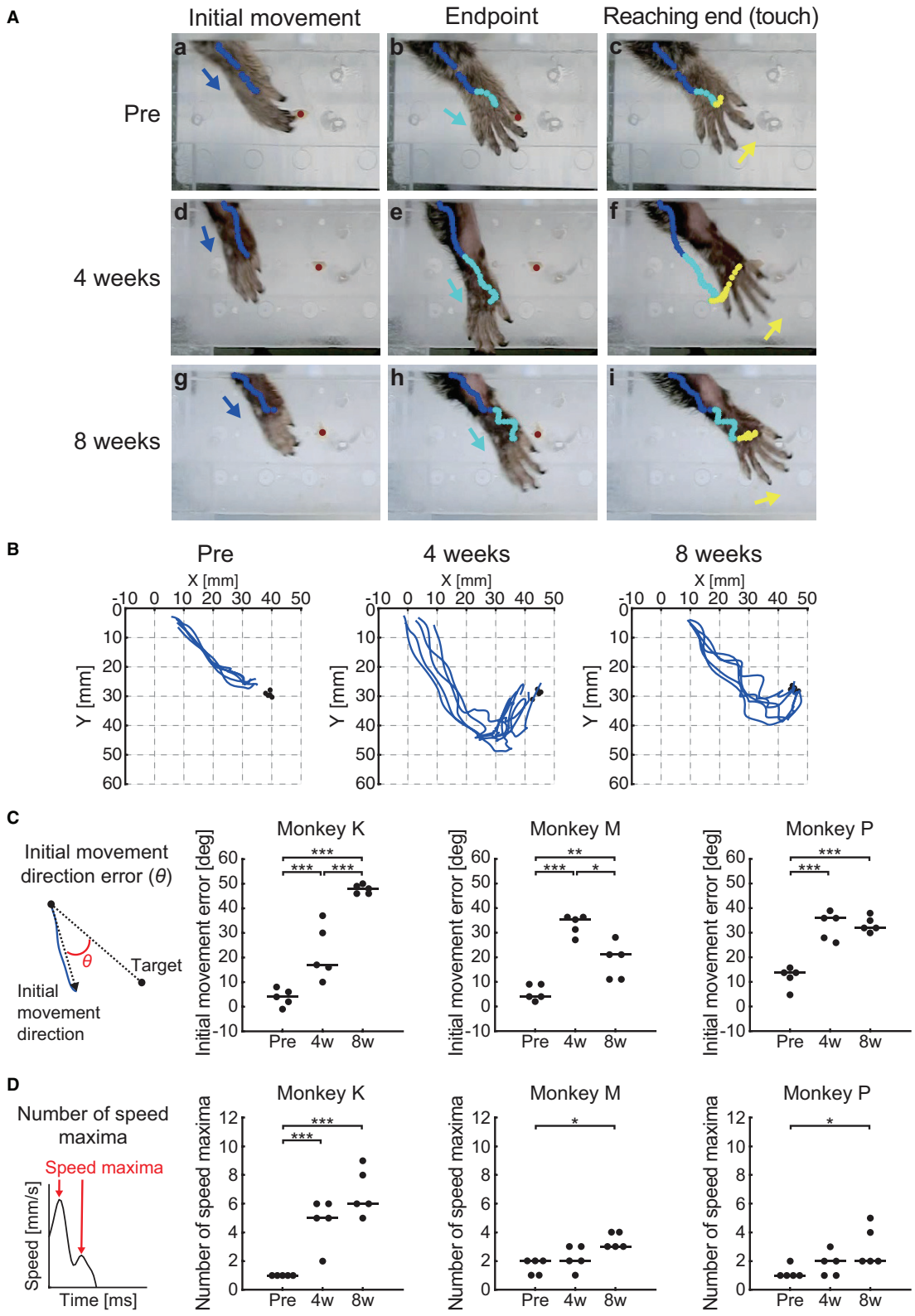

FIGURE 5
Behavioral results of reaching function. **(A)** Representative images of the reaching movement before, 4 weeks, and 8 weeks after creation of the lesion from one representative marmoset (Monkey M). Blue, cyan, and yellow lines indicate the trajectory of the index finger MP joint between movement phases (blue: from "Reaching start" to "Initial movement"; cyan: from "Initial movement" to "Endpoint"; yellow: from "Endpoint" to "Reaching end"). Blue, cyan, and yellow allows indicate movement direction between movement phases. Red circle indicates the position of the food pellet. **(B)** Typical example of reaching trajectories in the two-dimensional coordinates from one representative marmoset (Monkey M). Black circles indicate the positions at which food pellets were placed. **(C)** Time course of the transition in the initial movement direction error in each marmoset. Left inset indicates schema of the definition in the initial movement direction error, represented by θ. Black lines indicate median value of each time point. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. **(D)** Time course of the transition in the number of speed maxima in each marmoset. Left inset indicates schema of the definition in the number of speed maxima. Black lines indicate median value of each time point. *$p < 0.05$; ***$p < 0.001$.

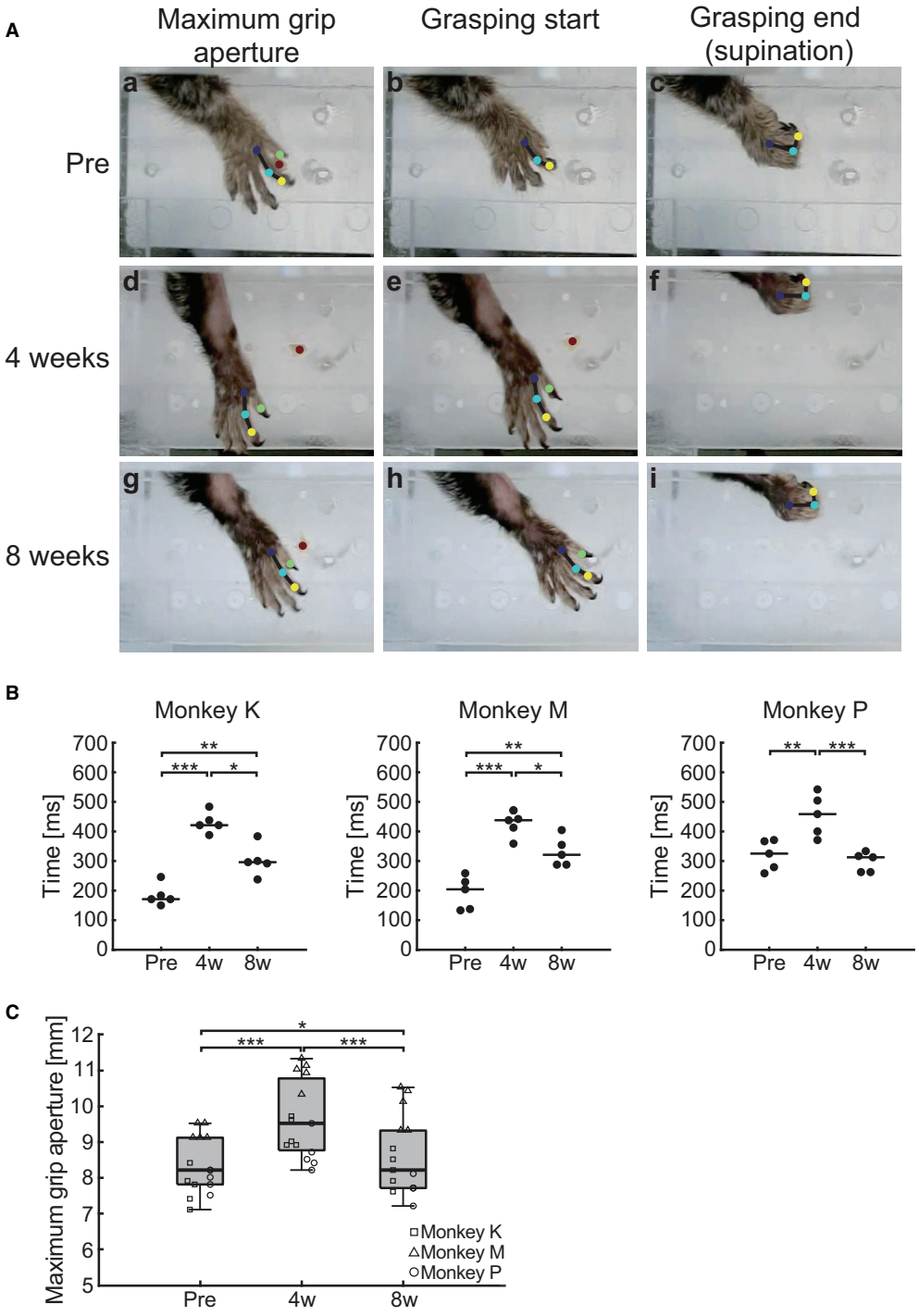

FIGURE 6
Behavioral results of grasping function. **(A)** Representative images of the grasping movement before, 4 weeks, and 8 weeks after creation of the lesion from one representative marmoset (Monkey M). Blue, cyan, yellow, and green circles indicate the position of the index finger MP, PIP, and DIP joint, and the thumb IP joint, respectively. Red circle indicates the position of the food pellet. **(B)** Time course of the transition in the grasping time in each marmoset. Black lines indicate median value of each time point. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. **(C)** Time course of the transition in the maximum grip aperture. Box plots indicate the median (black line in the box), interquartile range (IQR; gray box), and the lowest and highest data (error bars). *$p < 0.05$, ***$p < 0.001$.

number of speed maxima, grasping time, and maximum grip aperture. We found no differences between before and 2 weeks after creation of the lesion for initial movement direction error (Figure 7B; two-way ART-ANOVA, "time point" × "subject" interaction: $F_{(2,24)} = 2.03$, $p = 0.1526$; "time point" main effect: $F_{(1,24)} = 2.61$, $p = 0.1192$), number of speed maxima (Figure 7C; two-way ART-ANOVA, "time point" × "subject" interaction: $F_{(2,24)} = 0.02$, $p = 0.9793$; "time point" main effect: $F_{(1,24)} = 0.19$, $p = 0.6642$), grasping time (Figure 7D; two-way ART-ANOVA, "time point" × "subject" interaction: $F_{(2,24)} = 0.35$, $p = 0.7092$; "time point" main effect: $F_{(1,24)}$

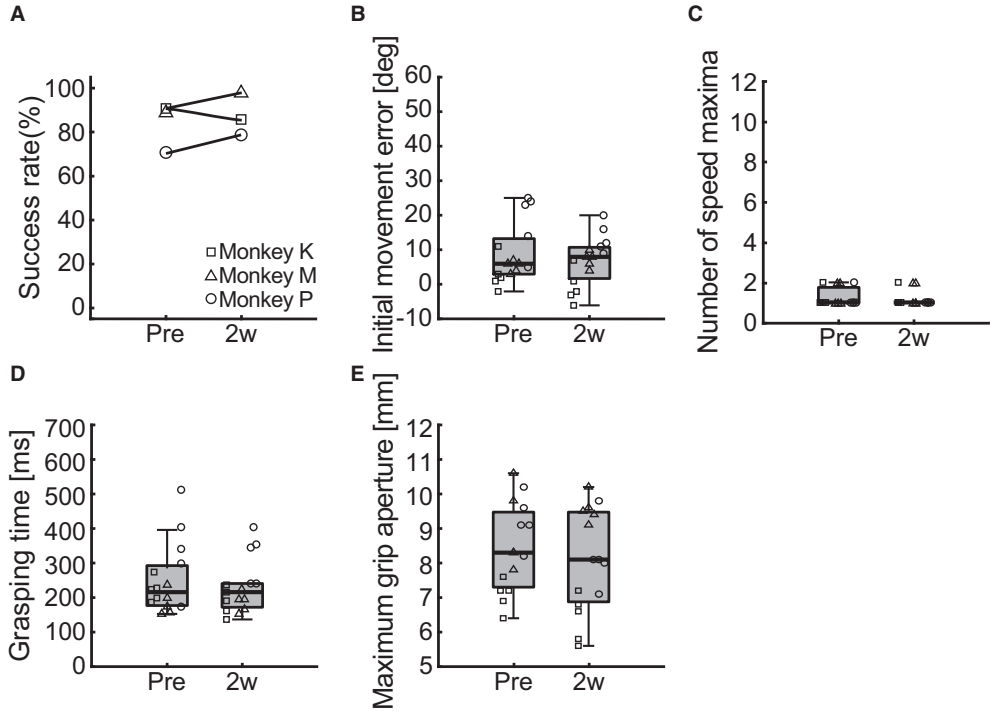

FIGURE 7
Behavioral results of the ipsilesional forelimb function. **(A)** Changes in the success rate of the pellet-reaching task. **(B–E)** Changes in the indices of reaching and grasping function including initial movement direction error **(B)**, number of speed maxima **(C)**, grasping time **(D)**, and maximum grip aperture **(E)**. Box plots indicate the median (black line in the box), IQR (gray box), and the lowest and highest data within 1.5 IQR of the lower and upper quartile (error bars), respectively.

$= 0.56$, $p = 0.4621$), or maximum grip aperture (Figure 7E; two-way ART-ANOVA, "time point" × "subject" interaction: $F_{(2,24)} = 2.39$, $p = 0.1132$; "time point" main effect: $F_{(1,24)} = 1.34$, $p = 0.2588$). These results indicated that the lesion only affected the forelimb function of the contralesional, but not the ipsilesional, side.

Discussion

Advantages of the present model

In this study, we used a photochemically induced cerebral infarction model. Other primate stroke models, such as occlusion of the middle cerebral (54) and anterior choroidal arteries (26), have variable ischemic lesions because of anatomical variations in the vascular architecture. Variability of lesions inevitably leads to variability in outcome measures, including lack of deficits, and highly reproducible lesions enable the prediction of motor deficits and lower variability of outcome measures (6). All the animals in the current study showed a lesion in motor-related cortical areas and exhibited more or less homogeneous motor impairment of reaching and grasping movements. Therefore, our animal model was more reliable and reproducible in terms of the motor disability. Moreover, most recovery of function was observed within 4 weeks of the lesion creation. For the time course of recovery, our model was comparable to human stroke patients, in whom the most dramatic recovery in motor function occurs during the first 30 days (55).

Although kinematic analysis to predict functional recovery after stroke has been used in human research (56), photothrombosis models in NHPs have never applied kinematic analysis to examine upper limb motor function (36–38). We found that the kinematic aspects of motor impairment were similar across humans and marmosets, as evaluated by kinematic indices commonly used for evaluating human stroke recovery (i.e., initial movement direction errors and the number of speed maxima). According to previous studies (57, 58), reaching movements can be broadly separated into two components: initiating movements (feed-forward control) and corrective movements (feedback control). The former is attributed to initial movement direction errors and the latter to the number of speed maxima. Therefore, we suggest that photochemically induced cerebral infarction is advantageous for reproducing the upper limb motor function impairment seen in human stroke survivors in an NHP model.

Initial movement direction errors

We observed an increase in initial movement direction errors after creation of the lesion. Previous human (45, 53, 59, 60) and macaque monkey (61) studies have shown comparable impairments in the initial phase of the reaching movement after stroke. Our results indicated that a lesion to the sensorimotor cortex causes impairment of the feed-forward control mechanism of upper limb movement.

We suggest that two potential mechanisms underlie cortically generated feed-forward malfunction. A lesion in the M1 may disrupt the cortical pathway involved in sensorimotor transformation, which is essential for reaching planning (62). Previous human and

animal studies have shown that a lesion or inactivation of the M1 disrupts not only motor execution but also sensorimotor planning; moreover, these disruptions are dissociable (62–65). This suggests that the M1 integrates somatosensory information about the limb and visual information about the target location to plan movement trajectories (63).

Another explanation is the dysfunction of the premotor cortex (PM). Our model showed a lesion in the PMd, in addition to the M1. In the PMd of marmosets, areas 6DC and 6DR correspond to areas F2 and F7 in macaques, respectively (52, 66, 67). Similar to the PM of macaques, area 6DC in the marmoset has strong connections to the M1 and is involved in the limb movements (52). Area 6DR is part of the parietofrontal network (52), which plays a role in visually guided reaching and grasping (21, 68). Previous marmoset studies have shown that impairment of the parietofrontal network without damage to M1 disrupts the feed-forward aspect of visually guided reaching and grasping (13, 19).

Number of speed maxima

We demonstrated that the speed maxima were also affected, which is in line with previous reports of human stroke patients (45, 53, 69–71). The number of speed maxima is considered an indirect measure of the efficiency of continuous corrective feedback control action to reach the target (60). Therefore, the increase in the number of speed maxima in the marmoset stroke model may be a clinically relevant outcome measure. The M1 has been proposed as a feedback controller (72, 73). The motor cortex receives somatosensory information from areas 3a, 3b, and 1/2 (51), and ongoing sensory input is used to refine and update descending motor commands (74, 75). In addition, a previous study of marmosets demonstrated strong motor–somatosensory cortical interactions during reaching (76) and suggests that damage to the M1 disrupts the updating of descending motor commands from sensory inputs. Although evidence has shown that sensory input is critical for motor execution, studies focusing on sensorimotor integration following stroke are limited (75). Our model raised interesting questions about the role of sensorimotor integration in motor recovery following stroke.

Difference between the recovery of reaching and grasping functions

Our result showed a more homogeneous and faster recovery of grasping than reaching function from 4 to 8 weeks after creation of the lesion in all animals. This result is consistent with previous reports on the recovery process of human stroke patients. Numerous measurements have been used to evaluate the recovery of motor function of the upper extremities in stroke survivors, and it is well established that the recovery process varies depending on the measurement (77–79). Among these, grip strength, a simple measure of power grip function, shows the fastest recovery of all the measures of grasping ability and can occur as early as 3 weeks (77). In contrast, the smoothness of trajectory for target-reaching recovers in 5 weeks (80); moreover, the index for accuracy at the endpoint of reaching, such as initial direction error, takes considerably longer (81).

Different recovery speeds for grasping and reaching in both species could be influenced by what extent the cortical control is required to properly execute each movement. Power grip requires the highly synergistic activity of multiple hand muscles (82). We previously reported that hand muscle synergy could be formed by the spinal interneurons (83). In line with this finding, it is known that activation of the sensorimotor cortex is less dominant during more synergistic power grip and more dominant during precision grip that requires individual finger control, in both human and NHPs (84–86). Therefore, power grip could be generated primarily by the contribution of the non-cortical area in the CNS. On the other hand, target reaching is highly dependent on cortical control, and that involves a widely distributed parietofrontal network (21, 68). In human stroke patients, for example, lesion in the parietofrontal cortex disrupts the target-reaching performance significantly (87, 88). Similarly, the experimental lesion on the comparable cortical area affects the target-reaching performance (17–19).

If the power grip could be generated primarily by the non-cortical area, then, it is reasonable to expect a faster recovery after stroke in the cortex. Because the function expected for the lesioned cortical area may be limited, it could be taken over by other areas in the CNS relatively easily. In contrast, because the lesioned cortical area had a significant contribution to the target reaching, it should take a longer period to be taken over, and thus, its slower recovery is also expected.

Limitations of the present model

One limitation of our model is the structural differences in the CNS between humans and marmosets. The CNS of marmoset is characterized by a lissencephalic brain (89, 90) and a lack of direct corticomotoneuronal projections to the motoneuron pools of distal hand muscles (91, 92) that are crucial for controlling independent finger movements (93, 94). Consequently, marmosets exhibit lower manual dexterity than humans (11, 76, 95). The corticospinal tract is more developed in humans than in marmosets; moreover, humans have greater cortical functional specialization (96), which may affect the time course of recovery of grasping movements. However, our marmoset model provides an advantage over rodents when assessing visually guided reaching movement that is impaired in human stroke patients.

A further technical consideration is related to the varied recovery time courses among animals for the success rate of the pellet-reaching task 8 weeks after creation of the lesion and initial movement direction errors. Specifically, Monkey M exhibited faster recovery for both measurements, which may be related to the extent of the lesion (the lesioned area of Monkey M was smaller than that of other animals; Table 1). As discussed earlier, reaching movements may be more sensitive to cortical lesions in marmosets. Although we controlled the infarction area using irradiation light, the difference in the extent of the lesion may have resulted in a difference in the time course of behavioral recovery.

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 animal study was reviewed and approved by Experimental Animal Committee of the National Institute of Neuroscience.

Author contributions

YS, TU, and KS designed the study. YS, MKu, MKo, and TU performed the study. AK and YS analyzed the data. AK and KS wrote the draft of the manuscript. All authors approved the final version of 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

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Prognostic role of dynamic neutrophil-to-lymphocyte ratio in acute ischemic stroke after reperfusion therapy: A meta-analysis

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Background: The prognostic role of the neutrophil-to-lymphocyte ratio (NLR), an inflammatory marker, in acute ischemic stroke (AIS) after reperfusion therapy remains controversial. Therefore, this meta-analysis sought to assess the correlation between the dynamic NLR and the clinical outcomes of patients with AIS after reperfusion therapy.

Methods: PubMed, Web of Science, and Embase databases were searched to identify relevant literature from their inception to 27 October 2022. The clinical outcomes of interest included poor functional outcome (PFO) at 3 months, symptomatic intracerebral hemorrhage (sICH), and 3-month mortality. The NLR on admission (pre-treatment) and post-treatment was collected. The PFO was defined as a modified Rankin scale (mRS) of >2.

Results: A total of 17,232 patients in 52 studies were included in the meta-analysis. The admission NLR was higher in the 3-month PFO (standardized mean difference [SMD] = 0.46, 95% confidence interval [CI] = 0.35–0.57), sICH (SMD = 0.57, 95% CI = 0.30–0.85), and mortality at 3 months (SMD = 0.60, 95% CI = 0.34–0.87). An elevated admission NLR was associated with an increased risk of 3-month PFO (odds ratio [OR] = 1.13, 95% CI = 1.09–1.17), sICH (OR = 1.11, 95% CI = 1.06–1.16), and mortality at 3 months (OR = 1.13, 95% CI = 1.07–1.20). The post-treatment NLR was significantly higher in the 3-month PFO (SMD = 0.80, 95% CI = 0.62–0.99), sICH (SMD = 1.54, 95% CI = 0.97–2.10), and mortality at 3 months (SMD = 1.00, 95% CI = 0.31–1.69). An elevated post-treatment NLR was significantly associated with an increased risk of 3-month PFO (OR = 1.25, 95% CI = 1.16–1.35), sICH (OR = 1.14, 95% CI = 1.01–1.29), and mortality at 3 months (OR = 1.28, 95% CI = 1.09–1.50).

Conclusion: The admission and post-treatment NLR can be used as cost-effective and easily available biomarkers to predict the 3-month PFO, sICH, and mortality at 3 months in patients with AIS treated with reperfusion therapy. The post-treatment NLR provides better predictive power than the admission NLR.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier: CRD42022366394.

KEYWORDS

acute ischemic stroke, neutrophil to lymphocyte ratio, endovascular therapy, reperfusion therapy, intravenous thrombolysis, prognostic

Introduction

Acute ischemic stroke (AIS) is one of the major causes of disability and death in the world (1). Reperfusion therapy after AIS, including intravenous thrombolysis (IVT) and endovascular treatment (EVT), has been shown to effectively improve neurologic outcomes in eligible patients with AIS (2, 3). Nevertheless, approximately 50% of patients remain disabled or die 3 months

after treatment (4). Age, infarct volume, hemorrhagic transformation, and baseline National Institutes of Health Stroke Scale (NIHSS) score are known major risk factors and predictors of adverse prognosis in patients with AIS (5, 6). However, the aforementioned risk factors as predictors of patient prognosis remain insufficient.

Recent studies have shown that inflammation plays an important role in stroke-induced injury, and elevated levels of inflammatory markers are associated with poor clinical outcomes (7–9). During the early stages of stroke, neutrophils accumulate in the ischemic area and release inflammatory mediators, leading to disruption of the blood–brain barrier (BBB), increased infarct volume, hemorrhagic transformation, and poor neurologic outcomes (8, 10). By contrast, lymphocytes as the brain's primary regulator may contribute to the repair of inflammatory damage as well as brain functional recovery (11). An increased infarct size and a worsening neurologic prognosis may be associated with the suppression of lymphocytes (8, 12). The neutrophil-to-lymphocyte ratio (NLR), a readily available serum biomarker for assessing the balance between neutrophils and lymphocytes, has been used to measure systemic inflammation (13, 14).

Previous retrospective cohort studies have shown that higher levels of admission or post-treatment NLR are associated with hemorrhagic transformation (HT) (15, 16), symptomatic intracerebral hemorrhage (sICH) (17–20), 3-month poor functional outcome (PFO) (17, 19, 21, 22), and mortality at 3 months (18, 19, 22) in patients with AIS treated with reperfusion therapy. Nevertheless, there is still no full understanding of the association between the dynamic NLR and clinical outcomes in patients with AIS treated with reperfusion therapy, owing to methodological limitations. In addition, several recent meta-analyses have also confirmed a link between the NLR and clinical outcomes in patients with AIS receiving reperfusion therapy (23–25). However, most of these reviews have certain limitations, such as the small number of included studies, inconsistent outcomes, different effect sizes, and different time points of NLR. Thus, we performed a meta-analysis to evaluate the association between the dynamic NLR and clinical outcomes in patients with AIS receiving reperfusion therapy.

Methods

This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The study protocol was registered with PROSPERO (number CRD42022366394).

Search strategy and study selection

PubMed, Embase, and Web of Science were searched from their inception to 27 October 2022. The language of publication was limited to English. The following search terms were used: ("stroke"[All Fields] OR "brain infarction"[All Fields] OR "cerebral infarction"[All Fields] OR "ischemic stroke"[All Fields] OR "acute ischemic stroke"[All Fields]) AND ("neutrophil lymphocyte ratio"[All Fields] OR "neutrophil-to-lymphocyte ratio"[All Fields] OR "NLR"[All Fields]) AND ("tissue plasminogen activator"[All Fields] OR "recombinant tissue plasminogen activator"[All Fields] OR "tPA"[All Fields] OR "t-PA"[All Fields] OR "rtPA"[All Fields] OR "rt-PA"[All Fields] OR "alteplase"[All Fields] OR "thrombolysis"[All Fields] OR "endovascular thrombectomy"[All Fields] OR "mechanical thrombectomy"[All Fields] OR "thrombectomy"[All Fields] OR "endovascular treatment"[All Fields] OR "endovascular therapy"[All Fields] OR "reperfusion therapy"[All Fields]). Two investigators (BW and FL) independently assessed the titles and abstracts of the records and excluded articles that did not meet the eligibility criteria. Subsequently, the reviewers assessed the full-text articles. In addition, we manually reviewed the reference lists and recent reviews to identify potentially relevant studies.

Eligible studies met the following inclusion criteria: (1) patients with AIS who received reperfusion therapy with IVT or EVT after the symptom onset; (2) assessed the relationship between NLR and 3-month PFO, sICH, or mortality at 3 months after reperfusion therapy; (3) PFO was defined as the modified Rankin scale (mRS) >2; (4) blood samples were collected on admission (pre-treatment) or post-treatment; (5) studies with sufficient data for calculating standardized mean difference (SMD) and/or odds ratio (OR) with corresponding 95% confidence interval (CI); and (6) full text was available. We excluded studies if they met any one of the following criteria: (1) studies focused on a specific population with inflammatory disorders, infectious diseases, or any other major illness (such as cancer); (2) articles in the format of abstract, letter, meta-analysis, review, comment, case report, or editorial; (3) cell or animal research; (4) designated outcome was unreported; and (5) duplicate publications. For duplicate reports, the study with the largest sample size was selected.

Data extraction and quality assessment

Two investigators (BW and FL) independently extracted the relevant data. The following information was extracted from each eligible study: first author, year of publication, country, study duration, study design, sample size, number of males, age, admission NIHSS score, treatment method, number and percentage of bridging therapy, blood collection time, study outcome, NLR cutoff, sICH definition, NOS scores, and whether the infection was excluded. If studies reported multiple post-treatment collection time points, we selected the time point closest to 24 h.

The methodological quality of the included studies was assessed using the Newcastle–Ottawa Scale (NOS) (26). The score ranges from 0 to 9, and studies with scores >7 are considered to be of high quality. Any disagreements regarding data extraction and quality assessment were resolved through consensus discussion.

Statistical analysis

The pooled OR and SMD with 95% CI were used to analyze the association between NLR and 3-month PFO, sICH, or mortality at 3 months after reperfusion therapy. If the study provided only the median, range, or interquartile range (IQR), the mean and standard deviation (SD) values were estimated using the methods

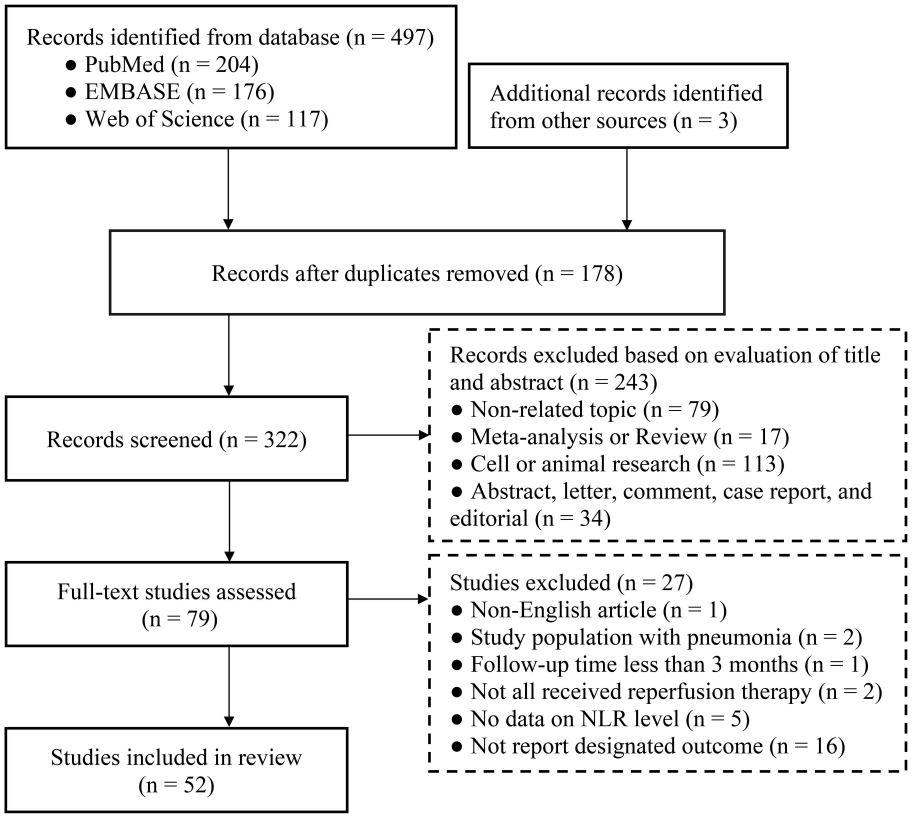

FIGURE 1
Flow diagram of the study search and selection process.

described by Luo et al. (27) and Wan et al. (28). When both adjusted and unadjusted OR were available, adjusted OR was used. The unadjusted OR was calculated when only count data were provided. The I^2 statistic and the chi-square test were used to assess statistical heterogeneity among studies (29). A *p*-value of the chi-square test <0.10 or $I^2 \geq 50\%$ was regarded as significant statistical heterogeneity. Considering the heterogeneity among the included studies, the random effects model (DerSimonian–Laird) was used to calculate the pooled effect sizes and the corresponding 95% CI. If sufficient studies were included (≥10) (30), subgroup analyses were conducted to explore the potential sources of heterogeneity according to the treatment method (EVT vs. IVT), study region (Asian vs. non-Asian), study design (prospective vs. retrospective), age (≥65 vs. <65 years), sample size (≥200 vs. <200), admission NIHSS score (≥15 vs. <15), bridging therapy (≥40 vs. <40%), OR (adjusted vs. unadjusted), NOS score (≥8 vs. <8), cutoff for NLR (yes vs. no), and infection excluded (yes vs. no vs. not reported). Sensitivity analyses were conducted to test the robustness of the results by excluding each study sequentially ($n \geq 10$). Egger's test and funnel plots were used to assess publication bias ($n \geq 10$) (31). An analysis of trim and fill was performed to further evaluate the potential existence of publication bias. An SMD of 0.2 was interpreted as reflecting small effects, 0.5 as reflecting medium effects, and 0.8 as reflecting large effects according to Cohen's rule of thumb (32). All tests were two-sided, and $p < 0.05$ were considered statistically significant. All statistical analyses were performed using Stata 17 software (Stata Corporation LP, College Station, TX, USA).

Results

Study selection and characteristics

A primary literature search identified 497 potentially relevant articles. Three additional records were obtained from other sources. After removing 178 duplicate publications, the titles and abstracts of 322 studies were reviewed. We excluded 243 studies based on title and abstract reviews. Next, the full text of the remaining 79 articles was reviewed. Finally, 52 articles with 17,232 patients were included in our analysis (15–22, 33–76). The article selection process is illustrated in Figure 1.

The characteristics of the included studies and the quality assessment results are presented in Table 1. The selected studies were published between 2013 and 2022. The study design included single-center ($n = 40$), dual-center ($n = 4$), multi-center ($n = 8$), retrospective cohort ($n = 48$), and prospective cohort studies ($n = 4$). This meta-analysis included 23 studies treating AIS with IVT, 28 studies treating AIS with EVT, and two studies treating AIS with IVT/EVT (including IVT alone, EVT alone, and combination therapy of IVT and EVT). The sample sizes of the included studies ranged from 51 to 1,227 participants. The mean and/or median ages of the participants ranged from 58 to 75 years. The mean and/or median admission NIHSS score ranged from 4 to 27 points. Three IVT studies and 26 EVT studies reported percentages of bridging therapy ranging from 6.6% to 30.7% and 7.5% to 77.6%, respectively. Infection was excluded in 24 studies, not excluded in 10 studies, and was not

TABLE 1 Basic characteristics of the studies included in the meta-analysis.

ID	References	Country	Duration	Design	Sample size (male)	Age (year)	Admission NIHSS	Treatment	Bridging therapy, n (%)	Blood; collection; time	Study; outcome	NRL cut-off	sICH; definition	Infection excluded	NOS; scores
1	Brooks et al. (21)	USA	2008–2011	R (S)	116 (NR)	67 (18–93)	17 (1–48)	EVT	27 (23.3)	Admission	(1) PFO; (2) Mortality	5.9	NR	No	7
2	Maestrini et al. (17)	France; Finland	NR	P (D)	846 (430)	71 (60–80)	10 (6–16)	IVT	56 (6.6)	Before IVT	(1) PFO; (2) sICH; (3) Mortality	4.8	ECASS II	No	9
3	Pagram et al. (33)	Australia	2009–2013	R (S)	141 (NR)	74.3 ± 10.7	10.1 ± 4.7	IVT	NP	Before IVT; after IVT	PFO	NR	NR	NR	8
4	Guo et al. (15)	China	2012–2015	R (D)	189 (123)	65.0 ± 10.6	12 (6–16)	IVT	58 (30.7)	Admission; after IVT	sICH	NR	ECASS II	Yes	9
5	Inanc et al. (34)	Turkey	2014–2015	R (S)	56 (35)	58.23 ± 11.94	16.09 ± 3.33	EVT	NR	Admission	(1) sICH; (2) Mortality	NR	NR	Yes	6
6	Goyal et al. (18)	USA	2012–2016	R (S)	293 (147)	62 ± 14	16 (13–19)	EVT	240 (70.0)	Admission	(1) PFO; (2) sICH; (3) Mortality	NR	SITS-MOST	NR	8
7	Wang et al. (35)	China	2014–2016	R (M)	199 (128)	64 (55–72)	16 (13–21)	EVT	85 (42.7)	Admission	PFO	NR	HBC	Yes	7
8	Duan et al. (36)	China	2014–2016	R (M)	616 (368)	66 (57–74)	16 (12–21)	EVT	216 (35.1)	Before EVT	(1) PFO; (2) sICH; (3) Mortality	7	HBC	Yes	8
9	Malhotra et al. (37)	USA	2011–2015	R (D)	657 (333)	64 ± 14	7 (4–13)	IVT	52 (7.9)	Before IVT	(1) PFO; (2) sICH; (3) Mortality	2.2	SITS-MOST	No	9
10	Shi et al. (38)	China	2009–2016	P (S)	372 (242)	64	10.9 ± 6.8	IVT	NP	Before IVT; after IVT	(1) PFO; (2) Mortality	NR	ECASS II	Yes	8
11	Semerano et al. (39)	Spain	2008–2017	R (M)	433 (266)	71 (61–80)	17 (11–21)	EVT	213 (49.0)	Before EVT; after EVT	(1) PFO; (2) sICH; (3) Mortality	NR	ECASS II	No	7
12	Pektezel et al. (40)	Turkey	2009–2018	R (S)	142 (62)	69 ± 13	13.9 ± 5.5	IVT	NP	Before IVT; after IVT	(1) PFO; (2) sICH	NR	ECASS II	No	6
13	Li et al. (41)	China	2017–2019	R (S)	156 (107)	64.43 ± 12.60	13 (11–17)	EVT	66 (42.31)	Admission	sICH	NR	ECASS III	NR	7
14	Ying et al. (42)	China	2016–2018	R (S)	208 (129)	67.3 ± 12.4	NR	IVT	NP	Admission; after IVT	(1) PFO; (2) sICH	NR	ECASS II	Yes	8
15	Meng et al. (43)	China	2015–2019	P (S)	302 (171)	69 ± 11	14.4 ± 4.6	EVT	115 (38.1)	Admission	(1) PFO; (2) Mortality	6.45	HBC	No	8
16	Lv et al. (44)	China	2016–2019	R (S)	564 (409)	63.0 (54.0–70.8)	8 (5–12)	IVT	NP	Before IVT	PFO	NR	NR	NR	7
17	Oh et al. (45)	Korea	2014–2019	R (S)	411 (222)	69.2 ± 13.4	10.4 ± 6.6	EVT	152 (37.0)	Before EVT	(1) PFO; (2) sICH; (3) Mortality	5.1	ECASS III	Yes	8

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TABLE 1 (Continued)

ID	References	Country	Duration	Design	Sample size (male)	Age (year)	Admission NIHSS	Treatment	Bridging therapy, n (%)	Blood; collection; time	Study; outcome	NRL cut-off	sICH; definition	Infection excluded	NOS; scores
18	Cheng et al. (46)	China	2016–2019	R (S)	381 (253)	68 (59–76)	7.0 (3.5–11.0)	IVT	NP	after IVT	PFO	NR	NR	Yes	7
19	Liu et al. (47)	China	2016–2019	R (S)	192 (138)	60.8 ± 11.7	5.0 (3.0–6.8)	IVT	NP	Before IVT	PFO	3.9	NR	Yes	7
20	Switońska et al. (48)	Poland	2017–2018	R (S)	51 (22)	67 (55–78)	11 (6–16)	IVT/EVT	17 (33.0)	Admission	sICH	NR	ECASS II	Yes	6
21	Ozgen et al. (49)	Turkey	2017–2018	P (S)	150 (83)	NR	NR	EVT	NP	Admission	(1) PFO; (2) Mortality	NR	NR	Yes	7
22	Pan et al. (50)	China	2016–2018	R (S)	151 (97)	68 (59–74)	9 (6–14)	IVT	NP	Admission; after IVT	PFO	NR	NR	Yes	7
23	Lux et al. (51)	UK	2016–2017	R (S)	121 (58)	66.4 ± 16.7	19 (1–28)	EVT	94 (77.6)	Admission; after EVT	PFO	NR	ECASS I	Yes	8
24	Aly et al. (19)	USA	2015–2019	R (S)	142 (69)	70 ± 16	17 (12–21)	EVT	70 (49.0)	Admission; after EVT	(1) PFO; (2) sICH; (3) Mortality	NR	ECASS III	Yes	7
25	Hu et al. (52)	China	2014–2019	R (S)	183 (123)	64.9 ± 10.5	4 (3–7)	IVT	NP	after IVT	PFO	NR	NR	Yes	8
26	Topcuoglu et al. (53)	Turkey	2011–2019	R (S)	165 (96)	70 ± 14	13 ± 5.6	IVT	NP	Before IVT; after IVT	(1) PFO; (2) sICH	NR	ECASS I	Yes	6
27	Majid et al. (54)	Pakistan	2015–2019	R (S)	98 (60)	58.0 ± 6.4	15.9 ± 7.7	IVT	NP	Admission	PFO	2.39	NR	Yes	6
28	Chen et al. (55)	China	2015–2019	R (S)	257 (186)	63.2 ± 12.6	NR	EVT	115 (44.7)	Admission	(1) PFO; (2) sICH	NR	ECASS II	Yes	8
29	Ören et al. (20)	Turkey	2016–2018	R (S)	133 (81)	66.56 ± 12.47	13 (10–17)	IVT	NR	Admission	sICH	NR	ECASS III	Yes	8
30	Guo et al. (56)	China	2014–2020	R (M)	1200 (756)	66.9 ± 12.5	NR	IVT	NP	Admission	sICH	NR	ECASS II	Yes	9
31	Yu et al. (57)	China	2018–2020	R (S)	102 (54)	66.9 ± 13.89	13.5 (9.75–17.00)	EVT	33 (32.3)	Admission	sICH	NR	HBC	NR	7
32	Weng et al. (58)	China	2016–2019	R (S)	291 (104)	67 (59–79)	8 (5–13)	IVT	NP	Admission; after IVT	Mortality	NR	NR	NR	7
33	Ferro et al. (59)	Portugal	2017–2019	R (S)	325 (161)	75 (66–83)	14 (8–19)	IVT/EVT	87 (26.7)	After IVT (EVT)	PFO	NR	NR	No	8
34	Yi et al. (60)	Korea	2015–2020	R (S)	440 (260)	70.2 ± 12.9	NR	EVT	159 (36.1)	Admission	PFO	3.7	ECASS III	Yes	7
35	Chen et al. (61)	China	2016–2019	R (S)	280 (179)	69 (59–77)	NR	IVT	NP	Admission	(1) PFO; (2) Mortality	NR	NR	NR	7
36	Xie et al. (62)	China	2014–2020	R (S)	462 (318)	63.3 ± 12.5	NR	IVT	NP	Before IVT	sICH	NR	ECASS II	NR	8
37	Yang et al. (63)	China	2016–2020	R (D)	623 (403)	67.36 ± 12.84	6 (4–10)	IVT	NP	Admission; after IVT	(1) PFO; (2) Mortality	NR	NR	Yes	6
38	Shi et al. (64)	China	2015–2017	R (S)	127 (65)	70.95 ± 12.24	20 (16–25)	EVT	53 (41.7)	Admission; after EVT	PFO	NR	HBC	NR	7
39	Gao et al. (65)	China	2016–2019	R (S)	283 (180)	NR	NR	IVT	NP	Before IVT	PFO	NR	NR	NR	7

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TABLE 1 (Continued)

ID	References	Country	Duration	Design	Sample size (male)	Age (year)	Admission NIHSS	Treatment	Bridging therapy, n (%)	Blood; collection; time	Study; outcome	NRL cut-off	sICH; definition	Infection excluded	NOS; scores
40	Li et al. (66)	China	2018–2020	R (S)	286 (167)	70 (63–77)	18 (12–30)	EVT	41 (14.3)	Before EVT	PFO	NR	ECASS II	NR	8
41	Lee et al. (67)	Korea	2015–2011	R (M)	282 (162)	69.5 ± 13.4	NR	EVT	148 (52.5)	Before EVT	PFO	NR	NR	NR	6
42	Sun et al. (68)	China	2017–2019	R (M)	147 (75)	67 (59–75)	16 (11–19)	EVT	302 (29.3)	Admission	PFO	4.1	HBC	NR	8
43	Zou et al. (69)	China	2017–2020	R (S)	160 (101)	70 (64–76)	15 (11–20)	EVT	12 (7.5)	After EVT	PFO	9.75	HBC	Yes	6
44	Weyland et al. (70)	Germany	2014–2019	R (S)	549 (273)	74.3 ± 12.6	NR	EVT	307 (55.9)	Admission	PFO	NR	NR	NR	8
45	Liao et al. (22)	China	2014–2019	R (M)	586 (443)	64 (56–73)	27 (17–33)	EVT	109 (18.6)	Admission	(1) PFO; (2) Mortality	NR	HBC	No	8
46*	Chen et al. (71)	China	2018–2020	R (S)	576 (379); 351 (249)	68 (59–76); 69 (60–76)	5 (3–10); 14 (11–19)	IVT; EVT	NP; 88 (25.1)	Before IVT/EVT; after IVT/EVT	(1) PFO; (2) sICH; (3) Mortality	NR	ECASS II	No	8
47	Shen et al. (72)	China	2019–2020	R (S)	369 (250)	66 (57–74)	15 (12–19)	EVT	80 (21.7)	Admission	sICH	5.48	HBC	NR	7
48	Sun et al. (73)	China	2017–2019	R (M)	1227 (776)	66 (56–73)	16 (12–20)	EVT	357 (29.1)	Admission	sICH	NR	ECASS II	NR	7
49	Sadeghi et al. (74)	Hungary	2016–2018	R (S)	285 (159)	66 ± 12.9	6.0 (5.0–9.1)	IVT	NP	Before IVT; after IVT	sICH	NR	ECASS II	NR	6
50	Kim et al. (75)	Korea	2013–2019	R (S)	128 (67)	68.9 ± 13.2	17 (13–21)	EVT	50 (39.1)	Before EVT; after EVT	sICH	NR	SITS-MOST	Yes	7
51	Li et al. (16)	China	2015–2021	R (S)	258 (156)	70 s(61–79)	NR	EVT	88 (34.1)	Before EVT; after EVT	PFO	NR	ECASS III	NR	8
52	Feng et al. (76)	China	2019–2021	R (S)	170 (115)	66.0 (58.8–74.3)	15 (12–19)	EVT	62 (36.5)	Admission; after EVT	PFO	NR	NR	No	7

NOS, Newcastle–Ottawa Scale; NIHSS, National Institute of Health Stroke Scale; sICH, symptomatic intracranial hemorrhage; PFO, poor functional outcome; ECASS, European Cooperative Acute Stroke Studies; SITS-MOST, Safe Implementation of Thrombolysis in Stroke-Monitoring Study; HBC, Heidelberg Bleeding Classification; R, retrospective cohort; P, prospective cohort; S, single-center; B, dual-center; M, multi-center; NP, bridging therapy not performed; NR, not reported; IVT, intravenous thrombolysis; EVT, endovascular treatment; NRL, neutrophil-to-lymphocyte ratio.

*This study reported separately on the IVT and EVT groups.

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reported in 18 studies. A total of 23 studies were considered high quality and reached a score of 8–9 points according to the NOS score.

Relationship between admission NLR and 3-month PFO

A total of 26 studies including 8,474 patients were used for the pooled OR analysis. Higher admission NLR levels were associated with an increased risk of 3-month PFO (OR = 1.13, 95% CI = 1.09–1.17, I^2 = 75.0%) (Figure 2A). The summary effect sizes in the IVT (OR = 1.17, 95% CI = 1.07–1.27) and EVT (OR = 1.11, 95% CI = 1.07–1.16) groups remained significant. Subgroup analyses indicated no evidence of heterogeneity among groups (Supplementary Table 1). A possible publication bias was detected by the visual inspection of the funnel plot (Figure 5A) and Egger's test ($P < 0.001$). The result remained significant for the association between admission NLR levels and PFO (OR = 1.10, 95% CI = 1.04–1.14) after the trim-and-fill analysis imputed 10 theoretical missing studies. Moreover, we performed sensitivity analyses, and the cumulative results remained steady after sequentially excluding each study (Figure 6A).

A total of 26 studies including 7,743 patients were used for the pooled SMD analysis. Patients with PFO had higher levels of

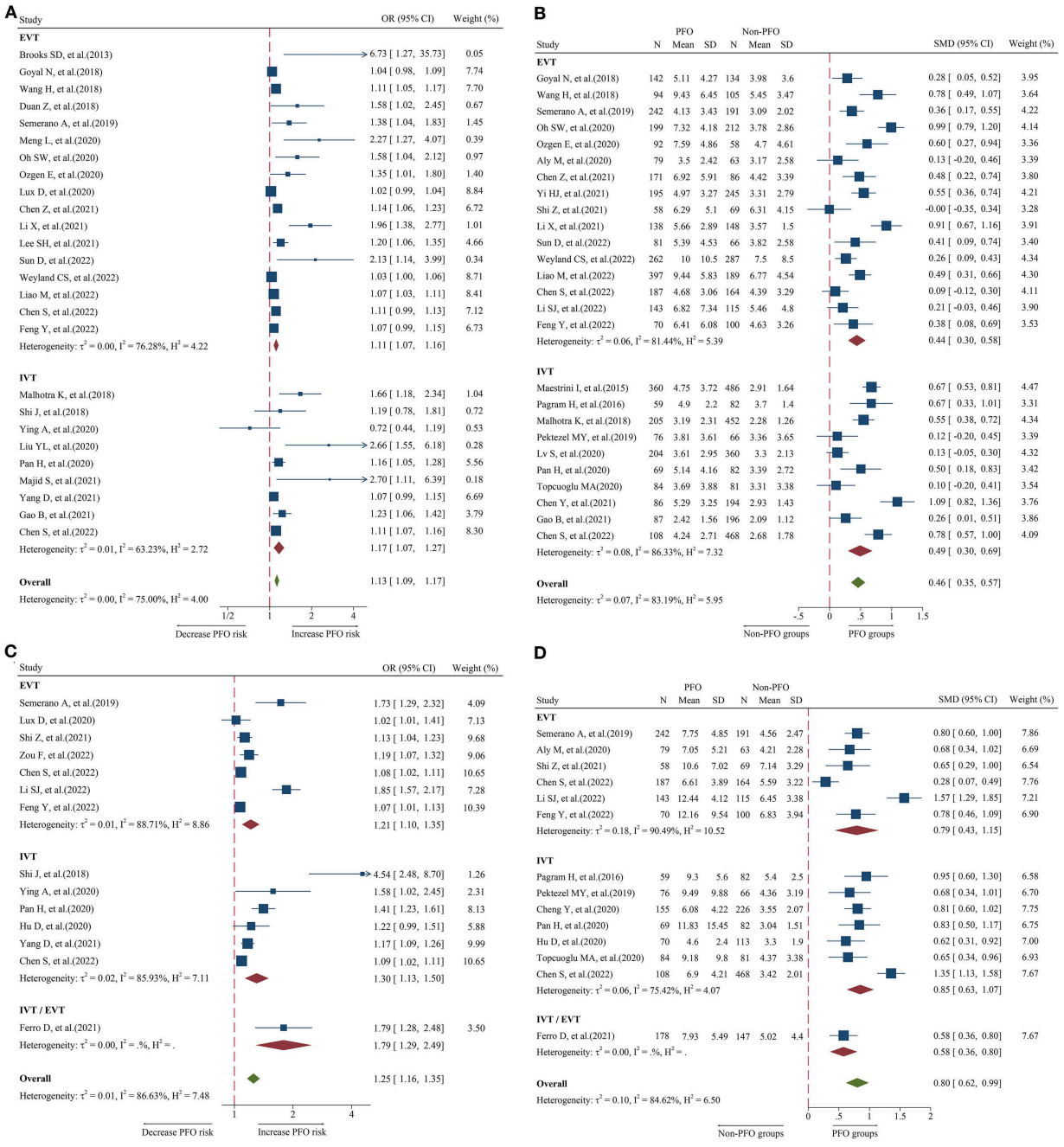

FIGURE 2
Forest plot showing the association of NLR and 3-month PFO; **(A)** forest plot of admission NLR based on OR; **(B)** forest plot of admission NLR based on SMD; **(C)** forest plot of post-treatment NLR based on OR; and **(D)** forest plot of post-treatment NLR based on SMD.

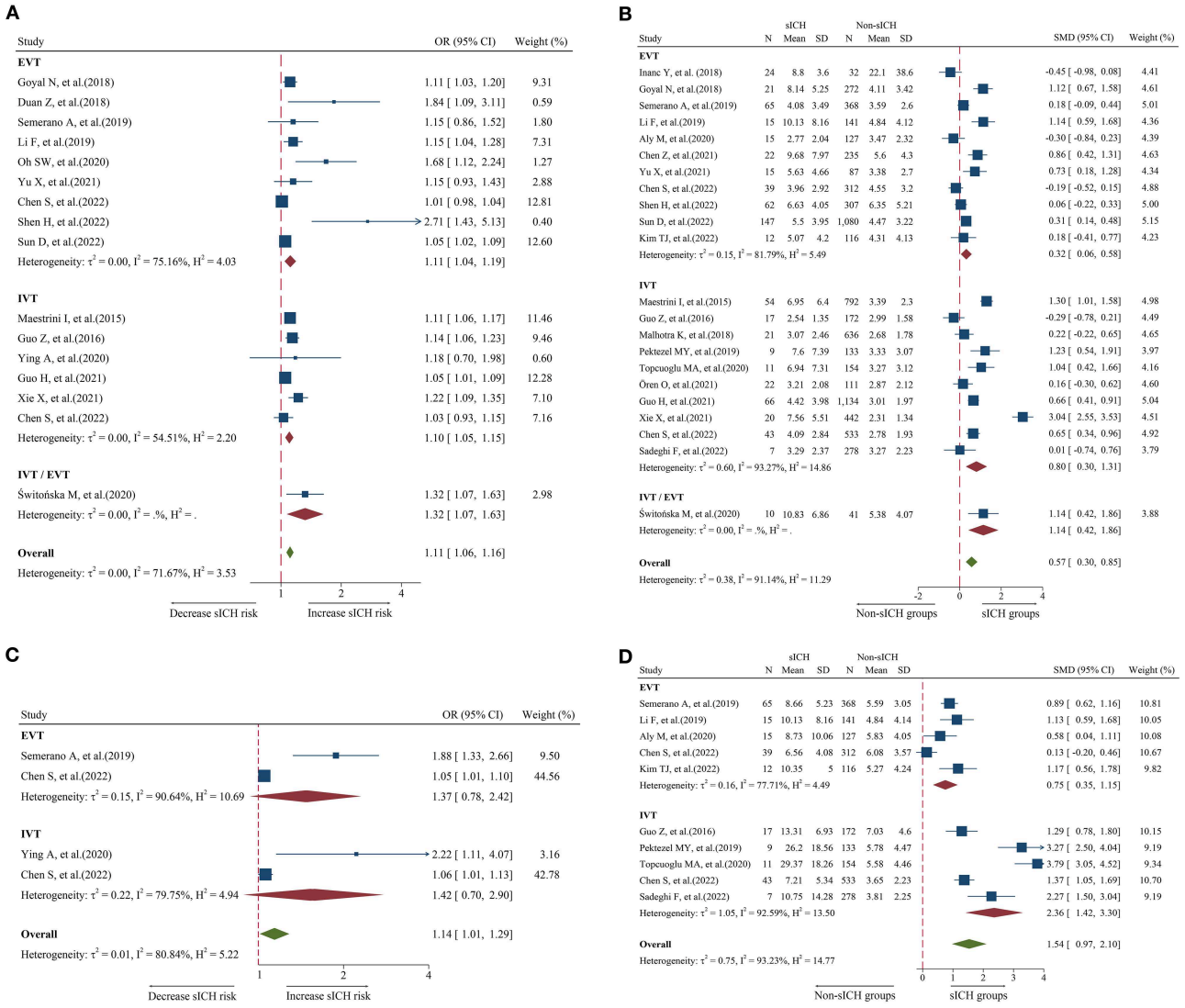

FIGURE 3
Forest plot showing the association of NLR and sICH; **(A)** forest plot of admission NLR based on OR; **(B)** Forest plot of admission NLR based on SMD; **(C)** Forest plot of post-treatment NLR based on OR; and **(D)** forest plot of post-treatment NLR based on SMD.

admission NLR than patients without PFO (SMD = 0.46, 95% CI = 0.35–0.57, I^2 = 83.1%) (Figure 2B). Similar results were observed in IVT (SMD = 0.49, 95% CI = 0.30–0.69) and EVT patients (SMD = 0.44, 95% CI = 0.30–0.58). We conducted several subgroup analyses and found no evidence of heterogeneity (Supplementary Table 2). The visual inspection of the funnel plot (Figure 5B) and Egger's test (P = 0.48) showed no evidence of publication bias. No significant change was observed in the pooled SMD after excluding each study (Figure 6B).

Relationship between post-treatment NLR and 3-month PFO

A total of 14 studies including 3,686 patients were used for the pooled OR analysis. Higher post-treatment NLR levels were associated with an increased risk of PFO (OR = 1.25, 95% CI = 1.16–1.35, I^2 = 86.6%) (Figure 2C). The relationship remained significant in the IVT (OR = 1.30, 95% CI = 1.13–1.50) and EVT groups (OR = 1.21, 95% CI = 1.10–1.35). A significant result was also obtained in one study that included IVT/EVT (OR = 1.79, 95% CI = 1.29–2.49). No source of heterogeneity was found in the subgroup analyses (Supplementary Table 3). According to the funnel plot (Figure 5C) and Egger's test ($P < 0.001$), there was a potential publication bias. After trimming and filling in four theoretically missing studies, the relationship between post-treatment NLR levels and 3-month PFO remained significant (OR = 1.15, 95% CI = 1.06–1.25). In the sensitivity analyses, the pooled OR was not significantly affected by excluding individual studies (Figure 6C).

A total of 14 studies including 3,380 patients were used for pooled SMD analysis. Post-treatment NLR levels were higher in patients with PFO than in those without PFO (SMD = 0.80, 95% CI = 0.62–0.99, I^2 = 84.6%) (Figure 2D). Similar results were also achieved in the IVT (SMD = 0.85, 95% CI = 0.63–1.07) and EVT groups (SMD = 0.79, 95% CI = 0.43–1.15). IVT/EVT was included in only one study (SMD = 0.58, 95% CI = 0.36–0.80), and the result was also

significant. According to the results of subgroup analyses, no source of heterogeneity was found (Supplementary Table 4). No substantial publication bias was found, according to the funnel plot (Figure 5D) and Egger's test ($P = 0.96$). In the sensitivity analyses, the results implied that no studies had a significant effect on the pooled SMD (Figure 6D).

Relationship between admission NLR and sICH

A total of 16 studies including 6,977 patients were used for the pooled OR analysis. Higher admission NLR levels were associated with an increased risk of sICH (OR = 1.11, 95% CI = 1.06–1.16, I^2 = 71.6%) (Figure 3A). Compared with the main analysis, the results of the IVT (OR = 1.10, 95% CI = 1.05–1.15) and EVT (OR = 1.11, 95% CI = 1.04–1.19) groups were generally consistent. Only one study included IVT/EVT (OR = 1.32, 95% CI = 1.07–1.63), and the result was also significant. No source of heterogeneity was found in subgroup analyses (Supplementary Table 5). Potential publication bias was detected by a visual inspection of the funnel plot (Figure 5E) and Egger's test ($P < 0.001$). There was still a significant relationship between admission NLR and sICH after trimming and filling in seven theoretically missing studies (OR = 1.08, 95% CI = 1.03–1.12). According to the results of the sensitivity analyses, no study had a significant effect on the pooled OR (Figure 6E).

A total of 22 studies including 8,055 patients were used for the pooled SMD analysis. The result suggested a difference between sICH and non-sICH groups (SMD = 0.57, 95% CI = 0.30–0.85, I^2 = 91.1%) (Figure 3B). The results of the IVT (SMD = 0.80, 95% CI = 0.30–1.31) and EVT (SMD = 0.32, 95% CI = 0.06–0.58) groups were generally in accordance with those of the main analysis. Only one study included IVT/EVT, and the result was significant (SMD = 1.14, 95% CI = 0.42–1.86). We performed several subgroup analyses and found no evidence of heterogeneity (Supplementary Table 6). Egger's test ($P = 0.53$) and a visual examination of the funnel plot (Figure 5F) indicated no evidence of publication bias. According to the results of the sensitivity analyses, none of the studies greatly impacted the pooled SMD (Figure 6F).

Relationship between post-treatment NLR and sICH

A total of four studies including 1,568 patients were used for the pooled OR analysis. There was an association between higher post-treatment NLR levels and a higher risk of sICH (OR = 1.14, 95% CI = 1.01–1.29, I^2 = 80.8%) (Figure 3C). However, these findings were not replicated in the IVT (OR = 1.42, 95% CI = 0.70–2.90) and EVT groups (OR = 1.37, 95% CI = 0.78–2.42). Publication bias, sensitivity, and subgroup analyses were not performed because of the small number of studies.

A total of 10 studies including 2,402 patients were used for the pooled SMD analysis. Post-treatment NLR was higher in patients with sICH than in those without sICH (SMD = 1.54, 95% CI = 0.97–2.10, I^2 = 90.9%) (Figure 3D). Meanwhile, the results were consistent with the earlier findings in the IVT (SMD = 2.36, 95% CI = 1.42–3.30) and EVT groups (SMD = 0.75, 95% CI = 0.35–1.15). There was no evidence of heterogeneity among subgroup analyses (Supplementary Table 7). Egger's test ($P = 0.005$) and a visual examination of the funnel plot (Figure 5G) indicated evidence of publication bias. The meta-analysis results did not change after adjusting for publication bias using the trim-and-fill method. The results of the sensitivity analyses showed that none of the studies had a significant effect on the pooled SMD (Figure 6G).

Relationship between admission NLR and 3-month mortality

A total of 14 studies including 6,473 patients were used for the pooled OR analysis. Higher admission NLR levels were associated with an increased risk of mortality (OR = 1.13, 95% CI = 1.07–1.20, I^2 = 79.0%) (Figure 4A). These findings were confirmed in the IVT (OR = 1.12, 95% CI = 1.04–1.20) and EVT groups (OR = 1.17, 95% CI = 1.06–1.29). Several subgroup analyses were conducted; however, no source of heterogeneity was found (Supplementary Table 8). There was possible publication bias according to the funnel plot (Figure 5H) and Egger's test ($P < 0.001$). After trimming and filling in three theoretically missing studies, admission NLR remained significantly related to mortality (OR = 1.11, 95% CI = 1.04–1.86). After excluding each study, the pooled OR did not change significantly (Figure 6H).

A total of 10 studies including 3,784 patients were used for the pooled SMD analysis. Admission NLR levels were higher in patients with mortality (SMD = 0.60, 95% CI = 0.34–0.87, I^2 = 86.7%) than in those without mortality (Figure 4B). The results remained significant in IVT (SMD = 0.91, 95% CI = 0.40–1.42) and EVT groups (SMD = 0.40, 95% CI = 0.22–0.57). No cause of heterogeneity was found in the subgroup analyses (Supplementary Table 9). The visual inspection of the funnel plot (Figure 5I) and Egger's test ($P = 0.56$) showed no potential publication bias. In the sensitivity analyses, no studies significantly impacted the pooled SMD (Figure 6I).

Relationship between post-treatment NLR and 3-month mortality

A total of six studies including 2,274 patients were used for the pooled OR analysis. Higher post-treatment NLR levels were associated with an increased risk of mortality (OR = 1.28, 95% CI = 1.09–1.50, I^2 = 91.7%) (Figure 4C). This finding remained significant in the IVT group (OR = 1.27, 95% CI = 1.05–1.54). However, this relationship was not significant in the EVT group (OR = 1.42, 95% CI = 0.66–3.04). Publication bias, subgroup, and sensitivity analyses were not performed because our analysis included <10 studies.

A total of four studies including 1,052 patients were used for the pooled SMD analysis. Post-treatment NLR levels were higher in patients with mortality (SMD = 1.00, 95% CI = 0.31–1.69, I^2 = 95.4%) than in patients without mortality (Figure 4D). The results did not change in the IVT (SMD = 2.03, 95% CI = 1.67–2.40) and EVT (SMD = 0.67, 95% CI = 0.12–1.22) groups. The number of studies was too small to conduct subgroup analyses, sensitivity analyses, and publication bias.

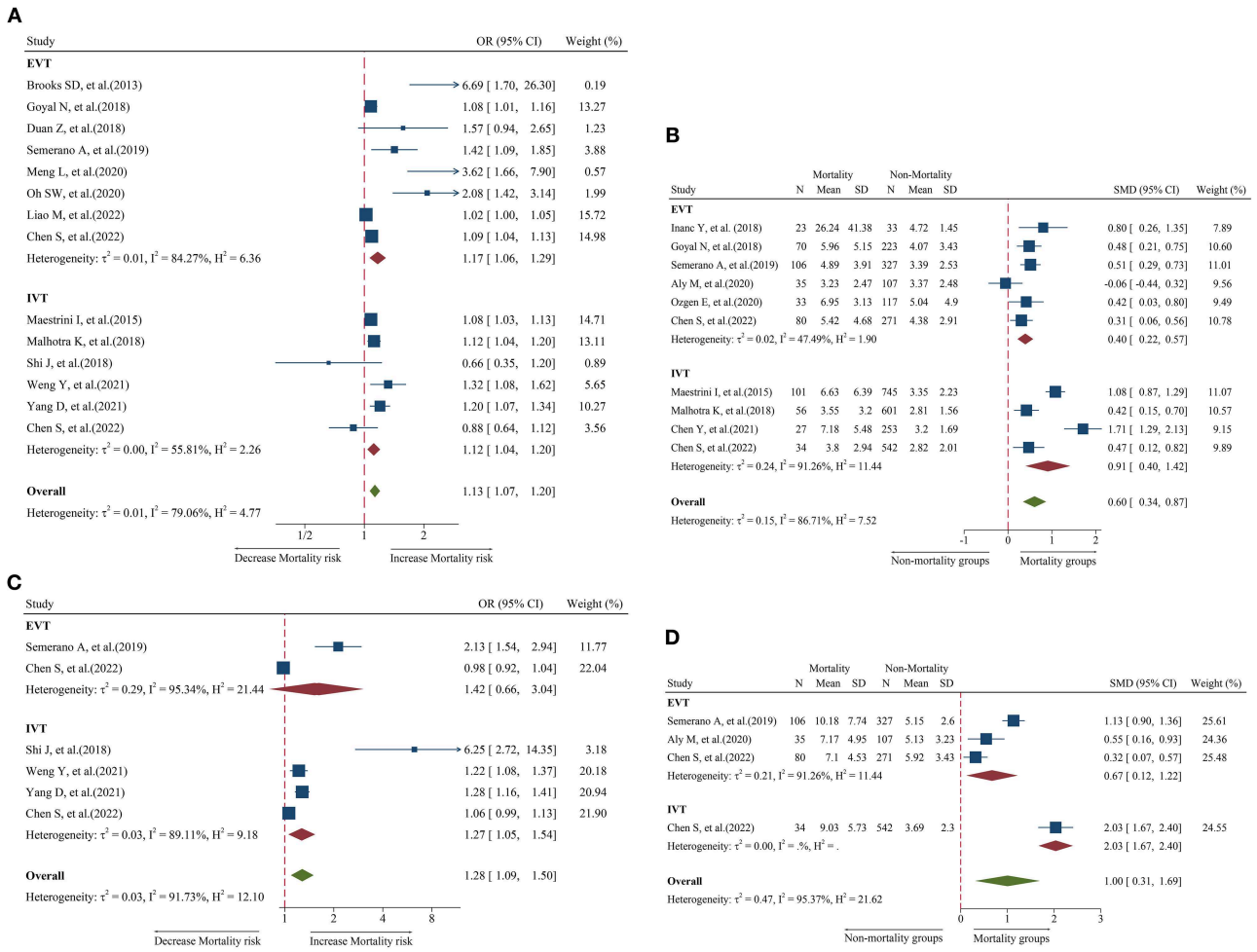

FIGURE 4
Forest plot showing the association of NLR and 3-month mortality; **(A)** forest plot of admission NLR based on OR; **(B)** forest plot of admission NLR based on SMD; **(C)** forest plot of post-treatment NLR based on OR; and **(D)** forest plot of post-treatment NLR based on SMD.

Discussion

This meta-analysis included 52 recent clinical studies with large sample sizes to investigate the association between the dynamic NLR and PFO at 3 months, sICH, and 3-month mortality in AIS after reperfusion therapy. We reported the results of both primary and secondary outcomes with effect sizes (OR or SMD) and 95% CIs. The results suggested that the higher levels of both admission and post-treatment NLR were associated with an increased risk of 3-month PFO, sICH, and mortality at 3 months, according to the pooled OR. Pooled SMD results showed that both admission and post-treatment NLR levels were higher in the PFO, sICH, and mortality groups than in the control group. Notably, post-treatment NLR showed better predictive capabilities for poor clinical outcomes of patients with AIS treated with reperfusion therapy than admission.

The role and mechanism of inflammation in the pathophysiology of AIS have been extensively studied (8). Ischemia and reperfusion damage can cause a marked inflammatory response, further increasing brain injury (8, 77). Recanalization treatment also leads to ischemia and reperfusion injury, which exacerbates acute brain injury and results in poor functional outcomes (77). After ischemic stroke, neutrophils migrate into cerebral ischemic regions within the first few hours after the onset of ischemia and activate the immune system (7, 9). Increased neutrophils destroy the BBB and increase cerebral edema and neurologic impairment by the activation of inflammatory mediators such as chemokines and cytokines, reactive oxygen species (ROS), and the release of adhesion molecules and proteolytic enzymes (7, 9). In summary, the pro-inflammatory activation of neutrophils increases infarct size, hemorrhagic transformation, and adverse neurologic outcomes (8, 9). Lymphocytes as the main leukocyte subpopulation may contribute to the repair of the ischemic brain tissue, in which regulatory T and B cells are important brain protective immunomodulators in ischemic stroke (11, 12). In addition, decreased lymphocyte counts may reflect a cortisol-induced stress response and a sign of reduced sympathetic tone, which may promote the secretion of pro-inflammatory cytokines, resulting in an increased risk of ischemia and reperfusion damage after ischemic stroke (75, 78). The NLR is considered to represent the balance between neutrophils and lymphocytes and has recently been reported as an easy computing, inexpensive, and stable comprehensive systemic inflammatory biomarker. Numerous studies have investigated the predictive and prognostic values of NLR in patients with AIS treated with IVT or EVT (15–22, 33–76). However, the conclusions of these studies are inconsistent. Meta-analysis

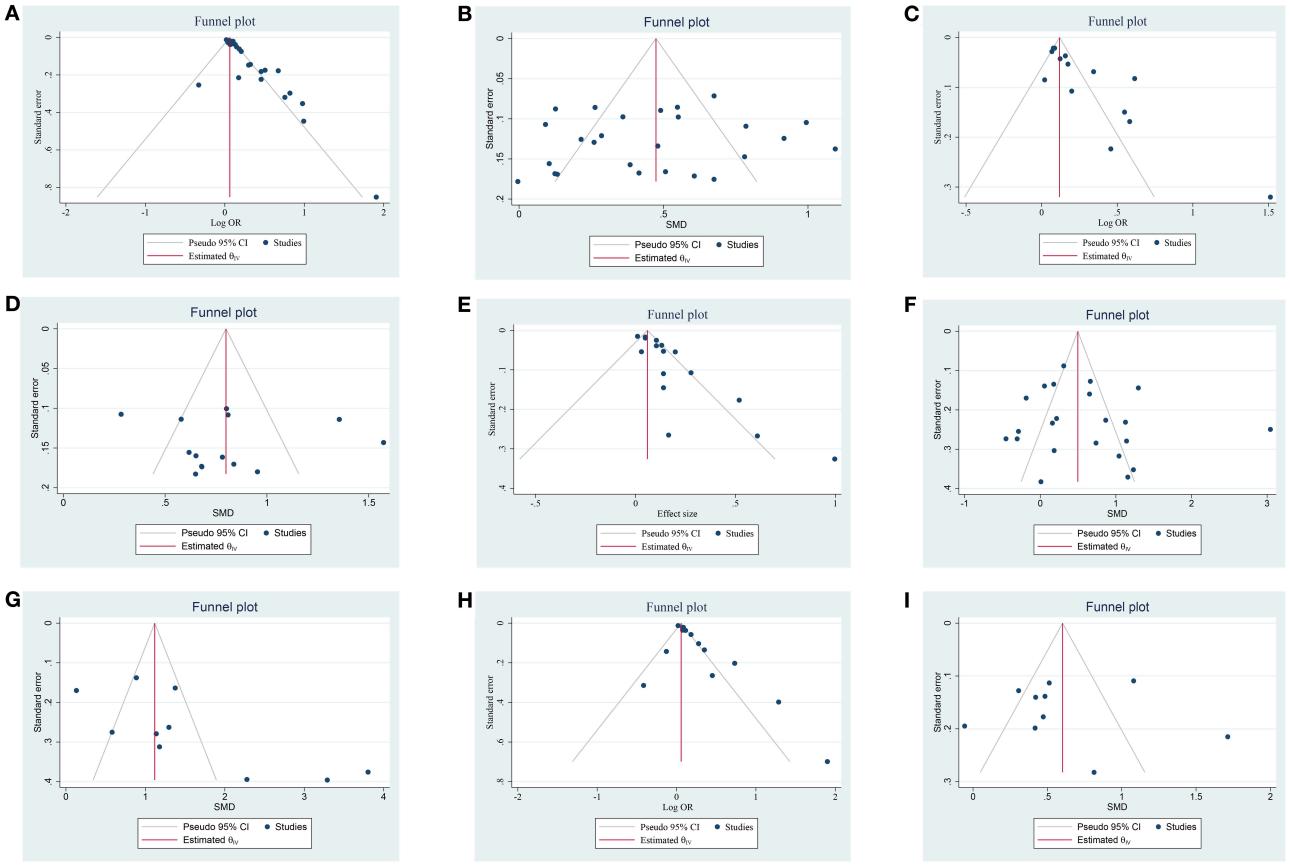

FIGURE 5
Funnel plot of the publication bias on the association of NLR and prognosis; **(A)** funnel plot of admission NLR and 3-month PFO based on OR; **(B)** funnel plot of admission NLR and 3-month PFO based on SMD; **(C)** funnel plot of post-treatment NLR and 3-month PFO based on OR; **(D)** funnel plot of post-treatment NLR and 3-month PFO based on SMD; **(E)** funnel plot of admission NLR and sICH based on OR, **(F)** funnel plot of admission NLR and sICH based on SMD; **(G)** funnel plot of post-treatment NLR and sICH based on SMD; **(H)** funnel plot of admission NLR and 3-month mortality based on OR; and **(I)** funnel plot of admission NLR and 3-month mortality based on SMD.

provides a much more likely approach for reaching reasonably strong conclusions.

Several previous meta-analyses have reviewed the predictive value of NLR for PFO after reperfusion therapy in patients with AIS (23–25). A meta-analysis conducted by Bi et al. (24) included six studies and showed that an increased baseline NLR was associated with 3-month PFO. Another study by Sharma et al. (25), which included 13 studies, showed that a lower admission NLR was associated with good functional outcomes (mRS 0–2). Our meta-analysis, which included 26 studies for the pooled SMD analysis, further verified the earlier results. Furthermore, the current study is also in line with the meta-analysis by Sharma et al. (25), which demonstrated that post-treatment NLR was related to 3-month PFO. However, the pooled SMD could not be interpreted as a risk measure because the effect sizes were not adjusted for potential confounders. Hence, we included 26 and 14 studies separately for the pooled OR analysis to investigate the relationship between NRL and 3-month PFO at admission and post-treatment. The results showed that higher admission NLR and post-treatment NLR increased the risk of 3-month PFO. Due to the large SMD and the higher OR of post-treatment NLR, our findings demonstrated that post-treatment NLR levels displayed a stronger predictive power for 3-month PFO than admission. There are several possible mechanisms to explain the earlier findings. First, neutrophils infiltrate the ischemic brain between 30 min and a few hours after infarction, peaking between days 1 and 3, and then declining steadily thereafter (79). Second, 24–48 h after reperfusion, BBB disruption leads to increased intracranial pressure and vasogenic edema (79). Third, the regulatory lymphocyte level in the ischemic brain parenchyma is low during the 1st day after stroke (80).

This study demonstrated that admission and post-treatment NLR levels were higher in patients with sICH than in those without sICH after reperfusion therapy in the pooled SMD analysis. The overall results were consistent with the meta-analysis by Bi et al. (24) and Sharma et al. (25). However, Sharma et al. (25) did not detect a significant association between post-treatment NLR and sICH in subgroup analyses stratified by the treatment method, and Bi et al. (24) did not investigate the relationship between post-treatment NLR and sICH. Compared with the previous meta-analysis, we included 22 and 10 studies separately to limit selection and publication bias, which may have influenced the results. Furthermore, subgroup analyses based on the type of treatment also showed that higher levels of admission and post-treatment NLR were observed in patients with sICH than in those without sICH. Similarly, for the pooled OR analysis, we found that the higher levels of admission and post-treatment NLR increased the risk of sICH. However, interestingly, we did not find a relationship between post-treatment NLR and sICH in

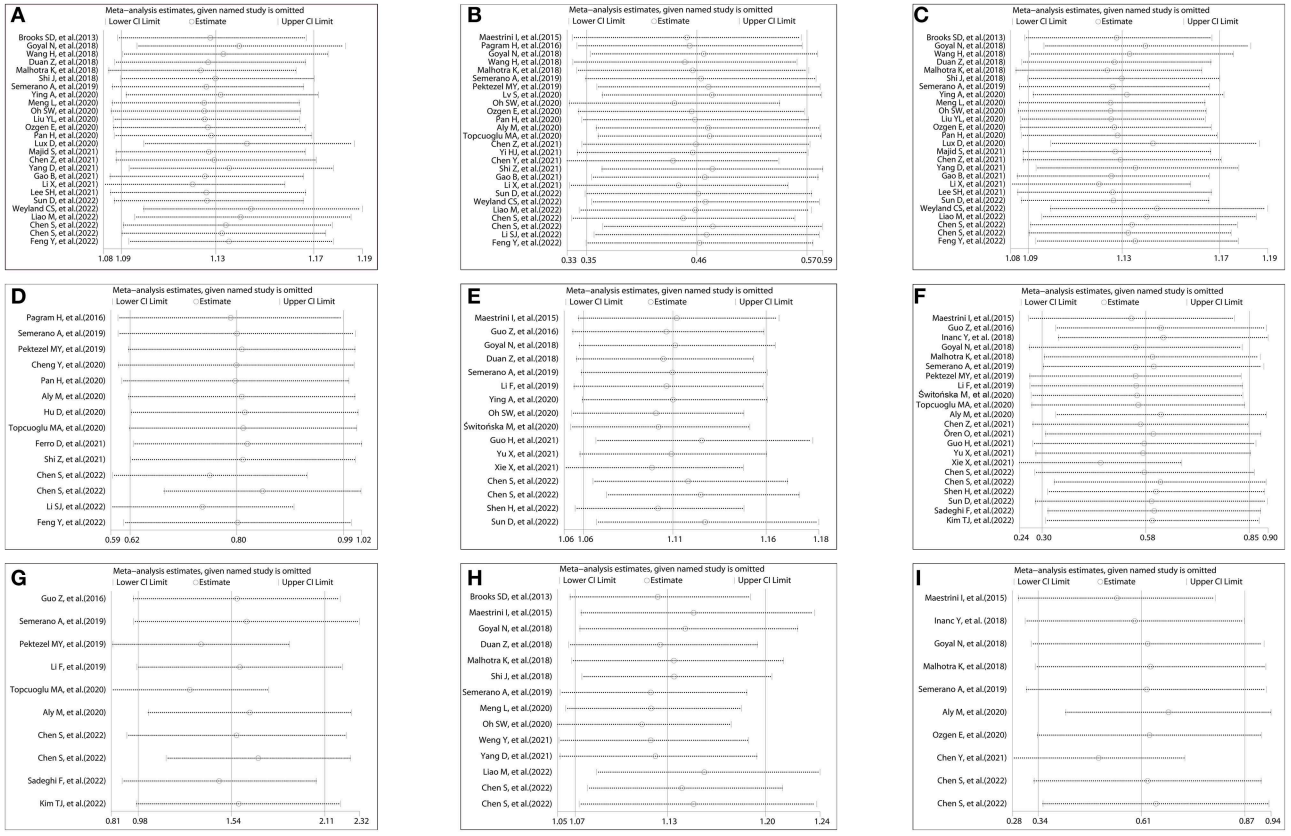

FIGURE 6
Sensitivity analysis on the relationship between NLR and prognosis; **(A)** sensitivity analysis of admission NLR and 3-month PFO based on OR; **(B)** sensitivity analysis of admission NLR and 3-month PFO based on SMD; **(C)** sensitivity analysis of post-treatment NLR and 3-month PFO based on OR; **(D)** sensitivity analysis of post-treatment NLR and 3-month PFO based on SMD; **(E)** sensitivity analysis of admission NLR and sICH based on OR; **(F)** sensitivity analysis of admission NLR and sICH based on SMD; **(G)** sensitivity analysis of post-treatment NLR and sICH based on SMD; **(H)** sensitivity analysis of admission NLR and 3-month mortality based on OR; and **(I)** sensitivity analysis of admission NLR and 3-month mortality based on SMD.

the subgroup analyses stratified by the treatment type. Fewer studies were included, and publication bias may explain this inconsistency. Further studies are needed to explore this causal relationship. Our findings suggested that post-treatment NLR levels had a stronger predictive power for sICH than admission due to its large SMD and higher OR. The possible underlying mechanism for these findings is that neutrophils enter the brain and release matrix metalloproteinase-9 (MMP-9), which may act on tight-junction proteins and then destroy the BBB from the lumen side of the blood vessels (77).

The present study also revealed that admission and post-treatment NLR levels were higher in patients with mortality at 3 months than in patients without mortality after reperfusion therapy in the pooled SMD analysis. These findings are consistent with those of the previous studies (25). However, in subgroup analyses stratified by the treatment type, Sharma et al. (25) did not find a statistically significant difference between admission NLR and 3-month mortality in patients with AIS treated with EVT ± IVT. In contrast, we included more studies than the aforementioned study, and the results indicated that a correlation exists between admission NLR and 3-month mortality in EVT patients. However, the pooled SMD only evaluated the differences in NLR levels between mortality and non-mortality. As a result, we also combined OR to assess whether higher NLR levels increased the risk of mortality. Our research showed that higher admission and post-treatment NLR levels were associated with an increased risk of mortality. However, higher post-treatment NLR was not associated with mortality in the EVT group. The possible reasons for this difference may include that only two studies met the inclusion criteria and had higher baseline NIHSS scores. Based on the large SMD and higher OR, we suggest that post-treatment NLR levels have greater predictive power for mortality than admission. These results may be related to the pathophysiological mechanisms described previously.

Several studies have shown that NLR as a dynamic variable is associated with HT (15, 16), sICH (15, 19, 40, 42, 71, 75), 3-month PFO (16, 19, 38, 40, 42, 51, 58, 63, 71, 75, 76), and death (19, 38, 58, 63, 71) in patients with AIS after IVT or EVT. These studies also showed that the post-treatment NLR has a more strong predictive ability for the poor prognosis of patients with AIS after reperfusion therapy than admission. The results of these previous studies are basically consistent with our findings.

Similar to other studies, this meta-analysis has some limitations. First, most of the included studies had a retrospective design, which made them vulnerable to selection bias and uncontrolled confounding factors. Therefore, future prospective cohort studies with adjustments for potential confounders are required to further explore the possible impact of the NLR on poor prognosis in patients with AIS treated with reperfusion therapy. Second, all analyzed studies were reported in English, which could have caused

publication bias and influenced the pooled results. Hence, we used a "trim-and-fill" approach to reduce its influence on the effect size. Third, most of the studies were conducted in Asia, which may result in a risk of selection bias in the patient population. However, subgroup analyses found no effect of the study region on the research findings. Fourth, the statistically significant heterogeneity among the included studies may have affected the reliability of the meta-analysis, and thus, the conclusion should be more conservative. In the stratified subgroup analysis, none of the included factors was confirmed to be a contributing factor. Meanwhile, the results of sensitivity analyses showed that no single study affected the estimated significance of pooled ORs or SMDs. Discrepancies in various adjustments and inadequate consideration of potentially confounding factors may also partially explain the heterogeneity. Fifth, owing to the statistical characteristics of SMD, it was not possible to adjust for confounding factors (e.g., baseline NIHSS severity, hypertension, and age). Therefore, we attempted to include adjusted ORs for analysis in our study as much as possible. Sixth, the adjusted risk factors for each study used to calculate ORs were different in the included studies. However, almost all studies included key factors such as age, sex, and NIHSS scores. Seventh, statistical methods were used to calculate the approximation of the mean and SD from the median and IQR. These methods have been proven stable and reliable in previous studies. Eighth, a few studies were unable or did not seek to exclude patients with existing infections, which may affect the accuracy of NLR application. Nevertheless, there was no substantial difference in the effect size according to the subgroup analyses regardless of whether the infection was excluded. Ninth, NLR data were limited to two different time points in this analysis: admission/pre-treatment and post-treatment (time point close to 24 h). However, NLR dynamically changes during the progression of AIS. Thus, future analyses with more time points might further explore the relationship between the dynamic profile of the NLR and prognosis. Therefore, our findings should be interpreted with caution because of the above limitations.

Conclusion

In summary, our findings show that both admission and post-treatment NLR can be used as cost-effective and easily available biomarkers to predict PFO at 3 months, sICH, and 3-month mortality in patients with AIS after reperfusion therapy. The predictive power of post-treatment NLR is better than that of admission. NLR as a stand-alone test or part of a risk prediction model may help clinicians easily and quickly identify patients after reperfusion therapy who have a poor prognosis and require more intensive monitoring during treatment. However, the prognostic value of the dynamic NLR is under investigation owing to the heterogeneity of the studies. Further studies are warranted to confirm the utility of the dynamic NLR in predicting the outcomes of patients with AIS treated with reperfusion therapy.

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.

Author contributions

BW and FL: literature search, data extraction, statistical analysis, and drafting of the manuscript. GS and SW: study design, quality evaluation, and comments on important intellectual content. All authors have reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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

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Risk factors of hemorrhagic transformation in acute ischaemic stroke: A systematic review and meta-analysis

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Background: Hemorrhagic transformation (HT) following reperfusion therapies for acute ischaemic stroke often predicts a poor prognosis. This systematic review and meta-analysis aims to identify risk factors for HT, and how these vary with hyperacute treatment [intravenous thrombolysis (IVT) and endovascular thrombectomy (EVT)].

Methods: Electronic databases PubMed and EMBASE were used to search relevant studies. Pooled odds ratio (OR) with 95% confidence interval (CI) were estimated.

Results: A total of 120 studies were included. Atrial fibrillation and NIHSS score were common predictors for any intracerebral hemorrhage (ICH) after reperfusion therapies (both IVT and EVT), while a hyperdense artery sign (OR = 2.605, 95% CI 1.212–5.599, I^2 = 0.0%) and number of thrombectomy passes (OR = 1.151, 95% CI 1.041–1.272, I^2 = 54.3%) were predictors of any ICH after IVT and EVT, respectively. Common predictors for symptomatic ICH (sICH) after reperfusion therapies were age and serum glucose level. Atrial fibrillation (OR = 3.867, 95% CI 1.970–7.591, I^2 = 29.1%), NIHSS score (OR = 1.082, 95% CI 1.060–1.105, I^2 = 54.5%) and onset-to-treatment time (OR = 1.003, 95% CI 1.001–1.005, I^2 = 0.0%) were predictors of sICH after IVT. Alberta Stroke Program Early CT score (ASPECTS) (OR = 0.686, 95% CI 0.565–0.833, I^2 =77.6%) and number of thrombectomy passes (OR = 1.374, 95% CI 1.012–1.866, I^2 = 86.4%) were predictors of sICH after EVT.

Conclusion: Several predictors of ICH were identified, which varied by treatment type. Studies based on larger and multi-center data sets should be prioritized to confirm the results.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=268927, identifier: CRD42021268927.

KEYWORDS

stroke, risk factor, intracranial hemorrhage, hemorrhagic transformation, reperfusion therapy, intravenous thrombolysis, endovascular thrombectomy

1. Introduction

Stroke is a leading cause of death and disability in Australia and around the world, with one in four people affected by stroke in their lifetime (1). Reperfusion therapies, including intravenous thrombolysis (IVT) and endovascular thrombectomy (EVT), can significantly improve patient outcomes (2) but are associated with complications, of which the most devastating is haemorrhagic transformation (HT). Haemorrhagic transformation after cerebral infarction is reported to occur in between 3.2 and 43.3% of strokes (3), and often results in a poorer prognosis. Aetiologically HT is a multifactorial phenomenon, and the ability to accurately predict the development of HT after reperfusion therapies has great potential to guide clinical decision making in order to maximize benefits and minimize harm.

Previous studies have identified many risk factors for HT, including (but not limited to) atrial fibrillation, higher baseline National Institute of Health Stroke Scale (NIHSS) score, advanced age, longer time from stroke onset to treatment (OTT), and lower baseline Alberta Stroke Program Early CT score (ASPECTS). Although a wide range of HT risk factors have been reported, findings have often been contradictory. For example, number of stent retriever passes at EVT has been variably reported to predict HT in comparable single-center cohorts (4–6), highlighting the heterogeneity of the evidence base.

Intravenous tissue plasminogen activator (tPA) improves outcome following ischaemic stroke when administered to appropriately selected patients up to 9 h after symptom onset (7–9). Endovascular thrombectomy (also known as mechanical thrombectomy), used either alone or in combination with IVT, has shown substantial benefit in patients with large vessel occlusion (10–14). Emerging evidence suggests that risk factors for HT vary considerably depending on the reperfusion treatment employed. In particular, higher rates of sICH have been reported following EVT (15), with certain imaging characteristics (occlusion site, ASPECTS) predicting HT in this setting (6, 16–19). Both individually and in combination, such predictors which are readily available in the hyperacute setting, have potential to guide clinical decision-making and prognostication.

This study reviews our current understanding of prognostic factors for HT in different treatment settings (IVT and EVT, respectively). Specifically, we aim to answer the review questions: (1) What are the baseline risk factors of haemorrhagic transformation after endovascular thrombectomy? (2) What are the baseline risk factors of haemorrhagic transformation after intravenous thrombolysis? (3) Is there any differences in risk factors of haemorrhagic transformation between endovascular thrombectomy and intravenous thrombolysis?

2. Methods

2.1. Search strategy

Electronic databases PubMed and EMBASE were used to identify relevant studies. The reference lists of eligible studies and systematic reviews were also checked and hand searching completed to find any additional relevant studies. The following search terms including their synonyms and available MeSH terms were used to retrieve relevant studies: Acute Ischemic Stroke, Hemorrhagic Transformation, Endovascular Thrombectomy, Intravenous Thrombolysis. The key search terms were combined using the Boolean operator "and" and "or" to retrieve the search results. Databases were searched from inception to August 2021.

2.2. Eligibility criteria

To be eligible for inclusion, studies were required to meet the following criteria: (1) Full-text publications in English. (2) Patients were diagnosed with acute ischaemic stroke. (3) Patient cohort aged 18 years old and over. (4) HT confirmed by CT/MRI scan within 48 hours after treatment. (5) Study included at least 50 patients. (6) Clinical or imaging data was measured prior to or during reperfusion treatment. (7) Treatment type of enrolled patients were either IVT or EVT, or bridging therapy (IVT plus EVT). (8) Predictors of HT were based on multivariate analysis and expressed as odds ratio (OR) with 95% CI.

2.3. Study screening and data extraction

Studies returned from the search results were screened using three steps. First, duplicate studies from across different databases were removed. Second, titles and abstracts of the search results were screened to check for eligibility by two independent reviewers (JS and CL), with disagreements resolved by discussion, and with a third reviewer (LC) if necessary. Finally, eligible full texts were screened by the same independent reviewers (JS and CL), with disagreements being resolved by discussion, and with a third reviewer if necessary.

For data extraction, two reviewers extracted the data independently using a predefined data extraction spreadsheet. Data were extracted from the selected studies guided by the CHARMS checklist (20), including authors and years, published journal, study type (randomized controlled trial or observational cohort), a single center or multi-center study, baseline characteristics of participants such as age, gender, onset-to-treatment time, NIHSS score, definitions of reported intracerebral hemorrhage (ICH) and the number of patients with HT, the HT confirmed timing after treatment, treatment type (intravenous therapy or endovascular therapy), risk factors identified and their type (continuous or categorized), regions and sample size. For performance measurements, odds ratio and 95% Confidence Interval (CI) and confounding variables adjusted in the multivariate analysis were extracted for prognostic factor studies.

2.4. Quality assessment

Two reviewers independently performed risk of bias assessments of the included studies. The two reviewers resolved any disagreements *via* discussion among themselves and with a third reviewer if required, until a consensus was reached.

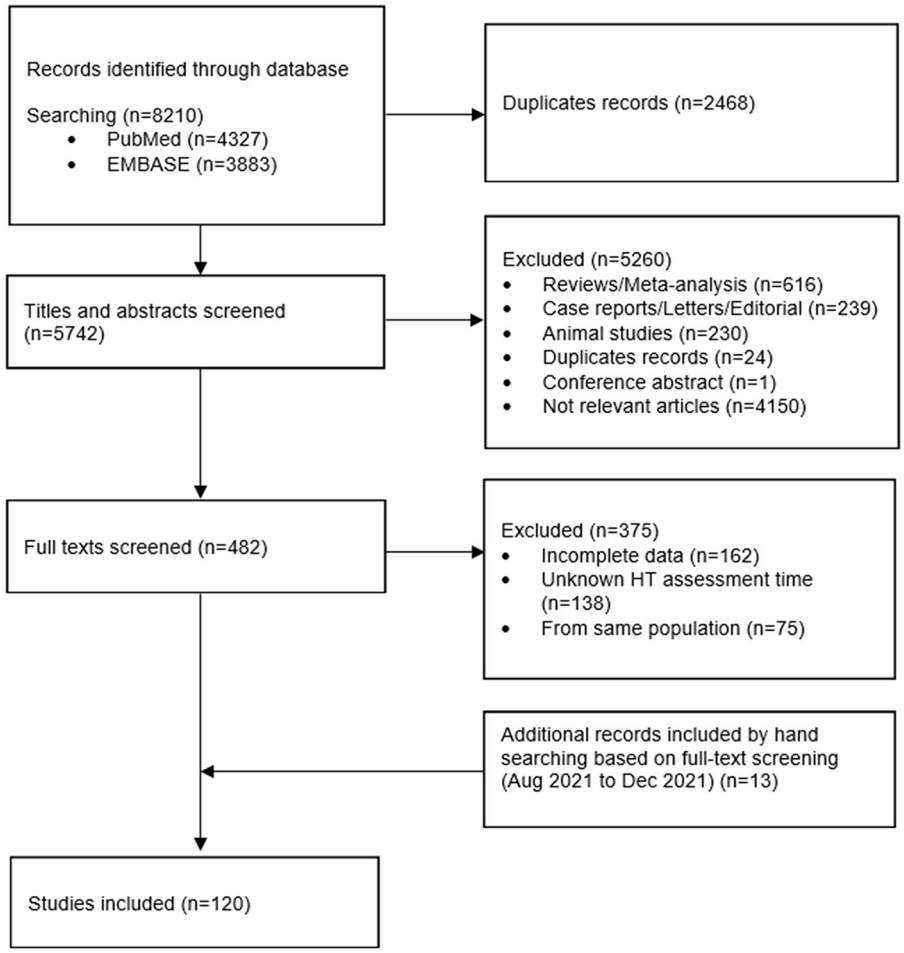

FIGURE 1
Screening flow diagram.

To assess risk of bias in the included studies, the Quality In Prognosis Studies (QIPS) tool was used to evaluate validity and bias across six domains: participation, attrition, prognostic factor measurement, confounding measurement, and account, outcome measurement, and analysis and reporting (21).

2.5. Statistical analysis

Combined hemorrhagic transformation rates with 95% CIs were computed for symptomatic ICH (sICH) and any ICH, respectively. A meta-analysis of risk factors using extracted OR with 95% CI from individual studies was conducted if the risk factor was reported in a minimum of two studies. Odds ratio is an appropriate measure for categorical outcomes (22) and is a preferable report measure in meta-analysis on outcome prediction models (23). As well as odds ratio was the most prevalent measure reported in the included studies, we only extracted the odds ratio that was adjusted for confounding factors, which is preferable to analyses based on summary statistics according to Cochrane guidelines (24).

The I^2 test was used to evaluate heterogeneity among included studies (25). For I^2 statistic, 25, 50, and 75% were the threshold for low, moderate, and high heterogeneity. The τ^2 was used to estimate the variance of the distribution of true effect sizes (26), and the confidence intervals around τ^2 were calculated to quantify the uncertainty of heterogeneity (27). Prediction intervals were calculated to estimate the effect sizes of future studies based on present evidence (28). A random-effects model was used to analyse the data, regardless of heterogeneity. Begg's funnel plots were used to test potential publication bias for those results with number of studies > 10. Sensitivity analysis was conducted by removing included studies one by one to detect the influence of individual studies on the estimate of the overall effect. All statistical analyses were conducted with Stata software package (V.13.1; Stata, College Station, Texas, USA) and R 4.1.2 (R Foundation), with a p-value of $p < 0.05$ considered statistically significant.

3. Results

3.1. Literature search and study characteristics

Literature search and screening processes are shown in Figure 1. Initially the search result included 5,742 articles after removing duplicates. After title and abstract screening, 482 articles

TABLE 1 Baseline characteristics of included studies.

	Total	Treatment type	
		IVT	EVT
Number of studies	120	67	53
Total sample size	345,477	300,979	44,498
Median sample size [range]	414 [204.5–1,125]	488 [235–1,475]	305 [199–751]

TABLE 2 Any ICH rates per treatment type.

	IVT	EVT
Number of studies	32	26
Total sample size	48,657	13,615
Median sample size	369.5 [199, 681.5]	271 [187, 633]
Range of HT rates	6.45%-31.77%	7.60–49.55%
Combined HT rates 95% CI	15.3% (13.8–16.9%)	30.7% (26.4–34.9%)

TABLE 3 sICH rates per treatment type.

	IVT	EVT
Number of studies	48	41
Total sample size	263,470	36,824
Median sample size	818 [404.5, 2,173]	314 [205, 915]
Range of sICH rates	1.27–15.75%	1.52–20.89%
Combined sICH rates 95% CI	4.1% (3.7–4.5%)	7.2% (6.3–8.1%)

remained. After full-text screening, 107 studies were included based on the search results, and another 13 relevant studies were identified *via* manual searching. In total, 120 studies (5, 6, 16–19, 29–142) were included in the meta-analysis.

Among the 120 included studies, 67 enrolled patients who were treated with IVT and 53 enrolled patients who were treated with EVT. Table 1 shows the characteristics of the included studies. The number of participants ranged from 71 (44) to 88,094 (55), with a total median sample size of 414 (Interquartile Range: 204.5–1,125). Further information on the characteristics of the included studies is summarized in Supplementary Table ("General characteristics").

The general study quality was good, with a lack of reporting in the "Study confounding" domain in ∼48% (58 out of 120) of the included studies. The results of quality assessment for each study are presented in Supplementary Table ("Quality assessment – QUIPS") and Supplementary Figures 1, 2 (143).

3.2. Event rates of ICH and sICH

Tables 2, 3 show the event rates of any ICH and sICH per treatment type. In total, there were 32 IVT-based studies and 26 EVT-based studies that reported any ICH rates. Among the reported studies, any ICH rate ranged from 6.45% (106) to 49.55% (65), with a combined any ICH rate of 22.0% (95% CI 20.0–24.1%). The number of studies reporting sICH rates was 48 for IVT and 41 for EVT respectively. The sICH rate ranged from 1.27% (55) to 20.89% (129) with a combined sICH rate of 5.2% (95% CI 4.8–5.6%). Four main sICH criteria were applied in the included studies: the Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS-MOST) criteria (144), the European Cooperative Acute Stroke Study (ECASS) criteria (145), the National Institute of Neurological Diseases and Stroke (NINDS) criteria (7) and the Heidelberg Bleeding Classification (HBC) (146). The proportion of studies using each sICH criteria is shown in Supplementary Figure 3. In cases of multiple sICH criteria, SITS-MOST criteria were used to calculate the sICH rate; if SITS-MOST criteria were not reported, ECASS criteria were used.

3.3. HT risk factors

In total, over 100 distinct risk factors were reported in the 113 prognostic factor studies. Since many risk factors were only reported in a single study, the meta-analysis included 24 risk factors that contributed to any ICH, and 32 risk factors that contributed to sICH. A summary of reported risk factors in the included study is shown in Supplementary Table ("Study results").

3.4. Meta-analysis of risk factors related to ICH

Figures 2, 3 show forest plots of risk factors for any ICH (147). A combined total of 16 risk factors for any ICH after IVT and 14 risk factors for any ICH after EVT were included in the meta-analysis. Meta-analysis showed that early ischemic changes, atrial fibrillation, hyperdense artery sign, hypertension and NIHSS score were predictors for any ICH after IVT, while atrial fibrillation, use of the Merci Device, diabetes mellitus, NIHSS score and number of thrombectomy passes were predictors for any ICH after EVT. Intraarterial tirofiban was associated with a lower risk of any ICH after EVT. Table 4 lists predictors for any ICH.

3.5. Meta-analysis of risk factors related to sICH

Figures 4, 5 show forest plots of risk factors for sICH. A combined total of 19 risk factors for sICH after IVT and 22 risk factors for sICH after EVT were included in the meta-analysis. Meta-analysis showed that atrial fibrillation, OTT within 3–4.5 h VS OTT within 3 h, statin use, NIHSS score, serum glucose level, age and onset-to-treatment time were predictors of sICH after IVT, while female gender, number of thrombectomy passes, serum glucose level, neutrophil to lymphocyte ratio, age and lower ASPECTS were predictors of sICH after EVT. Table 5 lists predictors for sICH.

3.6. Sensitivity analysis

In order to assess whether any particular study had a disproportionate influence on the meta-analysis results, heterogeneity assessment was done for the results with number of

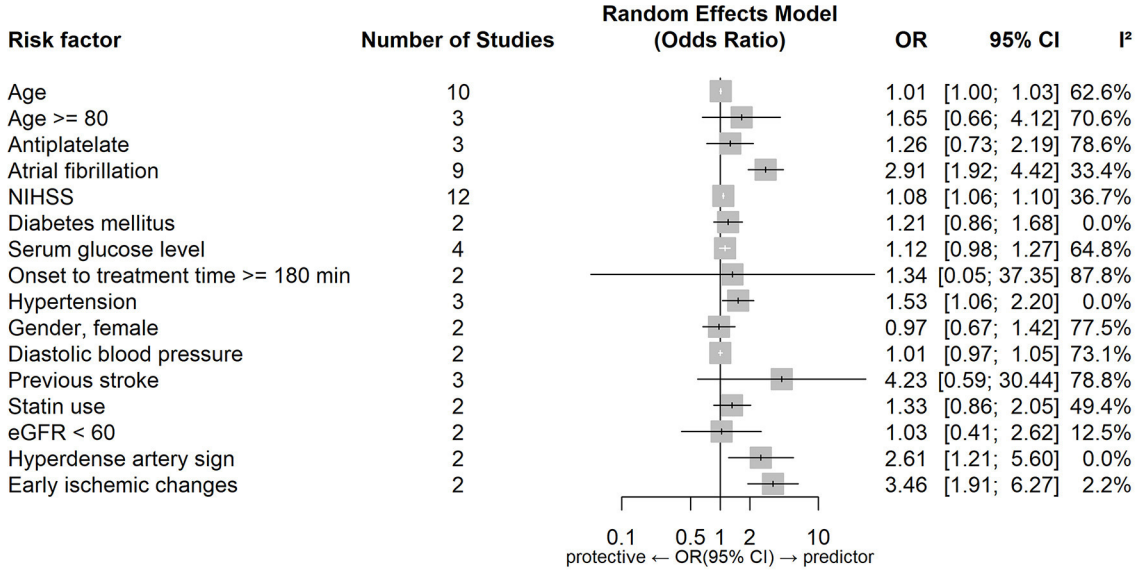

FIGURE 2
Forest plot of predictors for any ICH after IVT. OR, Odd Ratio; CI, Confidence Interval; NIHSS, National Institute of Health Stroke Scale; eGFR, estimated Glomerular Filtration Rate.

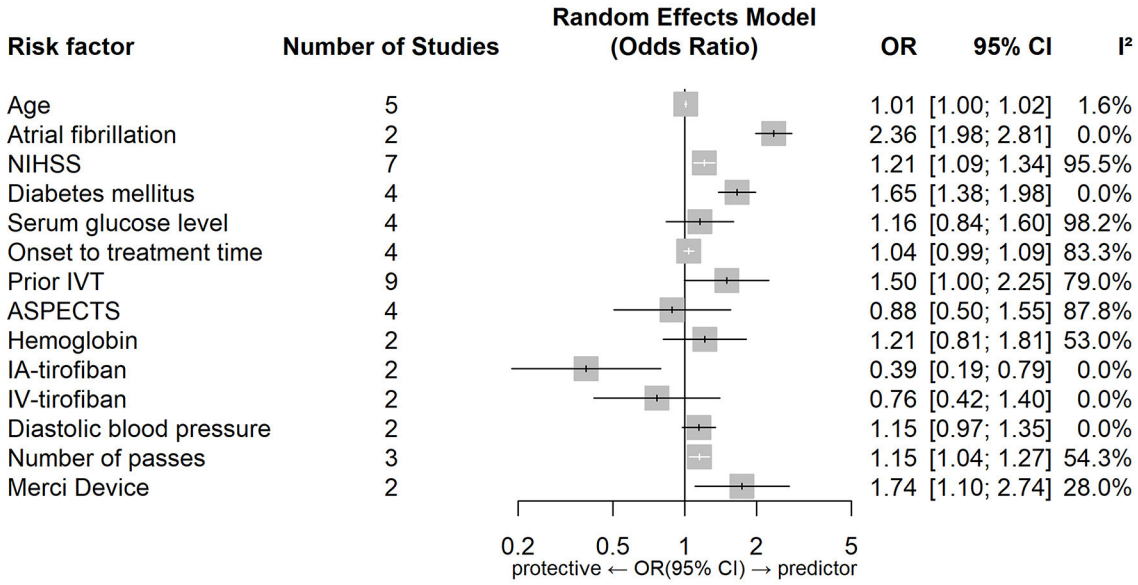

FIGURE 3
Forest plot of predictors for any ICH after EVT. OR, Odd Ratio; CI, Confidence Interval; NIHSS, National Institute of Health Stroke Scale; IVT, Intravenous Thrombolysis; ASPECTS, Alberta Stroke Program Early CT Score; IA, Intraarterial; IV, Intravenous.

studies ≥3 and I^2 ≥50%. For results where the confidence interval around τ^2 did not contain zero, a further sensitivity analysis was done by removing one study at a time. Three results showed a statistically significant association between prior IVT and any ICH after EVT, but with very high heterogeneity (I^2 ranged from 74–81%). The upper limit of the sensitivity analysis showed a statistically significant association between previous stroke and any ICH after IVT, with low-to-moderate heterogeneity but a very wide confidence interval (OR = 13.06, 95% CI 1.08–157.97, I^2 = 46%). The upper limit of the sensitivity analysis also showed a statistically significant association between antiplatelet use and sICH after IVT with low heterogeneity (OR = 2.17, 95% CI 1.50–3.14, I^2 = 0.0%), and also between antiplatelet use and sICH after EVT with high heterogeneity and a wide confidence interval (OR = 4.17, 95% CI 1.00–17.45, I^2 = 83%). The upper limit of the sensitivity analysis also showed statistically significant association between early ischemic changes and sICH after IVT, with low to moderate heterogeneity (OR = 2.98, 95% CI 1.37–6.49, I^2 = 44%).

TABLE 4 Predictors for any ICH in acute ischemic stroke patients: IVT vs. EVT.

Risk factors	Treatment type					
	IVT			EVT		
	Number of studies	Combined OR (95% CI)	I^2	Number of studies	Combined OR (95% CI)	I^2
Atrial fibrillation	9	2.912 (1.920–4.416)	33.4%	2	2.357 (1.978–2.809)	0.0%
NIHSS score	12	1.078 (1.058–1.099)	36.7%	7	1.208 (1.089–1.340)	95.5%
Diabetes mellitus	2	1.206 (0.865–1.683)	0.0%	4	1.655 (1.383–1.979)	0.0%
Hypertension	2	1.529 (1.060–2.205)	0.0%	N/A	N/A	N/A
Intraarterial tirofiban	N/A	N/A	N/A	2	0.386 (0.188–0.792)	0.0%
Number of passes	N/A	N/A	N/A	3	1.151 (1.041–1.272)	54.3%
Merci device	N/A	N/A	N/A	2	1.736 (1.101–2.739)	28.0%
Hyperdense artery sign	2	2.605 (1.212–5.599)	0.0%	N/A	N/A	N/A
Early ischemic changes	2	3.462 (1.912–6.268)	2.2%	N/A	N/A	N/A

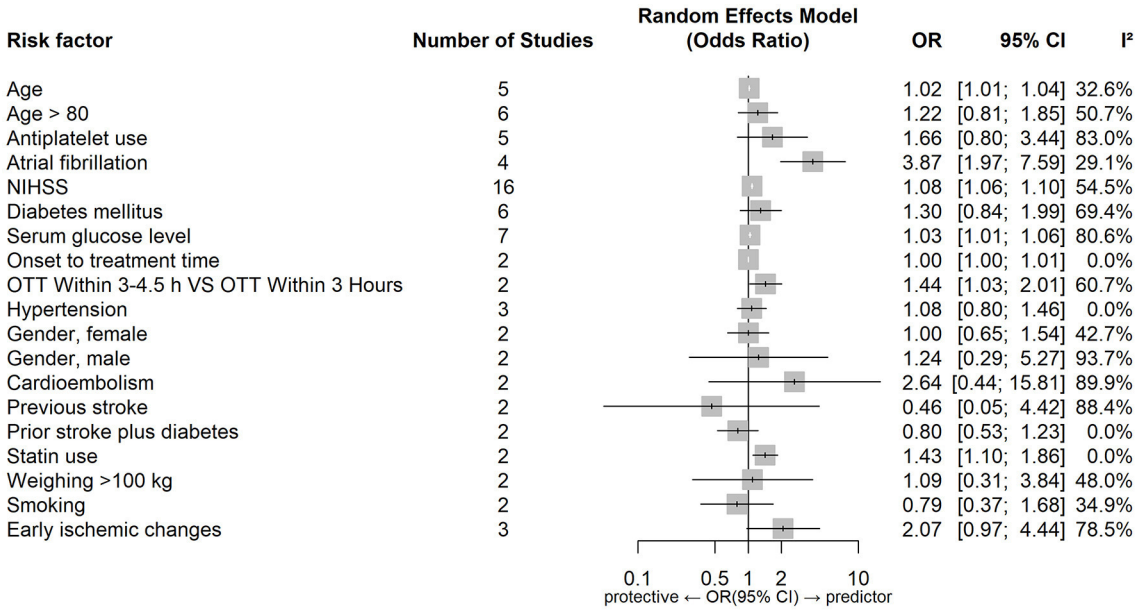

FIGURE 4
Forest plot of predictors for sICH after IVT. OR, Odd Ratio; CI, Confidence Interval; NIHSS, National Institute of Health Stroke Scale; OTT, Onset to Treatment Time.

A summary of heterogeneity assessment and sensitivity analysis is shown in Supplementary Table ("Heterogeneity assessment" and "Sensitivity analysis").

3.7. Assessment of publication bias

Funnel plots were used to assess publication bias for NIHSS score as a predictor of any ICH after IVT (number of studies $N = 12$) and sICH after IVT (number of studies $N = 16$). No evidence of publication bias was found for NIHSS score predicting any ICH after IVT (Egger's test, $P = 0.185$). However, there was evidence of significant small study bias for NIHSS score predicting sICH after IVT (Egger's test, intercept = 1.796, $t = 4.48$, $P < 0.001$), although one particular study (105) had a significant influence on this statistic.

4. Discussion

We performed a systematic review and meta-analysis to identify risk factors for HT after reperfusion therapies for acute ischaemic stroke. Although many factors have previously been reported as predictors of HT, findings are derived from widely varying studies.

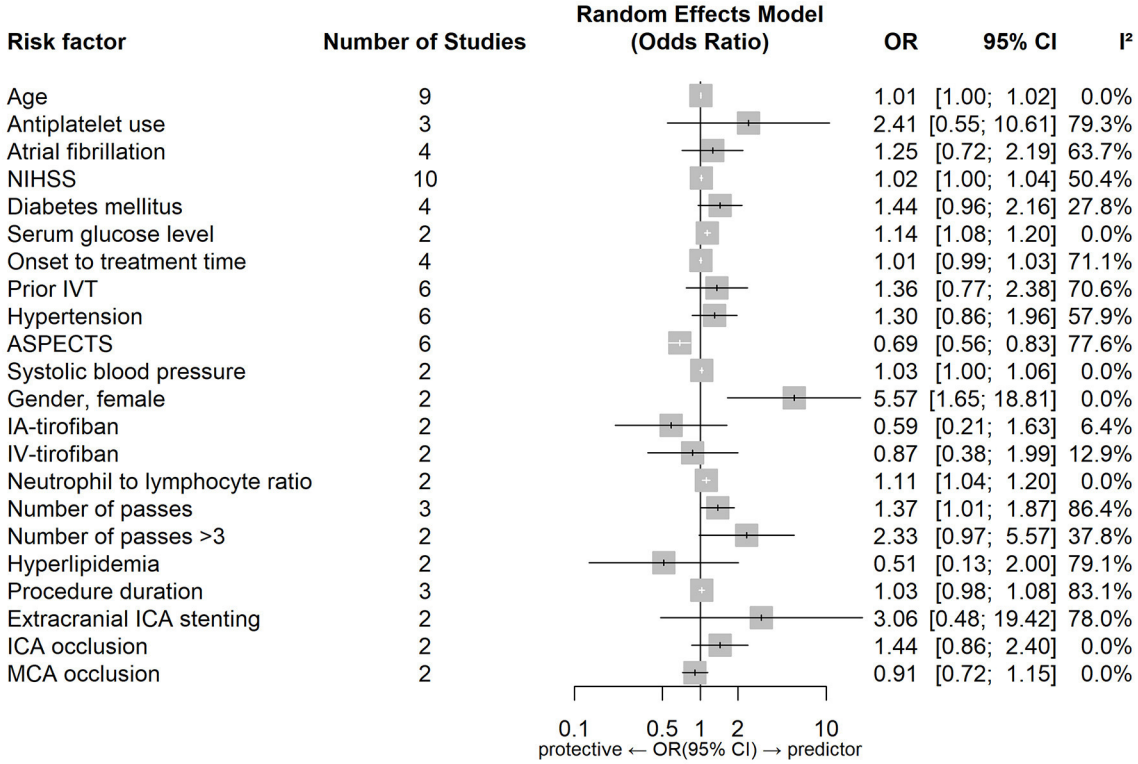

FIGURE 5
Forest plot of predictors for sICH after EVT. OR, Odd Ratio; CI, Confidence Interval; NIHSS, National Institute of Health Stroke Scale; IVT, Intravenous Thrombolysis; ASPECTS, Alberta Stroke Program Early CT Score; IA, Intraarterial; IV, Intravenous; ICA, Internal Carotid Artery; MCA, Middle Cerebral Artery.

TABLE 5 Predictors for sICH in acute ischemic stroke patients: IVT vs. EVT.

Risk factors	Treatment type					
	IVT			EVT		
	Number of studies	Combined OR (95% CI)	I^2	Number of studies	Combined OR (95% CI)	I^2
Age (continuous)	5	1.023 (1.010–1.037)	32.6%	9	1.008 (1.001–1.016)	0.0%
Atrial fibrillation	4	3.867 (1.970–7.591)	29.1%	4	1.254 (0.719–2.185)	63.7%
NIHSS score	16	1.082 (1.060–1.105)	54.5%	10	1.019 (0.995–1.043)	50.4%
Serum glucose level	7	1.034 (1.009–1.060)	80.6%	2	1.141 (1.081–1.204)	0.0%
Onset-to-treatment time	2	1.003 (1.001–1.005)	0.0%	4	1.007 (0.987–1.027)	71.1%
OTT within 3–4.5 h vs. OTT within 3 h	2	1.437 (1.027–2.011)	60.7%	N/A	N/A	N/A
ASPECTS	N/A	N/A	N/A	6	0.686 (0.565–0.833)	77.6%
Gender, female	2	1.000 (0.649–1.541)	42.7%	2	5.568 (1.649–18.807)	0.0%
Neutrophil to lymphocyte ratio	N/A	N/A	N/A	2	1.114 (1.037–1.197)	0.0%
Number of passes	N/A	N/A	N/A	3	1.374 (1.012–1.866)	86.4%
Statin use	2	1.428 (1.097–1.858)	0.0%	N/A	N/A	N/A

4.1. Disparities in HT rates

The combined rates of both any ICH and sICH after EVT (30.7 and 7.2%) and IVT (15.3 and 4.1%) in our study were lower than those reported in previous work (35% for any ICH and 8% for sICH after EVT and 6.5% for sICH after IVT) (148, 149). As well as having a larger sample size, our analysis also standardized the definition of ICH across all included studies that reported multiple sICH criteria using SITS-MOST criteria. The incidence of HT was generally lower when these criteria were applied (150). In contrast, Hao et al. (148) study did not standardize the identification of ICH, and only 5% of their included studies used SITS-MOST criteria (vs.

18.4% in our study). Tsivgoulis et al. (149) review had a relatively small sample size ($N = 12$ vs. N ranging from 63 to 1,643 in other included studies), with only one study using SITS-MOST criteria.

Our analysis found that the combined rate of any ICH and sICH were lower in patients treated with IVT, in comparison to those treated with EVT. This result is consistent with previous reports (15). Several factors are thought to be responsible for higher HT rates after EVT. First, EVT studies by their nature include only patients with large vessel occlusion (LVO) stroke, who usually have worse stroke severity with higher NIHSS scores (151–153) and larger areas of involved tissue. Resulting ASPECTS scores are generally lower, and indeed this factor was found to independently predict sICH after EVT in our study. Second, in a finding corroborated by our analysis for the Merci device, the use of thrombectomy devices themselves have several implications for HT risk that center on vessel abrasion, including those arising from the type of device used and the number of passes required to achieve satisfactory reperfusion. Third, EVT is more commonly performed in patients with atrial fibrillation (18, 44, 96, 154) and with longer onset-to-treatment times (17, 68, 96), and uses additional antiplatelet agents during the procedure (112). Finally, other procedure-related factors such as the use of general anesthesia (155), distal embolization and extracranial stenting (16) may also contribute to a higher HT rate after EVT. These factors were not identified in our meta-analysis, due to a lack of relevant studies.

4.2. Predictors of any ICH

Common predictors for any ICH after reperfusion therapy (both IVT and EVT) were atrial fibrillation and a higher NIHSS score. While these two factors also predicted sICH after IVT they did not do so after EVT, however the relevant data set for EVT was limited by moderate-to-high heterogeneity. Hypertension, a hyperdense artery sign, and early ischemic change on non-contrast CT were predictors of any ICH after IVT and diabetes mellitus, number of thrombectomy passes and use of the Merci device were predictors of any ICH after EVT. Intriguingly, intraarterial tirofiban was found to be protective for any ICH after EVT.

Hyperdense artery sign and early ischemic changes were found to be predictors of any ICH after IVT. There were insufficient studies included in the meta-analysis that examined the same predictors for EVT. Hyperdense artery sign is associated with a higher clot burden and cardioembolic stroke, both of which predict a potentially larger area of infarcted brain tissue and a diminished response to thrombolysis (156–158). Early ischemic changes (including hypodensity and swelling/effacement) indicate the presence of brain oedema arising from prolonged hypoperfusion, and possibly the development of irreversible injury (159). These imaging features have been shown to predict HT, in particular where a significant portion (>33%) of the involved vascular territory is affected (160). Both hyperdense artery sign and early ischemic changes were imaging-based predictors derived from non-contrast CT, the most widely studied (and quantitative) stroke imaging modality.

Number of thrombectomy passes and use of the Merci device were predictors of any ICH after EVT, although the former result was subject to significant heterogeneity (prediction interval 0.4183–3.1667). Successive thrombectomy passes are thought to damage the arterial intima and weaken the vessel wall, causing micro-perforations at the time of device deployment/retraction (96) and so increasing the likelihood of HT (161). Use of the Merci device may increase vessel injury, vasospasm, or arterial dissection (96).

The effect of intraarterial tirofiban and intravenous tirofiban on HT risk varied in a key report (162), promoting us to regard route of administration of tirofiban as an independent variable. Surprisingly we found that intraarterial tirofiban was protective for any ICH after EVT. Among previous studies only Sun et al. (112) concluded that intraarterial tirofiban significantly decreased the odds of any ICH, with others either reporting contradictory or inconclusive findings (79, 163, 164). The contradictory findings may be explained by different rates of adjunctive IVT in these studies (increased ICH risks with adjunctive IVT). Sun et al. (162) study had a relatively small sample size ($N = 195$), and selection bias was introduced because use of tirofiban was administered at the neuro-interventional specialists' discretion. This is likely to have led to the exclusion of patients with larger infarct sizes who were at higher risk of subsequent ICH (165, 166). In addition, in patients receiving tirofiban it is possible that more stringent post-procedural blood pressure management may have been pursued, and the use of antiplatelet and anticoagulant therapies may have been more aggressively rationalized to reduce the risk of ICH (164). Notably Sun et al. conclusions were specific to patients with stroke due to large artery atherosclerosis, and did not reach significance for cardioembolism. Zhao et al. (164), who specifically recruited patients with cardioembolic stroke, concluded that intraarterial tirofiban was not protective for any ICH after EVT. Taken together these findings suggest that tirofiban's effect may be specific to both route of administration and stroke etiology.

4.3. Predictors of sICH

Common predictors for sICH after reperfusion therapy were higher age and a higher serum glucose level. Atrial fibrillation, a higher NIHSS score, longer onset-to-treatment time and statin use were predictors of sICH after IVT, while lower ASPECTS, female gender, higher neutrophil-to-lymphocyte ratio and number of thrombectomy passes were predictors of sICH after EVT.

Lower APSECTS (indicating larger stroke volumes) were found to independently predict sICH after EVT in our study. As described above a hyperglycaemic environment can impair cell metabolism and reduce vasoreactivity, which may disrupt the blood brain barrier integrity and increase the permeability, leading to the development of HT (5, 44, 101, 139, 167).

OTT within 3–4.5 h VS OTT within 3 h and statin use were found only to be the predictors of sICH after IVT, with insufficient studies to examine these associations for EVT. Although statin use was found to be predictive of sICH after IVT this finding was derived from two studies, with several others reporting no association between statin use and sICH (168–171).

Atrial fibrillation, a higher NIHSS score and longer onset-to-treatment time were found to be predictors of sICH after IVT but not after EVT. This result was also confirmed by the heterogeneity

assessment, indicating there are different predictors of sICH after each treatment type.

A higher neutrophil-to-lymphocyte ratio was found only to predict sICH after EVT, there were insufficient results for IVT to examine the same factor. Neutrophil-to-lymphocyte ratio is a biomarker of systemic inflammation. Higher neutrophils lead to increased release of MMP-9 (matrix metalloproteinase-9) and disruption of neurovascular units and blood brain barrier integrity, increasing the risk of sICH (172–174).

4.4. Assessment of heterogeneity and publication bias

In this study, heterogeneity assessment and sensitivity analysis were done to explore the robustness of the results. Heterogeneity assessment was performed for 25 results and a further sensitivity analysis was done for 11 results, of which six differed from the original findings. The robustness of the meta-analysis was therefore generally good.

The between-study heterogeneity observed could be explained by several factors. First, different study designs with different inclusion criteria resulted in large variations in patient characteristics including stroke severity, average age, onset-to-treatment time, disease history and stroke etiology. There was also significant variation in the number and the ethnicity of enrolled patients. Second, definitions of the same risk factors were not standardized across different studies, and in some studies were ambiguous or not clearly stated. For example, there was no uniform method of defining hyperdense artery sign: a clot with a Hounsfield unit ratio of 1.1 indicated a hyperdense artery sign in one study (175), while in another a ratio of 1.5 was used to exclude a hyperdense artery sign (176). Furthermore, for drug related risk factors, different studies used different medication regimes with regard to type, dose, and timing of administration. For example, while prior antiplatelet use was identified as a predictor in multiple studies, the specific drug varied despite the fact that different agents are recognized to variably affect HT (177). Previous work has also demonstrated that different doses of tirofiban could have different effects on HT (163). Third, measurement bias, especially for biomarkers, is likely to have been a factor. A previous review reported that the definition of hyperglycaemia varied from study to study, and the measurement methods used included both random and fasting serum levels (178). Fourth, as previously mentioned, four different kinds of sICH criteria were used across the included studies, which is likely to have caused the rates of sICH identified to vary significantly. Lastly, studies went to different lengths to adjust for confounders in their multivariate analyses, or made no adjustments at all.

Finally, one of our two assessments for publication bias (NIHSS score predicting sICH after IVT) showed significant evidence of small study bias, possibly because we did not include abstracts or search the gray literature. As a result, some negative studies may have been omitted. However, the asymmetry of the funnel plots can also be caused by between-study heterogeneity (179), and heterogeneity assessment showed that the result of NIHSS score predicting sICH after IVT had moderate to high heterogeneity ($I^2 = 54.5\%$).

4.5. Strengths and limitations

This study is the first to compare risk factors for HT following different treatment types (IVT vs. EVT), an approach which has the potential to guide patient selection and clinical decision-making. It is also the first study to systematically review risk factors for sICH after IVT and the second to systematically review risk factors of HT after EVT (148). Although several systematic reviews have examined risk factors for HT after IVT (165, 180, 181), they did not differentiate "any ICH" from sICH. Several studies were also limited by the use of geographically restricted (often Chinese) patient groups. Compared to any ICH, sICH is more likely to predict a poor prognosis, making any study that identifies predictors of sICH of particular clinical relevance.

Our study has several limitations. First, we were unable to conduct a meta-analysis incorporating all reported risk factors because many were only reported in single studies. We also did not analyze risk factors for HT based on radiological criteria (hemorrhagic infarction and parenchymal hematoma) due to limited studies reported relevant information in our included studies. However, a systematic review (182) investigating predictors for different radiological degrees of HT had similar findings with this study. Second, there were large disparities in HT rates (6.45 to 49.55% in any ICH), indicating substantial differences of methodology across different incorporated studies. This possibly renders the meta-analysis results were very unstable and questions whether the pooled studies were homogenous. Although we performed heterogeneity assessment and sensitivity analysis to examine the robustness of the meta-analysis results, the meta-analysis results still need to be interpreted carefully. Third, we did not account for measures made to minimize the risk and extent of HT such as tight blood pressure and glucose control post procedure. Fourth, we did not differentiate treatment type of thrombectomy only and bridging therapy when analyzing the combined HT rates and predictors for HT. Fifth, we did not perform subgroup analysis by removing studies with a high risk of bias. However, results of sensitivity analysis demonstrated the general robustness of the meta-analysis findings and identified specific finding that needed to be interpreted with caution. Sixth, we only searched two electronic databases for the literature search. Nevertheless, initial search results returned nearly six thousand non-duplicated studies and additional manual searching was done to mitigate this potential risk of bias. Lastly, we did not search the gray (unpublished) literature in order to mitigate the risk of publication bias.

4.6. Implications for clinical practice

When treating patients with EVT, neuro-interventionalists should consider the impact of multiple retrieval attempts/device passes and be mindful of their choice of thrombectomy device, as both were found to be predictors of any ICH in this study. In

addition, patients with atrial fibrillation, a higher NIHSS score, higher age, or a higher serum glucose level should be considered in the highest risk category for HT in the hours and stays after hyperacute management, regardless of treatment.

4.7. Recommendations for future research

EVT studies are generally more recent than IVT studies (respectively 64.2 and 20.9% were published after 2020). EVT studies are also fewer in number ($N = 53$ vs. $N = 67$) and are generally smaller in size (median sample size 305 compared to 488). Meanwhile 27 studies proposed imaging-based predictors for HT, with 20 published after 2018. Improved imaging technology in recent years, particularly the advent of CT perfusion, have shown great potential to enhance patient selection by more accurately characterizing infarct core and penumbra. For example, CT perfusion-based predictors including those measuring infarct core volume (19, 53, 183, 184) and blood-brain-barrier permeability (6, 142, 185–188) haven been reported in previous work, and novel CT perfusion-based parameters such as net water uptake (189) continue to emerge. These techniques have largely emerged in tandem with EVT and indeed have enabled its application in the extended therapeutic window, meaning that the majority of published randomized trial data charactering HT has EVT as a focus. Conversely, data for IVT is in the main derived from trials using CT/CTA [although CTP-directed thrombolysis is an emerging evidence-based treatment approach (190)]. Furthermore, use of Tenecteplase, a newer thrombolytic agent with improved ease of use and a potentially more favorable safety profile, has been less widely studied. Core areas for future studies therefore include (a) novel imaging predictors of HT (particularly those using CT perfusion, given its use in the hyperacute setting) and (b) HT rates/characteristics after the administration of Tenecteplase (with and without EVT).

Both the use of multiple criteria for sICH and substantial variation in the timing of follow-up imaging introduced significant heterogeneity into the meta-analysis. Future studies should be harmonized to incorporate the use of SITS-MOST criteria to characterize HT in scans not performed more than 48 h after hyperacute therapy. These two simple steps would ameliorate much of the variability we found.

5. Conclusion

Hemorrhagic transformation is one of the most devastating complications of reperfusion therapy for patients with acute ischaemic stroke. This meta-analysis identified several predictors for HT, including atrial fibrillation, a higher NIHSS score, higher age, a higher serum glucose level number of thrombectomy passes, and lower ASPECTS. Key predictors for HT in the published literature, identified here, will form the basis for future studies. However, given the large disparities and heterogeneity across the included studies, the meta-analysis results need to be interpreted with caution, and studies based on larger and multi-center data sets should be prioritized to confirm the results.

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.

Author contributions

JS and CL screened abstracts and titles of potentially relevant studies, screened the full-text papers, and extracted data and assessed the quality independently. JS performed all statistical analysis, drafted the manuscript, and made critical revisions to the manuscript. LC contributed to the design of the study and made critical revisions to the manuscript. MP and AB conceived the study and made critical revisions to the manuscript. LL, CB, and XL critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

QY was employed by Apollo Medical Imaging Technology Pty Ltd.

The remaining 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.2023.1079205/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Risk of bias QUIPS summary.

SUPPLEMENTARY FIGURE 2
Risk of bias QUIPS traffic light.

SUPPLEMENTARY FIGURE 3
The proportions of sICH definitions in included studies.

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Low-dose vs. standard-dose alteplase for Chinese patients with acute ischemic stroke: A propensity score analysis

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Background and purpose: Previous studies have stimulated debates on low-dose alteplase administration in acute ischemic stroke (AIS) among the Asian population. We sought to evaluate the safety and efficacy of low-dose alteplase in Chinese patients with AIS using a real-world registry.

Methods: We analyzed data from the Shanghai Stroke Service System. Patients receiving alteplase intravenous thrombolysis within 4.5 hours were included. These patients were divided into the low-dose alteplase group (0.55–0.65 mg/kg) and the standard-dose alteplase group (0.85–0.95 mg/kg). Baseline imbalances were adjusted by using the propensity score matching. The primary outcome was mortality or disability, which was defined as the modified Rankin scale (mRS) score ranging from 2 to 6 at discharge. The secondary outcomes were in-hospital mortality, symptomatic intracranial hemorrhage (sICH) and functional independence (mRS score 0–2).

Results: From January 2019 to December 2020, a total of 1,334 patients were enrolled and 368 (27.6%) were treated with low-dose alteplase. The median age of the patients was 71 years, and 38.8% were female. Our study showed that the low-dose group had significantly higher rates of death or disability (adjusted odds ratio (aOR) = 1.49, 95% confidence interval (CI) [1.12, 1.98]) and less functional independence (aOR = 0.71, 95%CI [0.52, 0.97]) than the standard-dose group. There was no significant difference in sICH or in-hospital mortality between the standard-dose and low-dose alteplase groups.

Conclusions: Low-dose alteplase was related to a poor functional outcome without lowering the risk of sICH, compared with standard-dose alteplase for AIS patients in China.

KEYWORDS

ischemic stroke, thrombolysis, acute stroke therapy, recombinant tissue plasminogen activator, symptomatic intracranial hemorrhage

1. Introduction

Intravenous thrombolysis using alteplase (0.9 mg/kg) for acute ischemic stroke (AIS) within 4.5 hours of onset has been proven effective (1, 2). Despite the solid evidence on the efficacy of alteplase, a higher risk of symptomatic intracranial hemorrhage (sICH) after thrombolysis still cannot be ignored. Hence, some Japanese trials compared two alteplase dosages of low dose (0.6 mg/kg) and standard dose (0.9 mg/kg) in AIS patients and indicated that a low dose of alteplase might be more suitable for Asian people (3, 4). Furthermore,

the low-dose alteplase treatment has been strongly recommended for Japanese AIS patients within 4.5 hours by Japan Stroke Society Guidelines (5).

However, some studies in China suggested that the standard dosage might have better clinical outcomes than the low-dose alteplase without increasing the incidence of sICH (6, 7). The ENhanced Control of Hypertension And Thrombolysis strokE study (ENCHANTED) trial, as the only randomized controlled trial (RCT), has reported a lower incidence of sICH in the low-dose alteplase group but not achieved non-inferiority of low-dose to standard-dose alteplase for death or disability at 90 days (8). In our previous pool-data analysis, the low dosage was still considerable based on several regional databases (9).

Due to the high cost of a new RCT and the narrow gap expected to be found between dosages, we tried to investigate whether low-dose alteplase can bring better safety and a comparable functional outcome than standard dosage in Chinese patients with AIS using our real-world database.

2. Materials and methods

2.1. Study population

All data of this study were obtained from the Shanghai Stroke Service System (4S) registry, which was designed as a regional stroke network tracking real-time data on stroke care performance in Shanghai metropolitan area. The protocol of the 4S network has been reported previously and this study was approved by Institutional Review Board in Huashan hospital, with the waiver of consent as no identifying information was collected (10).

At all stroke centers in Shanghai, patients 18 years or older diagnosed with ICD-10 stroke codes (I63, I61, and G45) at discharge were registered in the 4S database. The eligibility criteria included: (1) aged 18 years or older; (2) had a clinical diagnosis of ischemic stroke with ICD-10 codes I63 at discharge; (3) received thrombolysis treatment within 4.5 hours of symptom onset; (4) no definite indication nor contraindication for either dose of alteplase. Patients with incomplete medical records were excluded. Based on the dosage of alteplase they used, the eligible patients were divided into low-dose alteplase group (0.55–0.65 mg/kg) and standard-dose alteplase group (0.85–0.95 mg/kg).

2.2. Data collection

Each stroke center in Shanghai used the electronic medical record with the web-based collection tools to document the stroke care procedures and automatically extract all medical data (10). Data of eligible AIS patients receiving thrombolysis with alteplase were analyzed in our study.

Abbreviations: 4S, Shanghai Stroke Service System; AF, atrial fibrillation; AIS, acute ischemic stroke; aOR, adjusted odds ratio; CI, confidence interval; ENCHANTED, The ENhanced Control of Hypertension And Thrombolysis strokE study; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; PSM, Propensity score matching; RCT, randomized controlled trial; sICH, symptomatic intracranial hemorrhage.

2.3. Outcomes

The primary functional outcome was death or disability, defined as modified Rankin scale (mRS) score 2-6 at discharge. The secondary functional outcome included in-hospital mortality, sICH, functional independence (mRS score 0–2) and distribution of scores on the mRS (ordinal shift analysis). The definition of sICH was Heidelberg Bleeding Classification (11).

2.4. Data analysis

Continuous data were expressed as mean (standard deviation) or median (interquartile range), and categorical data were described as frequency and percentage. Wilcoxon rank-sum test was used for continuous outcomes. All categorical variables were tested by Pearson χ^2 test or Fisher's exact test separately.

Propensity score matching (PSM) was used to adjust baseline imbalances. The propensity score was estimated with the multivariable logistic regression model, with the IV-alteplase dose (standard dosage and low dosage) as the dependent variable and all baseline characteristics in Table 1 as covariates. Patients with low-dose therapy were matched 1:4 to those with standard-dose therapy according to the propensity score, with replacement, using the nearest neighbor matching with a 0.2 caliper (propensity score-matched cohort). Bias reduction after PSM was evaluated using standardized mean differences in covariate means. We performed a sensitivity analysis to assess the robustness of the results with a 1:1 matched design.

Logistic regression was used to compare the functional outcome between the standard- and low-dose alteplase groups. When comparing the outcomes, cumulative incidences with a 95% confidence interval (CI) were presented and adjusted by patient features, including age, sex, history of ischemic stroke, hypertension, diabetes, dyslipidemia, atrial fibrillation (AF), myocardial infarction, baseline National Institutes of Health Stroke Scale (NIHSS) score, time from onset to treatment, premorbid modified Rankin scale (mRS) score and TOAST classification. The functional outcomes in subgroups were divided based on demographic variables (sex, age $\leq$ 70 vs. >70 years), TOAST classification, clinical severity (baseline NIHSS scores $\leq$ 10 vs. > 10), time from onset to treatment ($\leq$ 3 vs > 3 hours) and premorbid mRS (0–1 vs. 2–5). All tests were 2-sided and a p-value < 0.05 was considered statistically significant. Data analyses were conducted with Stata/SE 15.0.

3. Results

From Jan 2019 to Dec 2020, a total of 4995 patients received thrombolysis with alteplase. After excluding the cases with missing data (ie, the un-noted dosage of alteplase, treatment extended to the 4.5-hours window, absence of the mRS score or the NIHSS score at discharge), 3,179 patients were included in the study. The flowchart was shown in Figure 1. Baseline characteristics were shown in Supplementary Table 1. The patients in the low-dose group were older than those in the standard-dose group. Also, the low-dose group had a higher proportion of patients with a history of stroke,

TABLE 1 Baseline characteristics of the patients after propensity score matching.

	Standard-dose alteplase group, No. (%)	Low-dose alteplase group, No. (%)	*P*-value
Patients, No.	966	368	
Age, y, median (IQR)	71 (63, 79)	75 (64, 86)	**<0.001**
Men	1,775 (64.8)	268 (61.0)	0.44
Medical history			
Stroke	195 (20.2)	81 (22.0)	0.46
Diabetes	218 (22.6)	84 (22.8)	0.92
Hypertension	592 (61.3)	223 (60.6)	0.82
Dyslipidemia	30 (3.1)	10 (2.7)	0.71
Myocardial infarction	12 (1.2)	4 (1.1)	0.82
Atrial fibrillation	101 (10.5)	44 (12.0)	0.43
Smoking	302 (31.3)	102 (27.7)	0.21
TOAST classification			**<0.001**
Large-artery atherosclerosis	502 (52.0)	205 (55.7)	
Cardioembolism	149 (15.4)	59 (16.0)	
Small-vessel occlusion	264 (27.3)	70 (19.0)	
Other/unknown	51 (5.3)	34 (9.2)	
Premorbid mRS score $\leq$ 3	883 (91.4)	332 (90.2)	0.50
NIHSS, median (IQR)	4 (2,9)	4 (2,10)	0.79
LDL, mg/dL, median (IQR)	2.8 (2.2, 3.4)	2.8 (2.2, 3.4)	0.95
Time from stroke onset to intravenous thrombolysis, hr, median (IQR)	2.6 (1.9, 3.5)	2.5 (1.8, 3.4)	0.54

IQR, interquartile range; SD, standard deviation; CI, confidence interval; LDL, low-density lipoprotein; NIHSS, National Institutes of Health Stroke Scale [range, 0–42 (most severe)]; mRS, modified Rankin scale [range, 0-6 (most severe)]; y, years; hr, hours. P $\leq$ 0.05 was shown in bold.

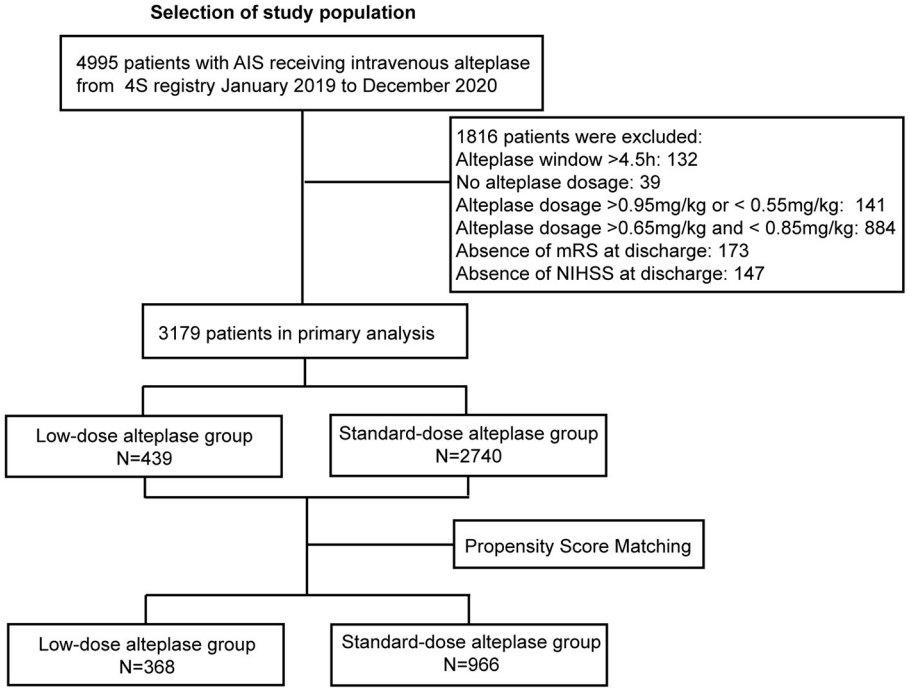

FIGURE 1
Study flow chart and numbers of eligible patients in each group. 4S, Shanghai Stroke Service System; AIS, acute ischemic stroke; NIHSS, National Institutes of Health Stroke Scale [range, 0–42 (most severe)]; mRS, modified Rankin scale [range, 0–6 (most severe)].

TABLE 2 Outcome according to assigned treatment in the propensity score matching dataset.

Outcome	Standard-dose alteplase, No. (%)	Low-dose alteplase, No. (%)	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)†	*P*-value
Primary						
mRS 2-6	373/966 (38.6)	179/368 (48.6)	1.51 (1.18, 1.92)	**0.001**	1.49 (1.12, 1.98)	**0.006**
Secondary						
sICH	30/966 (3.1)	8/368 (2.2)	0.69 (0.31, 1.53)	0.36	0.59 (0.25,1.37)	0.22
In-hospital mortality	5/966 (0.5)	4/368 (1.1)	2.11 (0.56, 7.91)	0.27	1.93 (0.47, 7.90)	0.36
mRS 0-2	718/966 (74.3)	243/368 (66.0)	0.67 (0.52, 0.87)	**0.003**	0.71 (0.52, 0.97)	**0.03**
mRS, median (IQR)	1 (0, 3)	1(1,3)	1.36 (1.10, 1.69)	**0.005**	1.21 (0.97, 1.51)	0.09

The propensity score was derived from the model with sex, age, history of ischemic stroke, hypertension disease, diabetes, dyslipidemia, atrial fibrillation, myocardial infarction, baseline NIHSS score, premorbid modified Rankin Scale, time from onset to treatment, baseline LDL-C and TOAST classification. IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale [range, 0–42 (most severe)]; mRS, modified Rankin scale [range, 0–6 (most severe)]; OR, odds ratio. †Adjusted for age, sex, history of ischemic stroke, hypertension disease, diabetes, dyslipidemia, atrial fibrillation, myocardial infarction, baseline NIHSS, time from onset to treatment, premorbid mRS and TOAST classification. P < 0.05 was shown in bold.

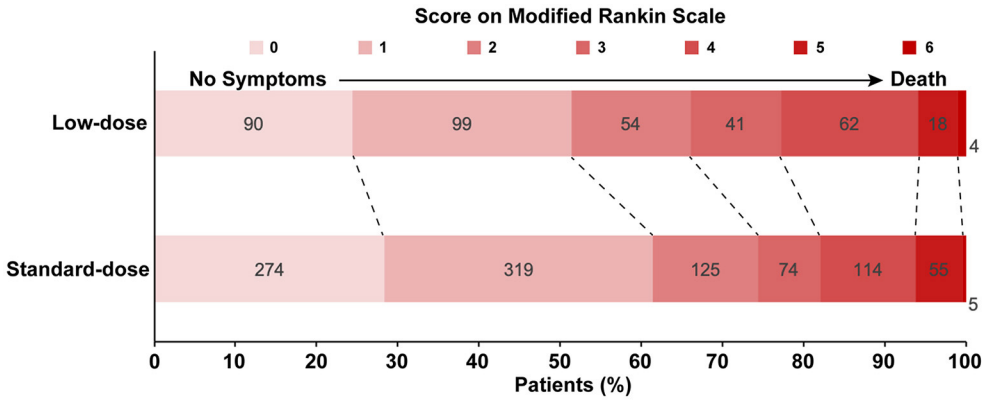

FIGURE 2
Distribution of Modified Rankin Scale Scores on discharge by low-dose vs. standard-dose alteplase in propensity score matching dataset.

AF, or disability. After adjustment of PSM, 1,334 AIS patients were included in the final analysis. Among them, 368 (27.8%) patients received low-dose alteplase. The median age of the patients was 71 years, and 38.8% were female. The baseline characteristics in both groups after PSM were presented in Table 1. After PSM, the baseline characteristics were balanced except for age and TOAST classification; the absolute standardized differences were basically within an acceptable margin of 0.1 (Supplementary Table 2).

The primary and secondary outcomes were summarized in Table 2. The low-dose group showed its association with a higher rate of mortality or disability (adjusted odds ratio (aOR) = 1.49, 95%CI [1.12, 1.98], $p = 0.006$) than the standard-dose group. Treatment with low-dose alteplase also resulted in a lower rate of mRS score 0-2 than that with standard-dose alteplase (aOR = 0.71, 95% CI [0.52, 0.97], $p = 0.03$). The results were similar to the univariate regression model. The distribution of mRS scores at discharge was shown in Figure 2. The common OR across the mRS scores between the low- and standard-dose alteplase was 1.36 (95% CI [1.10, 1.69]) in the univariate analysis, but without statistical significance after adjustment of baseline characteristics. In addition, no significant difference was found in the risk of in-hospital mortality or sICH for two groups. The sensitivity analysis for a 1:1 matched design showed generally similar results (Supplementary Table 3). Other serious adverse events during the hospitalization according to assigned treatment were presented in Table 3. There was a trend toward a higher incidence of pulmonary embolism in the low-dose alteplase group.

We performed subgroup analyses by age, gender, stroke subtypes, stroke severity, time from onset to treatment, and premorbid mRS score (Figure 3). All characteristics did not modify the treatment effect, except for stroke severity ($p = 0.02$). The patients with baseline NIHSS score ≤ 10 benefited more from the standard-dose alteplase therapy (OR = 1.56, 95%CI [1.16, 2.11], $p = 0.003$).

4. Discussion

Our study demonstrated that patients receiving low-dose alteplase were associated with a higher risk of death or disability than those receiving standard-dose alteplase. Moreover, patients treated with low-dose alteplase were not associated with a lower sICH rate, and tend to have a higher rate of pulmonary embolism. Mild and moderate stroke patients with a baseline NIHSS score

≤ 10 may have better outcomes when treated with standard-dose alteplase.

Despite that a dose of 0.9 mg/kg alteplase is widely recommended by most guidelines for AIS patients (12–14), the high risks of sICH after thrombolysis with alteplase cannot be ignored, particularly among Asians (15). The J-ACT trial showed that the rate of mRS 0-1 at 90 days and the incidence of sICH in Japanese AIS patients receiving 0.6 mg/kg alteplase were comparable to 0.9 mg/kg alteplase treatment of the NINDS trial (1, 4). The results of several non-randomized trials in Japan subsequently showed the similar efficacy and safety outcome of low dosage thrombolysis with alteplase when compared with the standard dosage (3, 16, 17). In 2016, the ENCHANTED trial, as the only RCT involving 3310 AIS patients (63% Asian), aimed to compare low-dose with standard-dose alteplase. Although the trial failed to achieve the non-inferiority of low-dose as compared to standard-dose alteplase concerning the outcome of death or disability at 90 days (8), it also did not prove the superiority of standard-dose alteplase. There were significantly fewer sICH and trends toward the lower mortality rate with low-dose alteplase. Therefore, low-dose alteplase performed well in the safety outcome, which provided an alternative approach for both doctors and patients to make the personalized decision for the individual patient with AIS.

Compared with the ENCHANTED trial, our study achieved a similar efficacy outcome. What is new in our study, clinical physicians were more likely to administrate low dosage due to elder age and combined with AF. Our study found no association between alteplase dosage and the rate of sICH or mortality, which was different from the ENCHANTED trial. However, our findings were consistent with the results of TIMS-China (6, 7). An Asian stroke registry study also found no association between alteplase dosage and sICH risk (9). Our results might be the reflection of the real-world or false-negative error, attributed to the confounding bias of the observational study.

TABLE 3 Other serious events during hospitalization according to assigned treatment.

Variable	Standard-dose alteplase group, No. (%)	Low-dose alteplase group, No. (%)	*P*-value
Recurrent stroke	44 (4.6)	16 (4.4)	0.87
Cardiac ischemia	5 (0.5)	2 (0.6)	1.00
Gastrointestinal Bleeding	14 (1.5)	2 (0.6)	0.26
Pulmonary embolism	6 (0.6)	7 (1.9)	0.06
Seizure	11 (1.1)	1 (0.3)	0.20
Hydrocephalus	1 (0.1)	0 (0)	1.0
Deep venous thrombosis	8 (0.8)	1 (0.3)	0.46

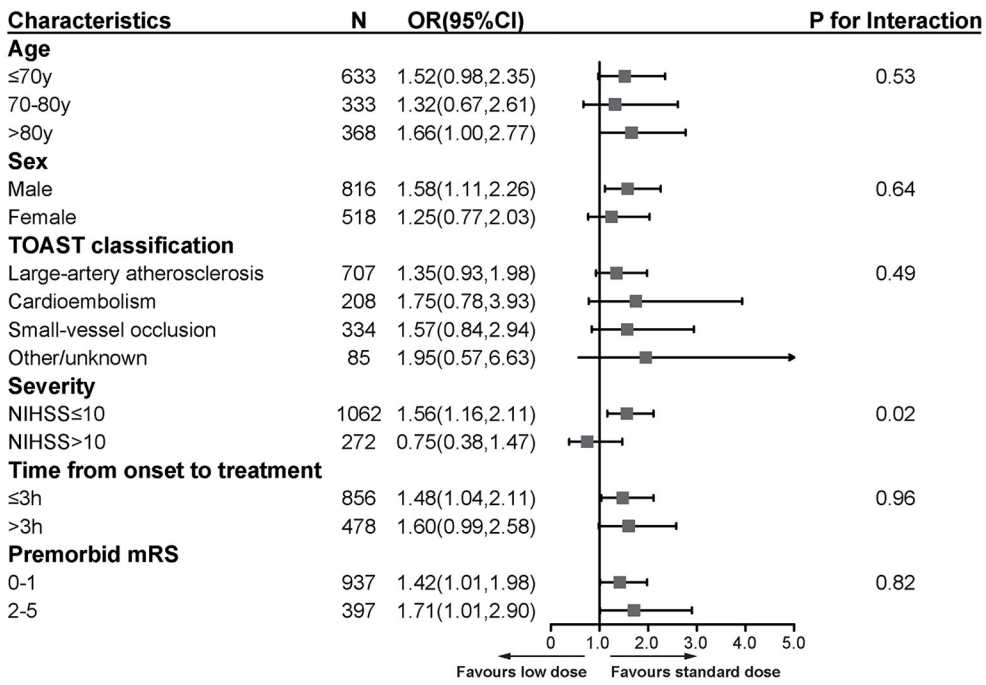

FIGURE 3
Subgroup analyses of primary functional outcome between low-dose and standard-dose group in propensity score matching dataset. The forest plot shows the difference in the primary functional outcome across all subgroups. The primary functional outcome was death or disability at discharge, defined by scores of 2 to 6 on the mRS [range, 0 (no symptoms) to 6 (death)]. The OR was calculated by using logistic regression, taking the following variables into account: age, sex, medical history (ischemic stroke, hypertension disease, diabetes, dyslipidemia, atrial fibrillation, myocardial infarction), baseline NIHSS, time from onset to treatment, premorbid score and TOAST classification. NIHSS, National Institutes of Health Stroke Scale [range, 0–42 (most severe)]; mRS, modified Rankin scale [range, 0–6 (most severe)]; OR, odds ratio.

In subgroup analyses, we found that patients with mild-moderate stroke (NIHSS $\leq$ 10) in the standard-dose group were more likely to show a favorable outcome. However, the significant heterogeneity of stroke severity might be caused by a small sample of patients with a baseline NIHSS > 10. Our previous study showed that in mild stroke patients (NIHSS $\leq$ 4), there was no difference in the sICH risk and clinical improvement between both dosages (18). Therefore, more studies on the effectiveness and safety of low-dose thrombolysis for patients with different stroke severity are still needed.

The ENCHANTED trial raised the concern of the high risk of sICH in standard-dose alteplase treatment. Considering the bleeding-prone constitution of the Asian population, a secondary analysis of the ENCHANTED trial showed similar efficacy and safety outcomes of both doses of alteplase in both Asians and non-Asians (19). Also, the clinical use of low-dose alteplase in AIS patients with older age and other high-bleeding risks remained uncertain. Our subgroup study suggested that low-dose alteplase might be related to unfavorable functional outcomes even in patients older than 80.

Our study had several limitations. Firstly, our research population was limited to the Shanghai metropolitan. The management of risk factors and other demographic characteristics may not extend to that of other regions in China. Secondly, it was an observational retrospective study based on a registry database, the confounding bias cannot be ignored even with PSM. Thirdly, the relatively small sample size of the low-dose group might have impacted the statistical results. Fourthly, our study lacked mortality and functional outcomes in the long-term follow-up. Considering these limitations, further well-designed studies for patients with specialized characteristics are needed to provide more reference to medical practice.

5. Conclusions

Our observational study indicated that Chinese AIS patients receiving low-dose alteplase might be associated with an unfavorable functional outcome without lowering the risk of sICH. Our findings from the real-world dataset might provide more evidence to support standard-dose alteplase regimen for AIS patients in clinical practice.

Data availability statement

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

Ethics statement

This study was approved by the Institutional Review Board in Huashan Hospital, with the waiver of consent as no identifying information was collected.

Author contributions

JX, XC, YX, YW, and SC performed the analysis. JX, XC, and YX drafted the manuscript. QD, YD, and KF revised the manuscript. YD and KF concepted this study, supervised the analysis, and finalized 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.

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Supplementary material

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Derivation and validation of a composite scoring system ($SAVED_2$) for prediction of unfavorable modified Rankin scale score following intracerebral hemorrhage

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Objective: To develop a composite score for predicting functional outcome post–intracerebral hemorrhage (ICeH) using proxy measures that can be assessed retrospectively.

Methods: Data from the observational ERICH study were used to derive a composite score ($SAVED_2$) to predict an unfavorable 90-day modified Rankin scale (mRS) score. Independent predictors of unfavorable mRS were identified *via* multivariable logistic regression and assigned score weights based on effect size. Area under the curve (AUC) was used to measure the score's discriminative ability. External validation was performed in the randomized ATACH-2 trial.

Results: There were 2,449 patients from ERICH with valid mRS data who survived to hospital discharge. Predictors associated with unfavorable 90-day mRS score and their corresponding point values were: age ≥70 years (odds ratio [OR], 3.8; 1-point); prior stroke (OR, 2.8; 1-point); need for ventilation (OR, 2.7; 1-point); extended hospital stay (OR, 2.7; 1-point); and non-home discharge location (OR, 5.3; 2-points). Incidence of unfavorable 90-day mRS increased with higher $SAVED_2$ scores ($P < 0.001$); AUC in ERICH was 0.82 (95% CI, 0.80–0.84). External validation in ATACH-2 ($n = 904$) found an AUC of 0.74 (95% CI, 0.70–0.77).

Conclusions: Using data collected at hospital discharge, the $SAVED_2$ score predicted unfavorable mRS in patients with ICeH.

KEYWORDS

cerebral hemorrhage, risk prediction, modified Rankin scale score, $SAVED_2$ score, hemorrhagic stroke

Introduction

Functional outcome following stroke is clinically meaningful and of major relevance to patients. Tools, such as the modified Rankin scale (mRS), have been developed for the accurate assessment of post-stroke functional outcome (1–3). However, functional outcome data are often unavailable or difficult to collect from real-world sources, such as electronic health records and administrative claims databases.

When long-term functional outcome data are not available, surrogate or proxy measures provide alternative methods to assess post-stroke functional outcome. Proxy measures have included discharge destination (4) and home-time calculations (5), both of which strongly correlate with functional outcome measures between 3 and 12 months post-stroke (6). For instance, in a systematic literature review, Costa et al. found that being discharged to a location other than home was associated with an unfavorable mRS score among the 2 studies that assessed this relationship and that increased home-time post-stroke was associated with improved functional outcomes (6). The majority of proxy measure evaluations have been performed in ischemic stroke populations, while proxy measures in hemorrhagic stroke have not been well characterized.

Composite risk models/scores are used to combine various known risk factors and translate them into a more easily interpretable risk assessment of an individual experiencing a particular outcome (7). Here, we sought to develop a composite scoring system to predict post-intracerebral hemorrhage (ICeH) functional outcome using various proxy measures and risk factors that can be assessed retrospectively with ease and accuracy. By developing a score to predict functional outcomes, we hope to support researchers in better characterizing outcomes post-ICeH among large populations where long-term measures of functional status may not be available.

Materials and methods

Study population

We analyzed individual patient-level data from 2 distinct, prospective clinical studies funded by the National Institute of Neurological Disorders and Stroke (Table 1).

The Ethnic/Racial Variations of Intracerebral Hemorrhage (ERICH) study was a multicenter, prospective, case-control study designed to recruit 1,000 non-Hispanic White, 1,000 non-Hispanic Black, and 1,000 Hispanic patients with spontaneous ICeH to identify risk factors among different races and ethnicities (8). The ERICH study allowed the inclusion of critically ill patients with ICeH, including those with Glasgow Coma Scale (GCS) score <5, intraventricular bleeding, and infratentorial bleeds (8–10). ERICH also included patients who had received anticoagulation prior to ICeH, and they comprised 13.9% of the White, 7.2% of the Hispanic, and 4.7% of the Black cohorts (11).

The Antihypertensive Treatment of Acute Cerebral Hemorrhage-2 (ATACH-2) multicenter, randomized, open-label trial evaluated the efficacy of early, intensive, antihypertensive intravenous nicardipine treatment in 1,000 patients with spontaneous ICeH (12). The study excluded patients with hemorrhage ≥60 mL, GCS score <5 at emergency department arrival, infratentorial bleeding (e.g., pons or cerebellum) and intraventricular extension, international normalized range (INR) >1.5, use of dabigatran or other oral anticoagulants, or pre-morbid disability requiring assistance in ambulation or activities of daily living (ADL). The primary outcome was death or disability (mRS score of 4–6, on a scale ranging from 0 [no symptoms] to 6 [death]) at 3 months after randomization. Details of the study design, patient populations, and methods, including those used to determine ICeH location and volume, have been previously described for both the ERICH (8) and ATACH-2 (12) studies.

Both ERICH (8) and ATACH-2 (12) reported mRS score at time points ranging from 1 month to ≥6 months post–hemorrhagic stroke. All patients in ERICH and ATACH-2 were eligible for inclusion in the current study if they survived to discharge and had a total hospital length of stay (LOS) <90 days. Patients were excluded if discharge destination data, age, mRS score, need for intubation/ventilation, hospital LOS, or prior stroke history were missing or recorded as "unknown." Attrition diagrams for ERICH and ATACH-2 are shown in Figure 1.

Outcomes

Based on proxy measures identified in a prior systematic literature review (6) and available ERICH and ATACH-2 data, potential proxy measures of functional status assessed in this study included discharge destination to home (including home healthcare or relative's/friend's home) vs. non-home locations; extended hospital LOS (defined as hospital LOS ≥8 days); and need for endotracheal intubation and ventilation. Functional outcome status was assessed using mRS at 30, 90, and 180 days after ICeH.

Statistical analysis

The association between proxy measures and unfavorable outcome classification (defined as a mRS score of 3–6) was assessed. The discriminative ability (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], and area under the curve [AUC]) of each proxy measure with unfavorable functional outcome was evaluated.

To derive a composite score to predict 90-day mRS score, we used proxy measures available at hospital discharge (i.e., need for intubation/ventilation, extended LOS, and discharge destination) from the ERICH study. Selected proxy measures were augmented with variables—advanced age (≥70 years) and prior stroke history—as they are included in well-known clinical risk prediction tools for patients with ICeH (i.e., the Intracranial Hemorrhage [ICH] and Functional Outcome in Patients With Primary ICeH [FUNC] scores) (13) and they are also available in real-world datasets.

We used multivariable logistic regression to identify independent predictors of unfavorable mRS score. Based on the effect size, score weights were assigned to significant proxies/covariates to create the prior **S**troke history per chart history, **A**ge ≥70 years, need for **V**entilation, **E**xtended hospital LOS ≥8 days, **D**ischarge to locations other than home (SAVED) score. A simplified score ($SAVED_2$) was also derived by assigning points to each covariate based on the relative weight of each predictor's beta-coefficient to make interpretation easier for clinicians at the bedside. Discriminative ability of $SAVED_2$ was assessed using AUC. Internal validation of the $SAVED_2$ score was performed using data from the ERICH trial and external validation

TABLE 1 Summary of the ERICH and ATACH-2 study designs and overall patient populations.

	ERICH (8, 10)	ATACH-2 (12)
Study design	Multicenter, prospective, case-control study	Randomized, multicenter, open-label trial
Key inclusion criteria	• Adults with spontaneous ICeH, including critically ill patients • Anticoagulation prior to ICeH was permitted	• Adults with spontaneous supratentorial ICeH • Volume <60 mL • GCS score ≥5 • INR <5
Key exclusion criteria	• Malignancies that lead to coagulopathy • Dural venous sinus thrombosis-associated hemorrhage • Hemorrhages attributable to vascular malformations, aneurysms, tumors, or hemorrhagic conversion of recent ischemic stroke	• ICeH related to trauma • ICeH located in infratentorial regions, such as the pons or cerebellum • IVH associated with intraparenchymal hemorrhage and blood completely fills 1 lateral ventricle or more than half of both ventricles • Use of oral anticoagulants within the past 48 h • Pre-morbid disability requiring assistance in ambulation or activities of daily living
Baseline characteristics	***N* = 2,568**	***N* = 1,000**
Mean age, years	62.4	61.9
Male, *n* (%)	1499 (58.4)	620 (62.0)
Race, *n* (%)		
Asian	0	562 (56.2)
White	860 (33.5)	287 (28.7)
Black	829 (32.3)	131 (13.1)
Hispanic ethnicity, *n* (%)	879 (34.2)	79 (7.9)
ICeH location, *n* (%)		
Deep	1,347 (52.5)	NA
Thalamus	NA	373 (37.8)*
Basal ganglia	NA	506 (51.2)*
Lobar	800 (31.2)	108 (10.9)*
Cerebellar	193 (7.5)	1 (0.1)*
Brainstem	134 (5.2)	0
IVH, *n* (%)	1,089 (42.4)	264 (26.7)*
GCS score		
Mean (SD) GCS score	mRS 0–3: 13.9 (2.5)	NA
	mRS 4–6: 10.8 (4.2)	NA
3–11 GCS score, *n* (%)	NA	147 (14.7)
12–14 GCS score, *n* (%)	NA	294 (29.4)
15 GCS score, *n* (%)	NA	559 (55.9)

*$N = 988$. GCS, Glasgow Coma Scale; ICeH, intracerebral hemorrhage; INR, international normalized range; IVH, intraventricular hemorrhage; mRS, modified Rankin scale; NA, not available; SD, standard deviation.

conducted using data from the ATACH-2 trial. All analyses were conducted with IBM SPSS, version 27 (IBM Corp., Armonk, NY).

Results

Derivation population (ERICH cohort)

Of the 3,000 patients with ICeH in the ERICH study, 2,449 patients were included in ≥1 of the analyses at 90, 180, or 365 days (Figure 1A). Of the 2,175 patients who survived to discharge and who had valid mRS data at 90 days after ICeH, 2,151 patients had data available at 180 days and 2,075 patients had data available at 365 days.

Nearly one-third of the patients were aged ≥70 years (30%), the median GCS score was 15 (range, 3–15), and 17% had a prior history of stroke (Table 2). Between 53.4 and 58.1% of patients had an unfavorable mRS score (3–6) at Days 30 through 365. Just over two-thirds of patients (68%) were discharged to a location other than home (Table 2). Using discharge destination as a proxy for unfavorable functional outcome yielded high sensitivity (86%), PPV (range, 67–73%), and NPV (range, 74–76%) at 90, 180, and 365 days (Table 3). More than half of the patients (53%) had an extended hospital LOS (Table 2). Extended

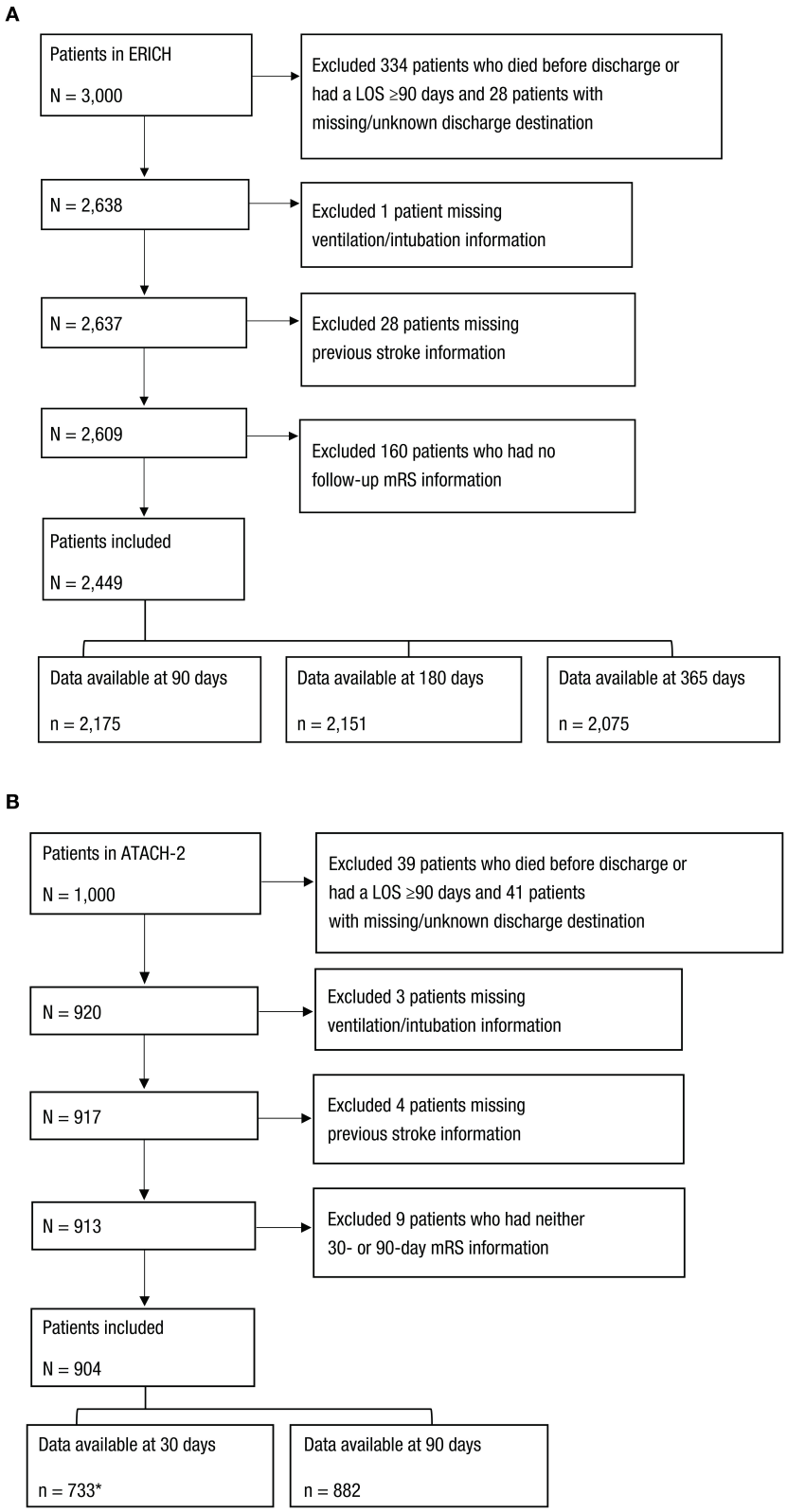

FIGURE 1
Selection of Patients From the **(A)** ERICH and **(B)** ATACH-2 Studies. *Excluded patients with a LOS ≥30 days. LOS, length of stay; mRS, modified Rankin scale.

TABLE 2 Demographics and clinical characteristics of the ERICH and ATACH-2 study populations included in the analysis.

Characteristic	ERICH (derivation cohort) $N = 2,449$	ATACH-2 (validation cohort) $N = 904$
Age ≥70 years, *n* (%)	729 (29.8)	240 (26.5)
Male sex, *n* (%)	1,429 (58.4)	559 (61.8)
White race *n* (%)	1,570 (64.1)	236 (26.1)
Country, *n* (%)		
United States	2,449 (100)	359 (39.7)
Japan	–	285 (31.5)
China	–	127 (14.0)
Other	–	133 (14.8)
Median GCS (range)	15 (3–15)	15 (3–15)
Median intracerebral bleed volume, mL (range)	9.5 (0.0–154.0)	9.7 (0.02–71.0)
Prior history of stroke, *n* (%)	420 (17.1)	142 (15.7)
Need for ventilation/intubation, *n* (%)	691 (28.2)	91 (10.1)
Hospital LOS ≥8 days, *n* (%)	1,306 (53.3)	673 (74.4)
Discharge to location other than home/relative's or friend's home, *n* (%)	1,667 (68.1)	633 (70.0)

GCS, Glasgow Coma Scale; LOS, length of stay.

LOS was modestly discriminative for predicting unfavorable functional outcome, ranging from 66 to 68% for sensitivity, 60 to 65% for specificity, 66 to 73% for PPV, and 59 to 61% for NPV across the different time points (Table 3). Twenty-eight percent of patients were ventilated/intubated during hospitalization for ICeH (Table 2). Using ventilation/intubation as a proxy for unfavorable functional outcome yielded high specificity (range, 86–87%) and PPV (77–82%), and lower NPV (range, 51–56%) and sensitivity (range, 41–42%) at 90, 180, and 365 days (Table 3).

Derivation of $SAVED_2$

To make a tool that clinicians can use at the patient bedside, we next derived a composite risk score to predict unfavorable outcome at 90 days for patients with ICeH who survived to discharge. Utilizing the ERICH population as the training database, we identified characteristics independently associated with unfavorable 90-day mRS score (3–6) and assigned points to each covariate based on the relative weight of each predictor's beta-coefficient using multivariable logistic regression. Among the 2,175 ERICH patients with available data, predictors independently associated with unfavorable 90-day mRS scores and their corresponding point values included: prior stroke history (odds ratio [OR], 2.8; 1 point), age ≥70 years (OR, 3.8; 1 point), need for ventilation (OR, 2.7; 1 point), extended hospital LOS ≥8 days (OR, 2.7; 1 point), and discharge to a location other than home (OR, 5.3; 2 points) (Table 4). The use of anticoagulation was also evaluated in the logistic regression model but was not found to be significantly associated with unfavorable mRS score (OR, 1.10; 95% confidence interval [CI], 0.77–1.56), potentially due to low utilization of anticoagulants (<10%) in the ERICH population.

The incidence of unfavorable 90-day mRS scores increased across neighboring and worsening $SAVED_2$ scores ($P < 0.001$) (Table 5). Unfavorable mRS scores at 90 days were seen in 36 of 289 patients (12.5%) with a $SAVED_2$ score of 0 and in 195 of 207 patients (94.2%) with a $SAVED_2$ score of 5 to 6. In the ERICH cohort, $SAVED_2$ had an excellent ability to discriminate between patients likely to have an unfavorable (3–6) vs. favorable (0–2) mRS score at 90 days (AUC, 0.82; 95% CI, 0.80–0.84; Table 6). A $SAVED_2$ score ≥3 predicted an unfavorable mRS score at 90 days with 81% sensitivity, 71% specificity, 80% PPV, 73% NPV, and 77% overall accuracy.

External validation population (ATACH-2 cohort)

Of 1,000 patients with ICeH in ATACH-2, 904 patients were included in this analysis (Figure 1B). There were 733 patients with mRS data available at 30 days and 882 patients with mRS data available at 90 days.

About one-quarter of the patients were aged ≥70 years (27%), the median GCS score was 15 (range, 3–15), and 16% had a prior history of stroke (Table 2). Unfavorable mRS scores of 3 to 6 were seen in 67% of patients at Day 30 and 54% of patients at Day 90. Most patients (70%) were discharged to a location other than home (Table 2). When using non-home discharge destination as a proxy for unfavorable mRS, sensitivity (83 and 85%) and PPV (80 and 65%) were high, and specificity (58 and 48%) was lower at 30 and 90 days, respectively (Table 3). Nearly three-quarters of patients (74.4%) had an extended hospital

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TABLE 3 Discriminative ability of proxy measures for unfavorable functional outcome (mRS Score 3–6) in ERICH and ATACH-2.

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
ERICH					
Non-home discharge destination					
90 days	86	56	73	74	74
180 days	86	52	69	74	70
365 days	86	50	67	76	70
Extended hospital LOS					
90 days	68	65	73	59	67
180 days	68	62	69	61	65
365 days	66	60	66	61	63
Ventilation/intubation					
90 days	41	87	82	51	60
180 days	42	87	80	54	62
365 days	42	86	77	56	62
ATACH-2					
Non-home discharge destination					
30 days	83	58	80	62	75
90 days	85	48	65	74	68
Extended hospital LOS					
30 days	81	37	73	48	66
90 days	81	32	58	59	58
Ventilation/intubation					
30 days	14	98	95	37	42
90 days	17	98	92	51	55

LOS, length of stay; mRS, modified Rankin scale; NPV, negative predictive value; PPV, positive predictive value.

TABLE 4 Derivation of $SAVED_2$ in ERICH: association of covariates with unfavorable mRS score (3–6) upon multivariable logistic regression and corresponding point assignment in $SAVED_2$.

	Beta-coefficient	OR	Lower 95% CI	Upper 95% CI	Point assignment
Stroke history (S)	1.04	2.82	2.09	3.81	1
Age ≥70 years (A)	1.33	3.76	2.95	4.81	1
Ventilation (V)	0.98	2.66	2.03	3.48	1
Extended hospital LOS (E)	1.01	2.75	2.18	3.47	1
Discharge: Not home (D)	1.67	5.32	4.23	6.69	2
Constant	−2.12	–	–	–	–

CI, confidence interval; LOS, length of stay; mRS, modified Rankin scale; OR, odds ratio.

LOS (Table 2). Using extended LOS as a proxy for unfavorable mRS yielded a high sensitivity (81%) and low specificity (37 and 32%) at 30 and 90 days, respectively (Table 3). Ten percent of patients were ventilated/intubated during the study (Table 2). When using ventilation/intubation as a proxy for unfavorable mRS, specificity (98 and 98%) and PPV (95 and 92%) were high, whereas sensitivity was low (14 and 17%) at 30 and 90 days, respectively (Table 3).

External validation of $SAVED_2$

External validation in 882 eligible patients from ATACH-2 suggested that $SAVED_2$ maintained good discriminative ability at 90 days (AUC, 0.74; 95% CI, 0.70–0.77; Table 6) within a dataset including patients with different baseline characteristics and bleed severity from the derivation dataset. At shorter (30-day) and longer (180-day) follow-up periods of functional outcome

assessment, $SAVED_2$ appeared to maintain its discriminative ability as evidenced by AUCs remaining in the clinically useful range, which is defined as ≥0.75 (Table 6).

Discussion

In this study, we identified proxy measures for assessing functional outcomes post-stroke and developed a novel scoring tool to predict 90-day functional outcomes based on data points that can be captured retrospectively. Our analysis confirmed that selected proxy measures, including discharge destination, extended hospital LOS, and need for mechanical ventilation, previously identified in the acute ischemic stroke literature (4, 14–16) may be useful in predicting functional outcome status in patients following ICeH. Discharge destination was highly sensitive but not as specific, whereas the need for mechanical ventilation had high specificity but lower sensitivity, suggesting that a composite measure including several of these proxy measures could create a stronger overall measure that accounts for the limited sensitivity or specificity of any given measure. We demonstrated that the use of these identified proxy measures as part of a composite risk score ($SAVED_2$) had a good-to-excellent ability to discriminate between patients likely to have an unfavorable (3–6) compared to favorable (0–2) mRS score at 90 days.

TABLE 5 Distribution of $SAVED_2$ scores in derivation cohort from ERICH.

$SAVED_2$ score	N	Unfavorable mRS score (3–6) at 90 days, *n* (%)
0	289	36 (12.5)
1	268	70 (26.1)
2	335	134 (40.0)
3	475	323 (68.0)
4	601	504 (83.9)
5	188	176 (93.6)
6	19	19 (100.0)

Kruskal-Wallis test: $P < 0.001$. mRS, modified Rankin scale.

These findings are important as accurate assessment of post-ICeH functional outcome is essential for both clinical and real-world evidence studies. $SAVED_2$ has the potential to serve as a tool to approximate functional outcome post-ICeH when standard outcome measures, such as mRS, are unavailable. The score could also be used to look at how factors, such as the hospital level, treatment patterns, and personal characteristics, relate to functional outcomes. In a clinical setting, $SAVED_2$ could be used to predict longer-term, 90-day outcomes at the time of hospital discharge.

While $SAVED_2$ appeared to have good discriminative ability at 30 days, there is additional need to confirm whether 1-month mRS score alone can be a dependable and more efficient outcome measure in clinical trials. Additional analysis of ATACH-2 trial data—including evaluation of agreement, weighted kappa, and assessment of utility-weighted mRS at 30- and 90-days after adjustment for potential confounding—could help address this question, as could developing a model to predict 90-day mRS based on 30-day mRS scores and potential covariates included in the $SAVED_2$ components.

This study is the first, to our knowledge, to report on the development and validation of a tool to assess functional outcomes among patients with ICeH using variables that can be assessed retrospectively. Unlike many studies that use split validation or just internal validation, we derived the $SAVED_2$ score using a large, diverse multiethnic cohort and validated it in a separate cohort, strengthening its generalizability to other external cohorts and populations. However, it is possible that differences in the ERICH and ATACH-2 study populations, such as the broader inclusion criteria used in ERICH to include critically ill patients, may have contributed to the slight discrepancy in AUCs observed between the 2 studies. Another limitation of our study is that the data were derived from and validated in populations with spontaneous ICeH and may not be applicable to patients with ischemic stroke or non-ICeH. Although we excluded patients with missing data on any of the key variables, data completion was high for these fields and few patients had missing data. Another limitation is that we derived $SAVED_2$ in the context of patients experiencing spontaneous ICeH in a pre–direct oral anticoagulant (DOAC) era, with fewer than 10% being on oral anticoagulants at the time of the bleed. Also, patients taking anticoagulants were excluded from the ATACH-2 validation cohort. It is unknown how anticoagulant use might impact results, as patients with anticoagulant-related ICeH may experience poorer

TABLE 6 Discriminative ability of $SAVED_2$ score ≥3 to predict unfavorable outcome (mRS 3–6) in the ERICH (derivation) and ATACH-2 (external validation) cohorts.

	ERICH		ATACH-2	
	90 days (derivation)	180 days (derivation)	30 days (external validation)	90 days (external validation)
Sensitivity, %	81	81	74	78
Specificity, %	71	67	66	58
PPV, %	80	75	82	68
NPV, %	73	73	55	69
Accuracy, %	77	74	72	69
AUC (95% CI)	0.82 (0.80–0.84)	0.80 (0.78–0.82)	0.76 (0.73–0.80)	0.74 (0.70–0.77)

AUC, area under the curve; CI, confidence interval; mRS, modified Rankin scale; NPV, negative predictive value; PPV, positive predictive value.

prognoses compared with patients not on anticoagulants at the time of ICeH (17).

Outcomes for patients with ICeH are very poor. Although it has been decreasing, the mortality burden post-ICeH remains high with a US National Inpatient Sample analysis reporting a 24% inpatient mortality rate (18). Anticoagulant use increases the risk of morbidity and mortality (17). Furthermore, patients who survive the ICeH have a high comorbidity burden. For example, in a German study in 61 patients with DOAC-related ICeH, 28 of 43 survivors (65%) had an unfavorable outcome of mRS 3 to 5, indicating moderate-to-severe disability at 3-month follow-up (19).

Functional outcomes are key for supporting ADL among patients, and we hope that this score can be used to better assess these outcomes among large population-based databases where these data were previously unavailable. To improve outcomes among patients, particularly among those taking anticoagulants, further research is needed to assess the relationship between ICeH and long-term functional outcomes. Utilizing proxy measures such as the $SAVED_2$ composite score may enable real-world studies of the long-term functional status associated with ICeH.

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 Institutional Review Boards/Ethics Committees at the participating sites. The patients/participants provided their informed consent to participate in those studies.

Author contributions

Contributed to the design, conceptualization of the study, and interpretation of data: CC, MCo, BK, BL, MCh, and AC. Statistical analysis: CC. All authors participated in drafting and revising the manuscript for intellectual content and approved the final manuscript.

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Conflict of interest

CC has received research funding from Alexion, AstraZeneca Rare Disease, Bayer AG, Global Blood Therapeutics, and Janssen; has received consulting fees from Alexion, AstraZeneca Rare Disease, and Global Blood Therapeutics; and has received honoraria from Medscape. MCo has received consulting fees from Alexion, AstraZeneca Rare Disease; and has received consulting fees from and served on the speakers' bureau for Portola Pharmaceuticals. BK and MCh are employees of Alexion, AstraZeneca Rare Disease. BL is a former employee of Alexion, AstraZeneca Rare Disease. AC has received research funding, consulting fees, and honoraria from Alexion, AstraZeneca Rare Disease, Bayer AG, and Bristol Myers Squibb/Pfizer. The authors declare that this study received funding from Alexion, AstraZeneca Rare Disease. The funder had the following involvement in the study: Bruce Koch, Belinda Lovelace and Mary J. Christoph are/were employees of Alexion, AstraZeneca Rare Disease and served as co-investigators for the study. They were involved in the study design, collection, analysis, interpretation of data, the writing of this article, and/or the decision to submit it for publication.

Publisher's note

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Liver fibrosis-4 score predicts outcome of patients with ischemic stroke undergoing intravenous thrombolysis

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Some evidence suggests a possible influence of liver disease on stroke prognosis. We investigated the association between fibrosis-4 (FIB-4) score, a marker of liver disease, and the 3-month outcome in patients with ischemic stroke undergoing intravenous thrombolysis. We also evaluated the rate of symptomatic intracranial hemorrhage after thrombolysis. In this prospective cohort study, we enrolled consecutive patients with ischemic stroke treated with thrombolysis who had a 3-month follow-up. The FIB-4 score was calculated and the validated cut-off values were used to indicate high/low risk of advanced liver fibrosis. The primary outcome was 3-month poor prognosis estimated as a modified Rankin scale score ≥3. Of the 264 included patients, 131 (49.62%) had a 3-month mRS ≥3, with a significantly higher FIB-4 score, compared to those with a mRS <3 score ($_{adj}p<0.001$). When adjusted for possible confounders by multivariate logistic regression, FIB-4 score remained a significant predictor of poor outcome (OR 1.894, $p = 0.011$), along with history of atrial fibrillation (OR 3.488, $p = 0.017$), admission NIHSS score (OR 1.305, $p < 0.001$), and low values of hemoglobin (OR 0.730, $p < 0.001$). Mechanical thrombectomy had a favorable effect on patients' outcome (OR 0.201, $p = 0.005$). The risk of poor 3-month outcome was significantly higher among the 32 patients (12.1%) with high risk of severe fibrosis ($p = 0.007$). FIB-4 score values were also related to symptomatic intracranial hemorrhage ($p = 0.004$), specifically among patients with high probability of advanced hepatic fibrosis ($p = 0.037$). FIB-4 score can be considered as a promising independent predictor of poor prognosis in patients with acute ischemic stroke undergoing intravenous thrombolysis.

KEYWORDS

liver fibrosis, acute ischemic stroke, symptomatic intracranial hemorrhage, stroke prognosis, personalized medicine, thrombolysis

1. Introduction

1.1. Background

According to the World Health Organization, 15 million people worldwide suffer from stroke each year. With a mortality rate of approximately one-third, it is the second most common cause of death and a leading cause of disability (1). Ischemic stroke is the most common type of stroke, accounting for approximately 80% of all acute strokes (2). Treatment approaches have been primarily directed at preserving neurons in the ischemic territory. The internationally approved treatments, recombinant tissue plasminogen activator (rt-Pa) and

endovascular intervention, aim at rapid arterial recanalization to restore oxygen and nutrient supply to the affected area (3). Early recanalization after stroke is associated with a greater likelihood of favorable outcome (4, 5).

Liver fibrosis, the histologic precursor of cirrhosis, is a chronic disease (6), often preceded and promoted by an inflammatory process in combination with the accumulation of extracellular matrix in the liver (7). Several biomarkers have been proposed for the assessment of liver fibrosis. Among them, the fibrosis index (FIB)-4 has shown the best diagnostic accuracy for advanced hepatic fibrosis, as demonstrated by ultrasonographic studies in nonalcoholic fatty liver disease (NAFLD) (8, 9), the most common cause of liver dysfunction in Western countries (10). In recent studies, liver disease has been shown to be a strong predictor of both in-hospital and long-term mortality in stroke patients (11, 12). Moreover, it is independently associated with an increased risk of hemorrhagic complications (13), the most threated complication of intravenous thrombolysis, leading to poor outcome and increased risk of mortality (14). It is not yet clear whether these findings can also be applied to subclinical liver disease, which may not be uncommon in patients with stroke (15). In a recently published study, Fandler-Höfler et al. (16) showed that stroke patients with higher FIB-4 score values had worse clinical outcomes 3 months after mechanical thrombectomy but they didn't find any increased risk of postoperative parenchymal hematoma, hemorrhagic infarction and symptomatic intracerebral hemorrhage.

1.2. Objectives

The aim of the present study was to investigate the association of FIB-4 score with 3-month neurological outcome and symptomatic intracranial hemorrhage in patients with acute ischemic stroke treated with IV rt-Pa.

2. Material and methods

2.1. Study design, setting, and participants

We retrospectively identified consecutive patients admitted to the Stroke Unit of the University Hospital of Ancona, Italy, from January 2017 to April 2021 for acute ischemic stroke treated with IV thrombolysis. Each patient underwent routine blood sampling at admission (within 24 h of admission). Supplementary Table 1 provides an overview of the eligibility criteria.

The study was approved by the ethics committee of the Marche Polytechnic University (ID 57/2020) and conducted according to the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study or their representatives.

2.2. Variables

Demographics, medical history, National Institutes of Health Stroke Scale (NIHSS) scores (17), and admission blood pressure were documented at baseline. Laboratory tests [including serum levels of creatinine, glucose levels, hemoglobin (Hb), platelet count (PLT), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), absolute monocyte count (AMC), alanine aminotransferase (ALT), aspartate aminotransferase (AST) total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, γ-glutamyltransferase (γGT), and creatine phosphokinase (CPK)] were determined by admission blood tests.

To quantify the extent of liver fibrosis, we used the noninvasive liver fibrosis score (FIB-4) for each patient at the time of admission.

The FIB-4 score was computed for every patient as follows:

$$FIB4 = \frac{Age\,(years) \times AST(\frac{IU}{L})}{PLT(\times \frac{10^9}{L}) \times \sqrt{ALT(\frac{IU}{L})}}$$

As validated in previous clinical trials, prediction of advanced liver fibrosis was indicated using a cut-off value ≥2.67, whereas a score value <1.30 was used to exclude severe liver fibrosis with high probability (18, 19).

2.3. Outcome measures

The primary outcome measure was functional status at 3 months, evaluated in the hospital's outpatient setting. Because of its ease of use and interpretability, the modified Rankin Scale (mRS) is a widely applied clinical measure of global disability. In particular, it is used to assess recovery from stroke and as a primary end point in randomized clinical trials of stroke treatments. In our study, poor outcome was defined as the occurrence of death or major disability (mRS ≥3) (20).

We also considered symptomatic intracranial hemorrhage (sICH) as a secondary outcome. We defined this hemorrhagic complication usually linked to rt-Pa, through the European Cooperative Acute Stroke Study (ECASS) III criteria, as follows (21). (1) Clinical deterioration: an increase of ≥4 points in NIHSS score or that led to death. (2) Radiographic features: any intracranial hemorrhage on CT/MRI performed at 22–36 h after stroke onset.

2.4. Biases and study size

We conducted this study on consecutive patients to avoid any selection bias. In order to address information bias, two aspects should be considered: the number of lost to follow-up was acceptable (Figure 1); the admission FIB-4 score was calculated only after the 3-month assessment, so the experimenter did not know the score value when assessing the 3-month mRS (primary outcome measure). Based on previous RCTs on Alteplase effectiveness (22), the minimum number of samples required to achieve a 95% confidence level with a marginal error of 0.05 was 241.

2.5. Statistical methods

We used standard statistical methods for descriptive statistics. Categorical variables were presented as frequencies and continuous

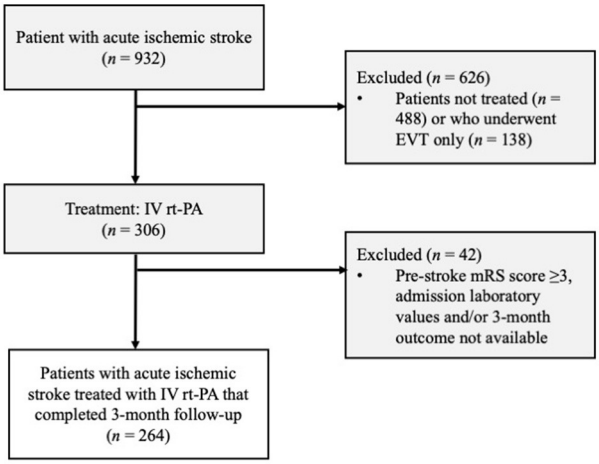

FIGURE 1
Patient selection flow diagram. EVI, endovascular treatment; IVT rt-PA, intravenous thrombolysis with recombinant tissue plasminogen activator; mRS; modified Rankin Scale.

variables as mean (standard deviation, SD) or median (interquartile range, IQR), when appropriate. Normality was assessed through the Shapiro–Wilk test. Depending on the normality of the distribution, comparisons were made by Student's *t*-test or Mann–Whitney test for continuous variables, and by Pearson χ^2 test for categorical variables. The multivariate logistic regression was used to identify whether the FIB-4 score could be an independent predictor of poor 3-month outcome, and to establish the real prognostic value of demographic, clinical, and laboratory variables that reached statistical significance in the univariate analysis. To prevent biases, we did not include the variables already used for calculating the FIB-4 score in the logistic regression. An equivalent analysis was carried out for the secondary outcome, and is available in the Supplementary material. A two-tailed *p*-value of <0.05 was considered statistically significant for all tests. False discovery rate (FDR) correction was applied to deal with the multiple testing problem (results are expressed as adjusted *p* or $_{adj}p$-values). Analysis was performed using JASP Team (2020). JASP (version 0.14.1).

3. Results

3.1. Participants and descriptive data

Of the 306 patients who suffered ischemic stroke treated with IV rt-Pa, 42 were excluded (Figure 1). Of the 264 enrolled patients, 131 (49.62%) had a modified Rankin Scale score of ≥3 after 3 months and 35 (13.3%) experienced a symptomatic intracranial hemorrhage (sICH; Table 1).

The mean FIB-4 score was significantly higher among patients with a poor prognosis compared with the other group (1,436 vs. 1,112, Student *t*-test −3.303, $_{adj}p < 0.001$).

3.2. Main results

Demographic, clinical, and laboratory characteristics of patients are presented in Table 1. In univariate analyses, patients with poor prognosis more frequently had the following characteristics (Table 1): older age, history atrial fibrillation, high admission-NIHSS scores, high blood levels of ANC and AST, and low blood levels of Hb. As shown in Table 1, in the baseline study, adjuvant treatment with mechanical thrombectomy was associated with poor outcome.

We performed a multivariate logistic regression to assess the true predictive value of variables that apparently had an influence on prognosis on univariate analysis. SICH rates (Tables 1, 2) were not included in the calculation because they were not obtainable at baseline assessment. Although age and AST values were statistically significant in univariate testing, they neither were included in the logistic regression since they were already factored into the FIB-4 score, in order to avoid a distortion of FIB-4 score effect on prognosis (i.e., a confusion bias).

On multivariate analysis (Table 3), FIB-4 score (OR 1.894, $p = 0.011$), history of atrial fibrillation (OR 3.488, $p = 0.017$), high admission NIHSS score (OR 1.305, $p < 0.001$) and low blood values of Hb (OR of high Hb levels OR 0.730, $p < 0.001$) remained significant predictors of poor prognosis. In spite of what was hypothesized with the univariate analysis, the regression demonstrated a protective effect of thrombectomy (OR 0.201, $p = 0.005$). Other variables (female sex, and ANC) were not significant prognostic predictors.

This statistical model showed good discriminatory power, with an area under the Receiver Operating Characteristic (ROC) curve of 0.877 (Supplementary Figure 1). It also produced a precision of 79.5% and an accuracy of 79.3%.

Considering the FIB-4 cut-off values, we observed that the 32 patients (12.1%) with a high risk of advanced fibrosis (i.e., FIB-4 score ≥2.67) were more frequently associated with a poor 3-month outcome ($_{adj}p = 0.021$), whereas the 137 patients (51.9%) with a high probability of exclusion of significant liver fibrosis (i.e., FIB-4 score <1.30) more frequently had a favorable 3-month outcome ($_{adj}p = 0.004$; Figure 2).

As with the primary outcome, univariate analyses were used to investigate the influence of variables on sICH (Table 2 and Supplementary Table 2). Regarding the secondary outcome, our study showed a statistically significant relationship between rates of sICH and FIB-4 values ($p = 0.004$), admission NIHSS ($_{adj}p = 0.035$), EVT ($_{adj}p < 0.001$), and serum levels of glucose ($_{adj}p < 0.001$) and ANC ($_{adj}p = 0.009$). However, multivariate analysis only confirmed the effect of admission NIH score on the secondary outcome (OR 0.901, $p = 0.035$, Supplementary Table 3).

Moreover, we divided the entire study population according to the cut-off values of the FIB-4 score, and we noted that patients with FIB-4 score <1.30 (exclusion of liver fibrosis) had lower probability of sICH (FIB-4 score <1.30, $p = 0.025$), whereas ischemic lesions from patients with high risk of advanced fibrosis (i.e., FIB-4 values ≥2.67) tended statistically to bleed more frequently ($p = 0.037$).

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TABLE 1 Baseline characteristics according to the 3-month outcome.

Baseline characteristics	Full cohort $n = 264$	mRS <3 $n = 133$	mRS ≥ 3 $n = 131$	$_{adj}p$-value
Demographics				
Female sex	123 (46.6%)	53	70	0.074[a]
Age, years	69.3 (13.8)	65.9 (14.0)	72.7 (12.7)	<0.001[b]*
Clinical history				
Hypertension	157 (63.3%)	73	84	0.080[a]
Diabetes mellitus	35 (14.6%)	18	17	0.963[a]
Actual smoking	59 (24.9%)	34	25	0.575[a]
Hypercholesterolemia	90 (36.6%)	51	39	0.337[a]
Atrial fibrillation	45 (19.2%)	11	34	<0.001[a]*
Ischemic heart disease	31 (13.3%)	16	15	0.986[a]
Prior stroke	32 (13.5%)	18	14	0.116[a]
Blood test variables				
FIB-4 score	1.284 (0.989)	1.112 (0.734)	1.436 (1.186)	<0.001[c]*
FIB-4 score ≥ 2.67	32 (12.1%)	9	23	0.021[a]*
FIB-4 score 1.31–2.66	95 (36.0%)	42	53	0.244[a]
FIB-4 score <1.30	137 (51.9%)	82	55	0.004[a]*
Total cholesterol, mg/dl	176.1 (40.34)	178.0 (39.63)	174.2 (41.14)	0.653[b]
HDL cholesterol, mg/dl	51.76 (14.67)	53.21 (14.81)	50.24 (14.44)	0.233[b]
LDL cholesterol, mg/dl	103.47 (32.64)	105.24 (34.04)	101.59 (31.13)	0.575[b]
Triglycerides, mg/dl	96.00 (63.00)	96.00 (62.00)	95.00 (59.25)	0.760[c]
Creatinine, mg/dl	0.860 (0.330)	0.850 (0.265)	0.880 (0.425)	0.575[c]
Glucose, mg/dl	108.00 (45.00)	107.00 (43.25)	109.00 (44.00)	0.080[c]
Platelets, $\times 10^9$/L	207.00 (80.50)	207.00 (80.00)	207.00 (79.00)	0.768[c]
Hemoglobin, g/dl	13.05 (2.425)	13.40 (2.10)	12.60 (2.65)	<0.001[c]*
ANC, $\times 10^9$/L	7.304 (3.338)	6.616 (3.228)	8.047 (3.312)	0.007[b]*
ALC, $\times 10^9$/L	1.670 (1.828)	1.722 (0.737)	1.614 (2.527)	0.768[b]
AMC, $\times 10^9$/L	0.697 (0.407)	0.642 (0.255)	0.757 (0.519)	0.090[b]
AST, U/L	17.00 (11.00)	16.00 (8.00)	19.00 (13.50)	0.021[c]*
ALT, U/L	22.00 (12.00)	22.00 (10.00)	22.00 (12.00)	0.768[c]
γGT, U/L	26.00 (24.00)	26.00 (23.00)	27.00 (23.00)	0.714[c]
CPK, U/L	99.00 (86.50)	94.00 (84.00)	108.50 (90.00)	0.389[c]
In-hospital variables				
Systolic blood pressure	139.0 (20.93)	139.0 (21.20)	139.0 (20.74)	0.986[b]
Diastolic blood pressure	76.4 (10.98)	76.4 (11.05)	76.4 (10.96)	0.986[b]
Admission NIHSS score	12.49 (6.16)	9.61 (6.17)	15.54 (4.49)	<0.001[b]*
EVT	146 (55.3%)	60	86	<0.001[a]*
sICH	35 (13.3%)	28	35	<0.001[a]*

mRS, modified Ranking Scale; FIB-4, fibrosis score 4; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; CPK, creatine phosphokinase; EVT, endovascular treatment; sICH, symptomatic intracranial hemorrhage. Categorical variables were presented as frequencies and continuous variables as mean (SD) or median (IQR).

[a] χ^2 test.

[b] Student t-test.

[c] Mann–Whitney test.

*adj p-value, p-values adjusted according to the false discovery rate (FDR) correction, < 0.05.

TABLE 2 sICH rates.

	Full cohort $n = 264$	No sICH $n = 229$	sICH $n = 35$	p-value
FIB-4 score	1.284 (0.989)	1.249 (0.796)	1.697 (1.348)	0.004[c]*
FIB-4 score ≥2.67	32 (12.12%)	24	8	0.037[a]*
FIB-4 score <1.30	137 (51.89%)	125	12	0.025[a]*

FIB-4, fibrosis score 4; sICH, symptomatic intracerebral hemorrhage. Categorical variables were presented as frequencies and continuous variables as mean (SD) or median (IQR).
[a] χ^2 test.
[c] Mann-Whitney test.
*p-value <0.05.

TABLE 3 Logistic regression: FIB-4 score influence on 3-month mRS (primary outcome).

Coefficients	Odds ratio	p-value	95% confidence interval	
			Lower bound	Upper bound
FIB-4 score	1.894	0.011*	1.160	3.094
Female sex	0.666	0.285	0.316	1.404
Atrial fibrillation	3.488	0.017*	1.253	9.710
Admission NIHSS	1.305	<0.001*	1.177	1.448
EVT	0.201	0.005*	0.066	0.609
Hb, g/dl	0.730	<0.001*	0.661	0.807
ANC, $\times 10^9$/L	1.000	0.223	1.000	1.000

mRS, modified Ranking Scale; FIB-4, fibrosis score 4 (continuous variable); EVT, endovascular treatment; ANC, absolute neutrophil count; Hb, hemoglobin. *p-value < 0.05.

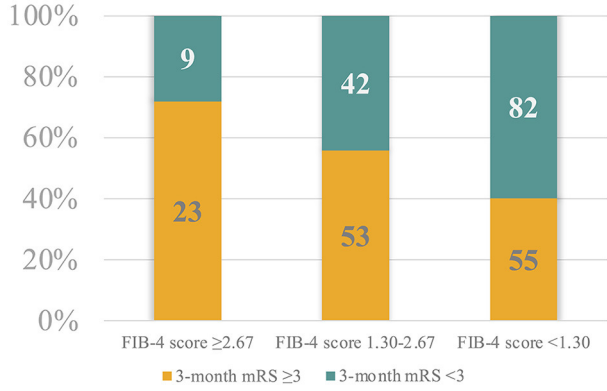

FIGURE 2
Patient proportional distribution based on the FIB-4 score cut-off values and the 3-month modified Ranking Scale.

4. Discussion

The extension of indications for intravenous rt-Pa in patients with stroke and, in particular, the lengthening of the time window, has stimulated the search for reliable predictors able to provide early information on the risk/benefit ratio of the treatment. The ability to predict the outcome shortly after hospitalization can play an important role in the decision-making process regarding the best therapeutic approach in stroke patients and to plan a proper overall therapeutic care. Recently, some of the main predictors of outcome in patients with ischemic stroke treated with rt-Pa have been described (23). High NIHSS scores, elevated systolic blood pressure values on admission, history of atrial fibrillation, and coronary artery disease were associated with poor outcome after 3 months. Another recent study on stroke patients undergoing rt-Pa reported that glycosylated hemoglobin blood levels were related to a poor early outcome but not to a poor functional prognosis at 3 months (24).

4.1. Interpretation of key results

In our study, in addition to clinical data, we considered using a simple and rapidly available index such as the FIB-4 score, based on laboratory parameters, to obtain prognostic information.

After adjustment for confounding factors by logistic regression analysis, we found that high values of FIB-4 score predicted outcome at 3 months in stroke patients treated with intravenous rt-Pa. Moreover, considering the validated cut-off values of this index, we were able to select a group of patients, characterized by a high risk of advanced liver fibrosis, who had a significantly higher probability of poor outcome than other patients. On the other hand, patients with exclusion of significant hepatic fibrosis had a higher probability of a favorable prognosis.

The FIB-4 score, which integrates blood levels of ALT, AST, and PLT, is not only a simple measure of patients' liver function but also reflects the complex systemic role of the liver itself. As highlighted by a cross-sectional study (11), liver dysfunction can lead to brain damage by several mechanisms, including small vessel disease or coagulopathy (25). In addition, NAFLD is associated with systemic inflammation (26, 27), vascular inflammation (28)

and atherosclerosis (25, 29–33). Advanced liver disease is associated with mixed coagulopathy (34), which increases the risk of both thrombotic and hemorrhagic stroke.

It's intuitive that worse outcomes may be the consequence of higher comorbidity in general, not a worse effect of thrombolysis. The selection of outcomes more specifically linked to this treatment should be considered in further dedicated works. For that very reason, we introduced the evaluation of the symptomatic intracerebral hemorrhage, a crucial mechanism involved in modulating the prognosis of patients with ischemic stroke undergoing fibrinolysis. Although the multivariate analysis would seem to exclude a role for the FIB-4 score in predicting bleeding complications, this hypothesis could not be entirely ruled out for two reasons: the limited number of patients with symptomatic cerebral hemorrhage, and the statistical model's inability to corroborate data from previous studies, which have also constantly indicated that admission hyperglycemia plays a significant role in predicting post-thrombolysis intracranial hemorrhagic events (35–37). Our findings from univariate analysis suggested that being affected by severe hepatic fibrosis may increase the risk of intracerebral hemorrhage. Based on these findings, the poor outcome at 3 months in patients with advanced hepatic fibrosis may be, at least in part, related to hemorrhagic complications. As demonstrated in previous studies, the intravenous use of rt-Pa significantly increases the risk of intracranial hemorrhage, which is otherwise uncommon in ischemic stroke (38). Therefore, we hypothesize that for patients with severe hepatic fibrosis and ischemic stroke, the option for intravenous thrombolysis should be carefully evaluated considering the possible related risks.

In the present study, other indicators able to predict outcome at 3 months were identified. The negative prognostic role of atrial fibrillation in our patients was not unexpected although its significance has not been fully elucidated (39). In the Virtual International Stroke Trials Archive, no significant association was found between atrial fibrillation and overall stroke outcome (40). However, some studies found that atrial fibrillation was associated with favorable outcomes after thrombolysis for severe stroke, probably because of the effect of the thrombolytic agent on embolic arterial occlusion (37). In agreement with our findings, most studies suggest that atrial fibrillation may increase the risk of symptomatic intracranial hemorrhage and early death, and decrease the likelihood of favorable outcome after thrombolysis (41, 42).

Our finding of negative predictive effects of high NIHSS scores (23, 43–45) and low serum levels of hemoglobin (46–49) on outcome confirm previous findings in patients undergoing thrombolysis for stroke.

The results of our multivariate analyses showing a favorable effect of endovascular therapy on stroke outcome are consistent with the results of a recent systematic review of 19 randomized clinical trials (RCTs) (50). In this review, endovascular thrombectomy in patients with acute ischemic stroke due to occlusion of large arteries in the anterior circulation increased the chance of survival with good functional outcome (3-month mRS <3) with no negative effect on the risk of intracerebral hemorrhage or death. The predictive influence of anamnestic and laboratory variables on patients undergoing mechanical thrombectomy was recently investigated in a 2021 publication (51).

Toh et al. published an article at the beginning of 2023 addressing the same topic as the current study, with impactful results that confirm the significant influence of the FIB-4 score on the outcome of stroke patients undergoing thrombolysis in a highly representative sample of Asian population (52).

4.2. Strengths and limitations

Our study has some limitations. Because of its observational nature, this retrospective investigation does not reach the quality of evidence needed to draw definitive conclusions. Therefore, future prospective studies with established time points for blood sampling need to be conducted to assess the true cause-and-effect relationship between liver injury and stroke. In the event of a demonstration of a causal relationship, it will be critical to understand whether any improvement in liver condition can lower the risk of poor prognosis in stroke. Furthermore, it is not sufficiently clear whether the calculation of FIB-4 on admission can be considered reliable in expressing chronic liver damage, or is too influenced by stroke-related changes in blood levels of AST, ALT, and PLT. Further investigation is needed to obtain a clear answer with simultaneous assessment of the FIB-4 score and other markers of chronic liver disease.

On the other hand, the large sample of patients included and the easy usability of the score in a clinical setting with ordinary and cost-effective laboratory tests are the most important strengths of the study. We also used the already validated cut-off values of the FIB-4 score, that are strong indicators for the presence/absence of advanced liver fibrosis, significantly simplifying the calculation of the risk of poor outcome.

5. Conclusion

The results of the present study suggested that the FIB-4 score, a rapidly available and cost-effective parameter, can be considered as an independent predictor of poor prognosis, with high predictive accuracy, in patients with acute ischemic stroke undergoing intravenous thrombolysis.

In the new perspective of patient-centered medicine, identification of simple factors that predict treatment response is crucial to guide physicians in providing therapeutic strategies tailored to each single patient.

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 study was approved by the Ethics Committee of the Marche Polytechnic University (ID 57/2020) and conducted according to the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study or their representatives.

Author contributions

Conceptualization, software, formal analysis, writing—original draft preparation, project administration, and had full access to all the data in the study and takes responsibility for its integrity and the data analysis: DN. Methodology: SL. Investigation: DN, SB, and CR. Resources: SL and MB. Data curation: MB, DN, SB, and CR. Writing—review and editing: DN, SB, CR, and MS. Supervision: SL and MS. All authors have read and agreed to the published version of the manuscript.

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.2023.1103063/full#supplementary-material

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Role of LDL-C level alteration in increased mortality risks in spontaneous intracerebral hemorrhage patients: Systematic review and meta-analysis

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Background: Current studies indicate a contradictory relationship between decreased mortality risks of spontaneous intracerebral hemorrhage (sICH) and elevated low-density lipoprotein cholesterol (LDL-C) levels. Thus, this meta-analysis was designed to examine the involvement of high LDL-C levels in a lower mortality risk of sICH patients.

Methods: PubMed, Cochrane, and Embase databases were searched up to the date of August 3rd, 2022. Pooled odds ratio (OR) with a 95% confidence interval (CI) was estimated for the higher vs. lower serum LDL-C level groups. Subgroup and sensitivity analyses were also carried out. Egger's test was applied to detect any potential publication bias.

Results: Of 629 citations reviewed, 8 eligible cohort studies involving 83,013 patients were enrolled in this meta-analysis. Compared with lower serum LDL-C levels containing patients, higher serum LDL-C patients exhibited significantly decreased risks of 3-month mortality (OR: 0.51; 95%CI: 0.33–0.78; $I^2 = 47.8\%$); however, the LDL-C level change wasn't significantly associated with in-hospital mortality risks (OR: 0.92; 95%CI: 0.63–1.33; $I^2 = 91.4\%$) among sICH subjects. All studies included were classified as high-quality investigations.

Conclusions: This meta-analysis suggests a higher LDL-C level may decrease the mortality risk in sICH patients. LDL-C level increase is inversely associated with the 3-month mortality risks in these patients but not significantly correlated with the in-hospital mortality risks. Further well-designed prospective studies with extended follow-up periods are needed to confirm these findings and explore underlying cross-talks.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022318318, identifier: PROSPERO 2022 CRD42022318318.

KEYWORDS

low-density lipoprotein cholesterol, intracerebral hemorrhage, mortality, meta-analysis, systematic review

1. Introduction

Spontaneous intracerebral hemorrhage (sICH) remains the most devastating form of stroke with high morbidity and mortality rates, accounting for over 10% of all stroke cases, and is estimated to affect nearly 2 million people worldwide each year (1–3). Despite many large-scale clinical trials and deeper insights into stroke mechanisms, proven effective

therapies to ameliorate post-sICH consequences are yet to come to clinics (4, 5). sICH occurrences are multi-factorial, and prognoses are challenging in most cases. Presently, ICH management is executed primarily focusing on the risk factors manipulation, medical measures to minimize post-hemorrhage adverse consequences, and surgical interventions in certain cases (6). Therefore, efforts are continuously made to re-evaluate risk factors for stratifying mortality risks of sICH patients and improving their prognosis predictions.

Low-density lipoproteins (LDLs) are the primary transporters of cholesterol across cells and tissues, contributing to atherosclerotic lesions of the blood vessel. Studies have consistently indicated that a high LDL-C level can elevate the risks of ischemic stroke and coronary heart dysfunctions, and the causal association between these two factors is well recognized (7–9). Interestingly, LDL-C seems to have opposite effects on the risk of ischemia and sICH, which may be protective against sICH (10). Some Mendelian randomization (11, 12) and meta-analyses (13–15) demonstrate that increased LDL-C levels can lower the risk of sICH. Nonetheless, several other epidemiological investigations could not consistently show the exact implication of higher LDL-C levels on sICH mortality (16–19). To obtain more comprehensive and objective insights into the outcome and prognosis of sICH subjects with higher LDL-C levels, we conducted this meta-analytical investigation, unveiling a significant association of baseline serum LDL-C level with mortality risks in sICH.

2. Methods

2.1. Search strategy

A systematic literature search was performed in the PubMed, Cochrane Library, and EMBASE databases for studies published up to August 3, 2022, by the pre-established search strategy, using the keywords "cerebral hemorrhage," "ICH," "intracerebral hemorrhage," "low-density lipoprotein cholesterol," and "LDL-C." The searches were conducted without language restrictions and adapted for each electronic database. The specific terms used for searching in each database, along with the number of records retrieved, are detailed in Supplementary Tables S1–S3. Besides, reference lists of retrieved articles were further screened to identify any eligible studies that didn't come up on the initial search.

2.2. Inclusion/exclusion criteria

Eligible studies satisfied the following selection criteria: (1) either retrospective or prospective cohort studies in nature; (2) enrolled sICH patients who were diagnostically confirmed by computed tomography (CT) or magnetic resonance imaging (MRI) examinations; (3) assessed the correlation between baseline serum LDL-C levels and the mortality risks in sICH patients, and (4) provided with univariate or multivariate-adjusted effect estimates [odds ratio (OR) with corresponding 95% confidence interval (CI)] for the association between LDL-C levels and sICH risks of mortality. The studies were excluded if: (1) these were either reviews, letters to the editor, comments, or meeting abstracts; (2) they included ICH participants with primary traumatic injuries; and (3) these were either duplicate publications or multiple articles based on the same cohort studies with overlapping data. In this case, the one with the most comprehensive results, or the largest sample size, was included.

2.3. Data extraction and quality assessment

Two investigators (JL and GL) independently searched and identified eligible literature for extracting relevant data for the meta-analysis, per the pre-determined selection criteria and data extraction strategies concerning the PRISMA recommendations (#PROSPERO CRD42022318318) as illustrated in Supplementary Table S5 (20). The extracted features included the first (co)author name(s), year of publication, study area and design, demographics of participants (gender variation, sample sizes, and mean/range of ages), clinical characteristics (scores on the NIHSS and GCS scales, and ICH volumes), outcome measurements, adjusted ORs with 95%CIs, and adjusted parameters in the multiple factor analysis (MFA). Once completed, investigators exchanged their data audit forms, and if there was any discrepancy, a group discussion was conducted to arrive at a consensus.

The Newcastle-Ottawa Scale (NOS) rating was applied to assess the methodological qualities of eligible articles (21). The scale includes three subscales of subject selection, comparability across groups, and ascertainment of exposure (Supplementary Table S4). Nine NOS stars referred to the maximum score for each article, of which 7–9 NOS stars corresponded to high, 4–6 stars to moderate, and $\leq$3 stars to low quality.

2.4. Statistical analyses

The ORs and 95%CIs were computed to measure the effects of altered LDL-C levels on mortality risks in sICH patients. The random-effect model (REM) was executed for meta-analyses. The Cochran's *Q*-test and I^2 index were used to determine the heterogeneity across studies (22). Results with I^2 values of $>$ 50% or *P*-values $<$ 0.05 indicated substantial heterogeneity. Subgroup analyses were performed by grouping studies based on the study area (e.g., China vs. Western) and design (prospective clinical study, PCS, vs. retrospective clinical study, RCS). The sensitivity analysis was conducted by sequential dropping of individual studies to measure the contribution of the respective study to the overall risk assessment. Egger's test was employed to determine any potential publication bias of enrolled studies (23). Stata v12.0 (Stata Corp., USA) was used for all statistical analyses.

3. Results

3.1. Literature search

The results of the literature search and the literature screening process are shown in Figure 1. Full-text documents were retrieved for 629 articles (226 in PubMed, 372 in EMBASE, and 31

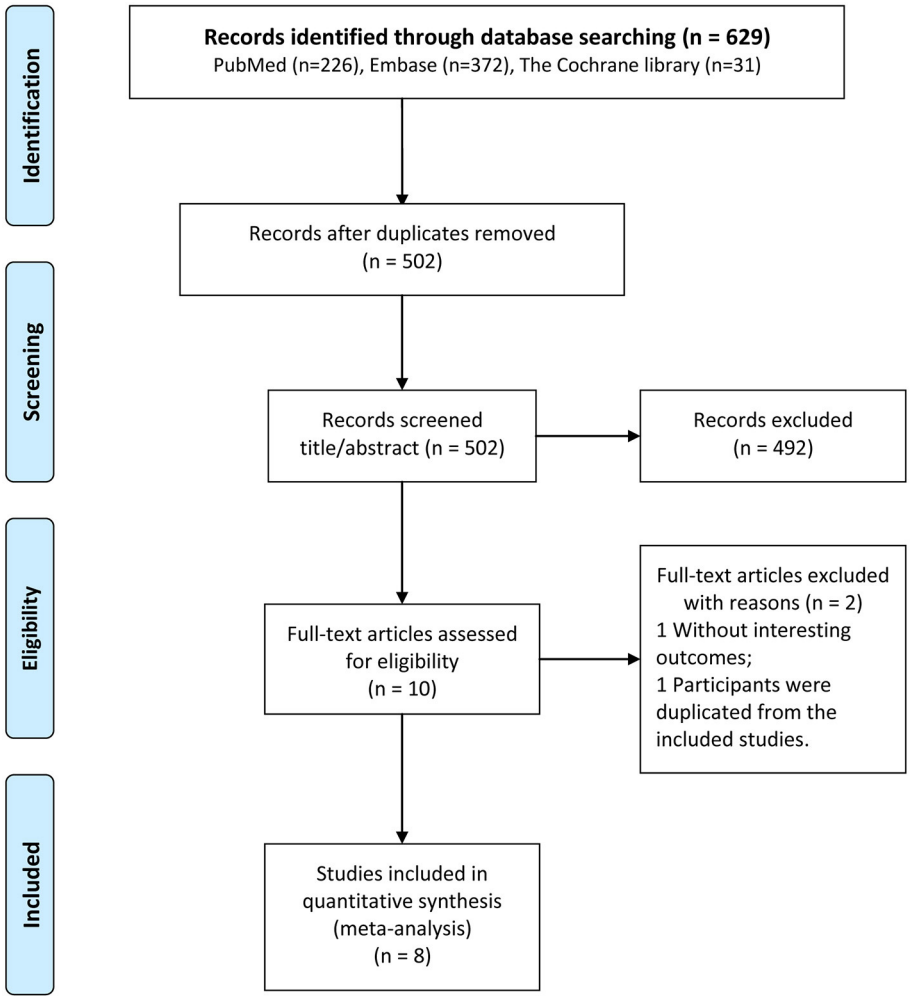

FIGURE 1
Flowchart of the selection process of included studies.

in Cochrane) from electronic databases for meta-analyses. No additional eligible study could be identified in the reference lists of included studies. After eliminating 127 duplicate articles, 502 studies were obtained in the initial screening. Of these, 492 irrelevant articles were excluded by reviewing their titles and abstracts. Then, 10 articles were subjected to full-text evaluation. Finally, 8 cohort studies (16–19, 24–27) were selected for the analysis.

3.2. Study characteristics

Table 1 summarizes the baseline characteristics of eligible studies. Eight selected articles published between 2009 and 2022 were analyzed. Four studies were from China (17, 25–27), and the rests were from the USA (16), Finland (18), and Spain (19, 24). There were two retrospective and six prospective cohort studies, having sample sizes ranging from 88 to 75,433 and a total of 83,013 participants. The study subjects were all acute sICH patients diagnosed by imaging methods such as CT or MRI. For each included study, the subjects' ages were in the range of 59 to 74 years. Additionally, the in-hospital mortality risk was reported in 4 studies (16–18, 26) and 5 studies (18, 19, 24, 25, 27) documented the 3-month mortality risk in sICH patients. The lower or higher LDL-C level cutoff values varied obviously across the original studies. Multivariate analyses revealed the association between LDL-C level changes and ICH mortality risks. The NIHSS and GCS scores, ICH volumes, OR (95%CI), and adjusted factors are listed in Table 1.

Most studies were rated with NOS scores ranging between 7 and 9 stars (Supplementary Table S4), with six studies scoring ≥8 stars, indicating all finally included studies were of high quality. The main sources of bias in these studies were recall bias and confounding bias.

3.3. Results of the in-hospital and 3-month mortality risk assessments

Figure 2 shows the forest plot for in-hospital and 3-month mortality risks. Four studies including 77, 855 patients, estimated

TABLE 1 Characteristics of eight included studies in this meta-analysis.

Study (Area, design)	n, M/F	Age, years	Admission NIHSS score	Admission GCS score	ICH volume, ml	Exposure of LDL-C, mg/dL	Mortality	OR (95%CI)	Adjusted factors
Chang et al. (16) (USA, PCS)	672, 379/293	61.6 ± 14.0	8 (2,18)	NR	NR	Per 10 unit increase	In-hospital	0.68 (0.57, 0.80)	BMI, Hypertension, Hyperlipidemia, CAD, CHF, CKD, Smoking, Admission glucose/HDL-C/Creatinine/SBP/NIHSS
Ding et al. (17) (China, PCS)	75433, 47079/28354	63.0 ± 12.8	NR	14 (8,15)	NR	>100 vs. ≤100	In-hospital	1.13 (1.01, 1.26)	Age, sex, BMI, SBP, DBP, smoking, drinking status, hypertension, diabetes mellitus, previous ICH, medication history, creatinine, GCS score
Mustanoja et al. (18) (Finland, RCS)	964, 550/414	66 ± 13	7 (3,14)	14 (10,15)	7.3 (2.7, 16)	Per Quartile increase	In-hospital	0.55 (0.32, 0.95)	Age, NIHSS, GCS, ICH volume, IVH, Statin use
							3-month	0.81 (0.54, 1.21)	
Ramírez-Moreno et al. (24) (Spain, PCS)	88, 50/38	73.8 ± 8.9	10.2 ± 7.6	13.0 ± 3.1	24.9 ± 35.0	>100 vs. ≤100	3-month	0.33 (0.11, 0.96)	Age, sex, hypertension, prior antihypertensive treatment, prior anticoagulation, ICH volume, ventricular extension, GCS, NIHSS, glucose
Rodriguez-Luna et al. (19) (Spain, PCS)	108, 62/46	71.6 ± 11.5	17 (10,20)	15 (11, 15)	27.4 ± 33.2	≥95 vs. <95	3-month	0.16 (0.03, 0.78)	Age, baseline ICH volume, intraventricular extension
Wen et al. (25) (China, PCS)	4606, 3087/1519	61.7 (51.9, 72.8)	9 (3,18)	15 (11,15)	NR	>100 vs. ≤100	3-month	0.54 (0.38, 0.78)	Age, sex, lipid-lowering drugs
Yang et al. (26) (China, RCS)	786, 486/300	59 (51, 68)	8 (4,12)	NR	15-45	Per 1 unit increase	In-hospital	1.47 (1.07, 2.01)	Age, NIHSS, Bleeding volume, Blood glucose, Serum Albumin, Fasting, Bleeding position, SBP lowering
You et al. (27) (China, PCS)	356, 236/120	64.1 ± 13.7	6 (3,10)	NR	9.3 (4.9, 20.0)	Per 1 unit increase	3-month	0.27 (0.08, 0.97)	Age, Gender, Smoking, hypertension, diabetes mellitus, Stroke, SBP, DBP, TC, TG, HDL-C, NIHSS, Bleeding volume

F, female; M, male; NR, not reported; PCS, prospective clinical study; RCS, retrospective clinical study; ICH, intracerebral hemorrhage; NIHSS, National Institutes of Health Stroke Scale; DBP, diastolic blood pressure; SBP, systolic blood pressure; CAD, Coronary artery disease; CHF, Congestive heart failure; CKD, Chronic kidney disease; BMI, body mass index; GCS, Glasgow coma scale; IVH, intraventricular hemorrhage; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol.

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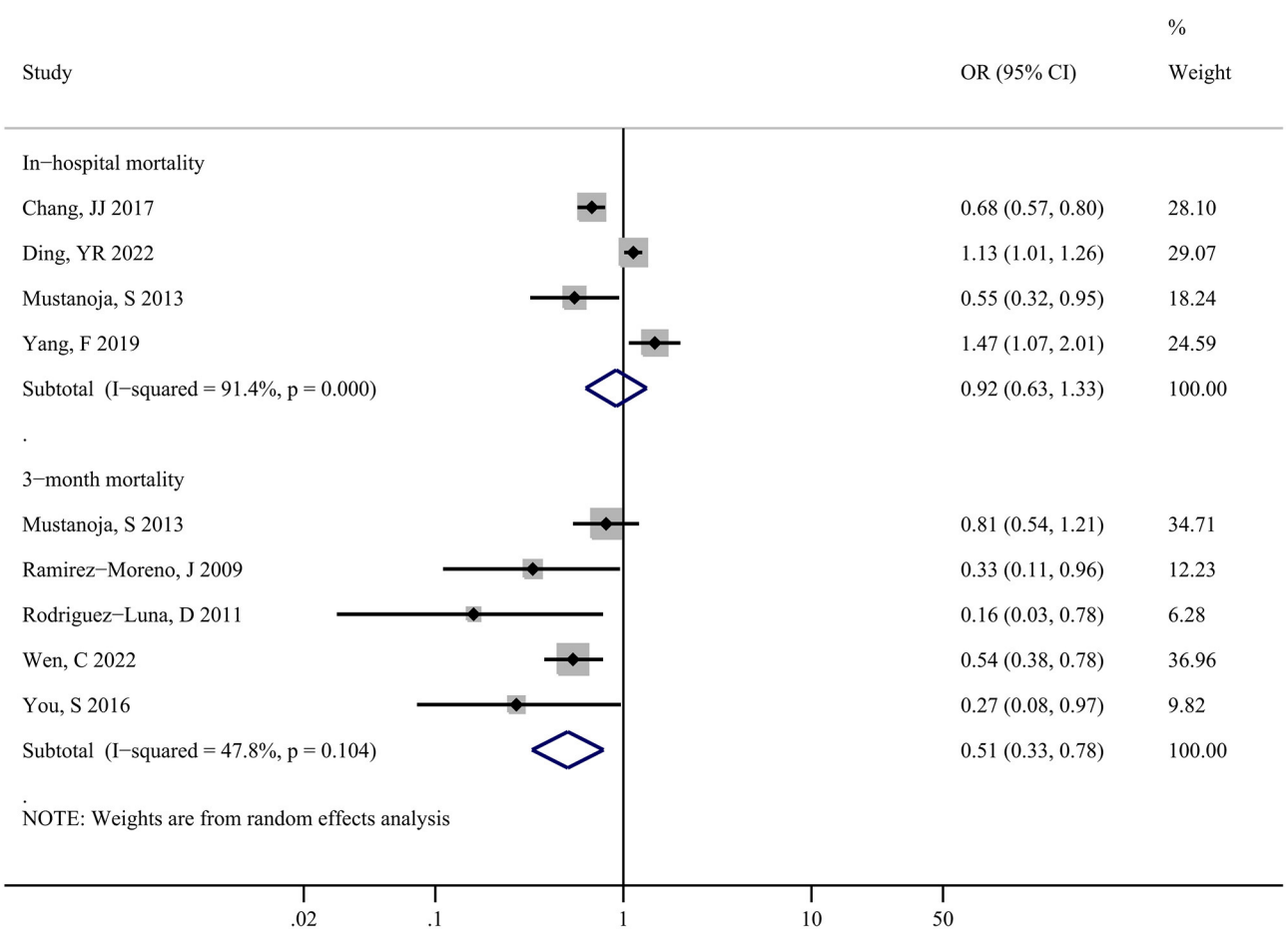

FIGURE 2
Forest plots showing pooled odds ratios (ORs) with 95% confidence intervals of in-hospital and 3-month mortality risk comparing the higher vs. lower serum LDL-C level in patients with sICH.

the risk of in-hospital mortality and the patients' LDL-C levels, indicating no significant association between the elevated LDL-C levels and risks of in-hospital mortality (OR: 0.92; 95%CI: 0.63-1.33; $P = 0.647$) in sICH patients, with substantial heterogeneity across studies ($I^2 = 91.4\%$; $P < 0.001$).

Moreover, five studies including 6,122 patients showed that alteration in LDL-C levels and the risk of 3-month mortality had a strong association, with a pooled risk estimate of 0.51 (0.33, 0.78; $P = 0.002$) and no statistically significant heterogeneity ($I^2 = 47.8\%$; $P = 0.104$) across studies. In summary, a higher serum LDL-C level may have a beneficial effect on decreasing the risk of death in sICH patients.

3.4. Results of subgroup analyses

For the in-hospital mortality, the pooled results of the three subgroups (China, PCS, and RCS) were not statistically significant and highly heterogeneous ($P > 0.05$; $I^2 > 50\%$). However, the pooled results for the Western subgroup were significant (OR: 0.67; 95%CI: 0.57–0.78; $P < 0.001$), with almost no heterogeneity among the studies ($I^2 = 0\%$, $P = 0.466$) (Table 2).

For the 3-month mortality, the pooled estimates and size of heterogeneity for both subgroups of China and PCS were consistent with the original pooled results, whereas the pooled results for the Western and RCS subgroups were not significant ($P > 0.05$) (Table 2).

Overall, the study design variation did not have any effects on the LDL-C level's association with the in-hospital mortality risks of sICH patients, unlike the association with the 3-month mortality risk. Interestingly, the region of the study conducted seemed to influence the association of LDL-C level modulation with either in-hospital or 3-month mortality risk in sICH individuals. In other words, a higher LDL-C level may be associated with decreased in-hospital mortality risks in the West and 3-month mortality risks in China.

3.5. Sensitivity analysis and publication bias

As shown in Table 3, the sensitivity analysis revealed that the pooled risks were significant for both in-hospital and 3-month mortality risks in sICH, and no major change was observed in the pooled estimation even if any individual study was eliminated at

TABLE 2 Subgroup analyses of baseline LDL-C level with the risk of in-hospital mortality and 3-month mortality among sICH patients.

Outcomes	No. of study	OR (95%CI)	*P*-value	Heterogeneity test	
				I^2 (%)	P_H
In-hospital mortality					
Overall	4	0.92 (0.63, 1.33)	0.647	91.4	<0.001
Area					
China	2	1.23 (0.97, 1.57)	0.090	58.0	0.123
Western	2	0.67 (0.57, 0.78)	<0.001	0	0.466
Design					
PCS	2	0.88 (0.54, 1.45)	0.616	95.9	<0.001
RCS	2	0.92 (0.35, 2.42)	0.870	89.4	0.002
3-month mortality					
Overall	5	0.51 (0.33, 0.78)	0.002	47.8	0.104
Area					
China	2	0.50 (0.32, 0.77)	0.002	8.7	0.295
Western	3	0.44 (0.17, 1.12)	0.085	63.4	0.065
Design					
PCS	4	0.43 (0.28, 0.66)	<0.001	12.3	0.331
RCS	1	0.81 (0.54, 1.21)	0.306	NA	NA

OR, odds ratio; CI, confidence interval; PCS, prospective clinical study; RCS, retrospective clinical study; NA, not available.

TABLE 3 Outcomes of the sensitivity analysis and test of publication bias.

Outcomes	No. of studies	Sensitivity analysis		Egger's test
		OR (95% CI)	Robust	*P*-value
In-hospital mortality	4	0.78 (0.51, 1.22) to 1.05 (0.82, 1.52)	Yes	0.724
3-month mortality	5	0.47 (0.34, 0.65) to 0.62 (0.44, 0.89)	Yes	0.100

OR, odds ratio; CI, confidence interval.

each turn. Moreover, the change in the pooled effect size for in-hospital mortality risks, from 0.78 (0.51, 1.22) to 1.05 (0.82, 1.52), was not significant ($P > 0.05$). Likewise, the leave-out one study sensitivity analysis of 3-month mortality also indicated a significant ($P < 0.05$) stable pooled OR (95%CI), ranging from 0.47 (0.34, 0.65) to 0.62 (0.44, 0.89).

The Egger's test could not identify any publication bias for both in-hospital ($P = 0.724$) and 3-month mortality ($P = 0.100$) risks for analyzed studies (Table 3). The funnel plot asymmetry analysis could not be performed for each subgroup due to the inclusion of fewer than 10 articles (28).

4. Discussion

This comprehensive meta-analysis of eight cohort studies involved 83,013 individuals with ICH to correlate the mortality risks of these patients with serum LDL-C levels. The sICH patients exhibited a robust influence of baseline LDL-C levels on the 3-month mortality risks, whereas no discernible effect was noticed for in-hospital mortality risk prediction. Sensitivity analyses also repeatedly confirmed this finding. However, values of LDL-C levels in predicting 3-month mortality risks were not significant in both the western and retrospective study subgroups, yet became statistically significant in the western subgroup when predicting in-hospital mortality risks, which might be respectively explained by significantly higher heterogeneity and a limited number of eligible studies. Thus, larger sample sizes are required to validate the extrapolation of these findings.

Furthermore, considerably higher heterogeneity ($I^2 = 91.4\%$) might mask the exact association of altered LDL-C levels with in-hospital mortality risks (OR: 0.92; 95%CI: 0.63–1.33). To overcome that, we conducted subgroup and sensitivity analyses, revealing no considerable heterogeneity without any adjustment for significance thresholds. The observed heterogeneity for in-hospital mortality risk might stem, in part, from a broad range of confounding factors that different studies had adjusted for at the cost of power reduction. Besides, the heterogeneity might correlate with participants' characteristics (e.g., age, geographic, and racial differences), as well as genetic polymorphisms (e.g., allelic variations of *APOE*, *PMF1,* and *SLC25A44* genes) (29–31). Also, differences in threshold values of LDL-C, diet, and exercise habits across studies could introduce heterogeneity. Interestingly, the Western subgroup analysis showed that the in-hospital mortality

risk had a significant association with patients' LDL-C levels (OR: 0.67; 95%CI: 0.57–0.78). Pooled results of subgroups, however, showed inconsistency with the original results, suggesting that more high-quality studies are needed to establish this association, irrespective of the study area and participants' characteristics.

The effect of serum LDL-C level on ICH mortality risk may vary depending on the follow-up duration, which could partially explain the observed association of LDL-C level variation with the 3-month mortality risk but not in-hospital mortality, which could be related to severe ICH-associated higher mortality than LDL-C-induced ICH risk in the early stage. Hence, under aggravated ICH conditions, the correlation between mortality risks and LDL-C levels may not be crucially important (17, 32). It's noted that for patients with GCS scores between 9 and 15, low LDL-C levels can exacerbate hematoma expansion and in-hospital mortality risk, but not in coma patients (GCS scores 3–8). Most studies had a maximum follow-up period of 3 months, so the predictive value of LDL-C level for 1-year mortality could not be estimated in this meta-analysis.

Above all, our findings indicated that sICH patients having lower serum LDL-C levels could be at higher mortality risks. Our study supported the potential of LDL-C level alteration as an independent predictor for 3-month mortality risks in sICH patients and suggested outlines for risk stratification and clinical outcomes in ICH in designing patient-specific therapy.

4.1. Possible explanations for underlying mechanisms

Several different explanations regarding underlying mechanisms may be considered in this context.

First, LDL-C plays key functions in many physiological processes, such as maintaining vessel wall integrity. Reportedly, most healthy individuals are born with LDL-C levels ranging from 40 to 60 mg/dL (33). While lower LDL-C levels can be independently related to brain microhemorrhages under acute ICH conditions (34), which can further contribute to poor treatment response, stroke recurrence (35), and perihematomal edema expansion (36). The possible mechanisms are as follows: (1) disintegration of the endothelium (37); (2) necrotic death of medial smooth muscle cells (MSMCs) (38); (3) inhibited platelet aggregation (39); (4) accelerated osmotic membrane rupture in erythrocytes (40), and (5) impaired synaptic reconstruction (41). ICH patients with genetically reduced levels of LDL-C are more likely to carry APOE ε2/ε4 allele, indicating recurrent ICH (42, 43). Theoretically, high LDL-C levels could rescue ICH patients from hematoma enlargement and mortality risks. Further prospective studies are essential to assess an optimum LDL-C level to prevent adverse circumstances in ICH.

Second, the inclusion of observational studies prevented establishing a causal relationship in this meta-analysis. An excessively low LDL-C level can reversibly increase the mortality risk (44), but it may not necessitate a causal relationship in all cases (25). Whether LDL-C levels change over time due to physiological and clinical reasons is still unclear. A longitudinal study shows a subacute reduction in LDL-C levels preceding sICH (45), and both LDL-C and triglyceride levels can decline at the disease onset and restore to normal levels during the recovery phase (46–48). Debilitation and/or disease also can lower LDL-C levels (49, 50), which might be a surrogate for malnutritional or a sign of severe disease (51, 52), thus predisposing the individual to increased stroke mortality (53), indicating lower LDL-C level could induce chronic health problems and higher mortality, especially in the elderly (54). Hypocholesterolemia can lead to sepsis, adrenal failure, and increased mortality risks in critically ill patients (46). It is hypothesized that the pathophysiological abnormality of ICH patients causes higher mortality and reduction in LDL-C levels, in parallel, which may not be rescued by treatments directed to normalizing a single dysfunction (55). Thus, adjusting the LDL-C level to an optimum may not likely modify the death risk in sICH subjects. Furthermore, elderly pre-ICH statin users are at higher risk of comorbidities due to their lower LDL-C levels, just like individuals under antithrombic medications with an elevated risk of bleeding and stroke (56). Due to insufficient data on pre-ICH statin use, we could not evaluate the relationships between statins, sICH outcomes, and low LDL-C levels. However, we investigated the relationship between sICH outcomes and serum LDL-C levels. Besides, the pre-statin exposure rate among sICH is relatively low, without any independent correlation with worse treatment responses in sICH patients (18, 57), which is consistent with other meta-analyses suggesting that pre-ICH statin application might not have any association with post-ICH mortality risks (56, 58–60). Above all, the reciprocal correlation between the LDL-C level at admission and mortality risks in sICH patients should be interpreted with caution. Further large-scale studies are needed to further elucidate the impact of low LDL-C levels and ICH death risk.

4.2. Statin treatment after ICH

Although conventionally lowering lipid levels is considered the best to control atherosclerotic cardiovascular diseases (61), the effect of statins on optimally regulating LDL-C levels still needs more validation to gauge the risk of sICH in atherosclerotic patients. Since chronic use of statins increases bleeding complications, while its sudden discontinuation can predispose to ischemia, the application of statins in acute sICH patients is highly debated (62). Similarly, a recent study (10) advised against abruptly discontinuing statin medication in cases of acute ICH without first conducting a thorough health assessment. In contrast, a prior Markov decision analysis endorsed that avoiding statins is expedient in all ICH survivors after weighing these competing risks and benefits (63). However, individualized treatment decisions based on expert consultation have been advocated by recent guidelines (64). Furthermore, due to the limited data availability, our meta-analysis was unable to identify the threshold value, and we could not comprehend the linear or non-linear association of serum LDL-C with mortality risk in sICH patients. The previous report showed low LDL-C levels (<100 mg/dL) containing subjects had enhanced 3-month mortality and were more prone to hematoma enlargement than their counterparts with higher LDL-C levels (110–129 mg/dL) (25). Particularly, <70

mg/dL can increase the risk of hematoma expansion and in-hospital mortality by several folds compared to subjects with higher LDL-C levels (17, 19, 65). Statin treatment in ICH patients with relatively low LDL-C should be approached with caution due to the increased lifetime risk. Hence, randomized controlled trials are urgently necessary to resolve the complicacy of statin use in patients with higher risks of ICH.

4.3. Strengths and limitations

We found that the elevated level of LDL-C exhibits an inverse relationship with the in-hospital and 3-month mortality risks, respectively, among post-ICH patients in western countries and China. To the best of our knowledge, this is the first meta-analysis reporting an assessment of LDL-C level variation in relation to sICH mortality risk. The strengths of the study include its extensive literature search, stringent criteria, and thorough scrutinization of included studies. Despite the limited number of articles, the sample size was considerably large, and the accuracy of the meta-analysis results was relatively satisfactory. Moreover, the methodological quality control for included reports matched the international standard. Despite the influence of confounding factors, like pre-treatment medications, ICH volumes, admission NIHSS scores, and hypertension, all studies produced highly objective and reasonable observations after multiple factor adjustments. Furthermore, the sensitivity analyses and Egger's test suggested the good reproducibility and high credibility of the pooled results across the included studies. Finally, we strictly adhered to the PRISMA recommendations for study screening, data extraction, quality control checking, and analyses to reduce recall bias, especially for observational studies.

Yet, we note that this review does have some limitations. First, being a study-level meta-analysis, this study could not consider methodological variations in analyzed articles. It wasn't possible to adopt a uniform adjustment of variables for all studies. Also, a standardized analysis of the age and sex of the participants could not be performed to identify potential confounding factors for heterogeneity. An individual-level meta-analysis will be appropriate to evaluate the relationship between ICH mortality risks and low LDL-C levels in terms of demographics, existing medications, and comorbidities.

Second, in all cases, only one-time measured post-ICH serum LDL-C level values were included for analysis, which might introduce classification bias. There were no pre-ICH onset data for these patients, and possible variations in LDL-C levels during the hospital stay and follow-up periods were not considered. It remains unclear whether an acute sICH condition induces LDL-C level alteration and/or whether the reverse phenomenon is the actual culprit in elevating the mortality risk. Time-average serum LDL-C level analysis can be useful to verify the association of pathological LDL-C levels with higher mortality risks.

Third, different cutoffs for LDL-C levels were used in the prediction of mortality risks across studies, and the methodological heterogeneity might lead to the discrepancy in results, which prevented us from determining the optimal value of LDL-C level in better predicting ICH survival. And the enrolled studies were ineligible for the dose-response analysis because they lacked the necessary data (e.g., the number of cases in each LDL-C level). Additionally, abnormally low LDL-C levels might have underpowered our results. We expect future studies to analyze clinically uncommon subsets of patients critically for better understanding.

Fourth, all eligible studies involved cohort analyses, which had a higher susceptibility to bias from confounding factors than randomized controlled trials. Residual confounding biases are sometimes unavoidable due to the omission of or insufficiently measured variables. Some important confounding factors related to mortality risk should be adjusted as comprehensively and consistently as possible in the statistical model, while the severity of ICH (GCS and NIHSS scores), hypertension, pre-medications, divergent inciting causes of ICH (blood pressure, cerebral amyloid angiopathy, anticoagulation, and vascular anomalies), complete neuroimaging data (hematoma shape, volume, and site), and time from ICH occurrence to cranial CT scan were not fully captured in several of these studies. Inadequate adjustment may have resulted in an overestimation of the risk estimate.

Fifth, most participants were Han Chinese and Caucasians, which limited the generalizability of our observations to the general population, but it might have some reference values. Caution should be paid to extrapolating these results to other ethnic groups. The association and optimal range of LDL-C level change may differ across ethnic groups due to their varying baseline LDL-C levels, environments, and individual risk factors.

5. Conclusions

In conclusion, an increase in LDL-C level is inversely associated with 3-month mortality risks in sICH patients but not significantly correlated with in-hospital mortality risks. Serum LDL-C level can be a potential independent biomarker of mortality risk evaluation in sICH patients and may be helpful in early decision-making in clinical practices and contribute to identifying those at higher risks of mortality. Nevertheless, the subgroup analyses revealed inconsistencies with the original pooled results, indicating further well-designed studies with stringent quality control and larger sample sizes are recommended to validate the stability and extrapolation of these results as well as to determine an appropriate LDL-C range.

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.

Author contributions

XS and JL were involved in the conception and design of the study. JL, GL, and YZ contributed to the literature screening, data acquisition, statistical analysis, and interpretation. JL and GC wrote the manuscript, which was revised, and approved by all the authors

for publication. XS and JZ participated in the review, editing, and supervision of the article. All authors contributed to the study and approved the submitted 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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1114176/full#supplementary-material

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Improving the models for prognosis of aneurysmal subarachnoid hemorrhage with the neutrophil-to-albumin ratio

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Objective: Many peripheral inflammatory markers were reported to be associated with the prognosis of aneurysmal subarachnoid hemorrhage (aSAH). We aimed to identify the most promising inflammatory factor that can improve existing predictive models.

Methods: The study was based on data from a 10year retrospective cohort study at Sichuan University West China Hospital. We selected the well-known SAFIRE and Subarachnoid Hemorrhage International Trialists' (SAHIT) models as the basic models. We compared the performance of the models after including the inflammatory markers and that of the original models. The developed models were internally and temporally validated.

Results: A total of 3,173 patients were included in this study, divided into the derivation cohort (n =2,525) and the validation cohort (n =648). Most inflammatory markers could improve the SAH model for mortality prediction in patients with aSAH, and the neutrophil-to-albumin ratio (NAR) performed best among all the included inflammatory markers. By incorporating NAR, the modified SAFIRE and SAHIT models improved the area under the receiver operator characteristics curve (SAFIRE+NAR vs. SAFIRE: 0.794 vs. 0.778, p =0.012; SAHIT+NAR vs. SAHIT: 0.831 vs. 0.819, p =0.016) and categorical net reclassification improvement (SAFIRE+NAR: 0.0727, p =0.002; SAHIT+NAR: 0.0810, p <0.001).

Conclusion: This study illustrated that among the inflammatory markers associated with aSAH prognosis, NAR could improve the SAFIRE and SAHIT models for 3month mortality of aSAH.

KEYWORDS

neutrophil-to-albumin ratio, intracranial aneurysm, subarachnoid hemorrhage, mortality, prediction model

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a fatal disease (1). Between 25 and 30% of patients with aSAH die within 3 months of onset (2), and 40% of aSAH patients do not regain independent function (3). Consequently, establishing an accurate and straightforward prediction model for the early prognosis of aSAH has always been a priority in aSAH clinical research.

Two externally validated predictive models, the SAFIRE model (4) and the SAHIT model (5), have been developed using data from prospective cohort studies. The derivation cohort of the SAFIRE model included 1,215 patients, while the validation cohort included 2,143; for the SAHIT model, 10,936 and 3,355 patients were included in the respective cohorts. The area under the receiver operating characteristic curve (AUC) for SAFIRE was 0.83 (95%CI 0.80–0.85), while AUC values for SAHIT remained between 0.76–0.81 in external validation. However, R^2 in SAHIT was only 23–31%, signifying that the included predictors explained only 23–31% of the variability in outcome, whereas the SAFIRE model does not report its R^2. As neither model incorporated laboratory tests, the potential of baseline biomarkers to enhance the model has yet to be explored.

Recent studies have confirmed that inflammation in the initial phase of aSAH is implicated in its pathological process (6). Several peripheral inflammatory markers, including the neutrophil-to-albumin ratio (NAR) (7), neutrophil-to-lymphocyte ratio (NLR) (8), platelet-to-lymphocyte ratio (PLR) (9), monocyte-to-lymphocyte ratio (MLR) (10), and systemic immune inflammation index (SII) (11), have been reported to be associated with short-term outcomes of aSAH, raising the possibility that they may serve as promising prognosticators. However, there has yet to be a systematic study on whether these markers can improve the predictive capabilities of existing models. This study sought to investigate if these validated inflammatory markers could bolster the predictive power of SAFIRE and SAHIT models, in addition to selecting the marker that conferred the greatest enhancement.

Methods

Study design and source of data

Patient data were derived from a large observational cohort study at Sichuan University West China Hospital. Patients were divided into the derivation cohort (February 2009 to December 2017) and the validation cohort (January 2018 to July 2019). Treatment of patients was carried out according to standardized guidelines (12).

Patients were enrolled only when they were diagnosed with SAH by computed tomography, magnetic resonance imaging, angiography, or cerebrospinal fluid test, and aneurysm were identified precisely. Exclusion criteria included (1) aneurysms were caused by trauma or arteriovenous malformations and (2) aneurysms were treated before ictus. We also excluded patients whose personal identification numbers were wrong or whose household registrations were not found in the Household Registration Administration System. We used personal identification numbers to identify death records from this system.

The study was approved by the West China Hospital Institutional Review Board (No. 20211701), with a waiver of informed consent due to minimal risk to patients. Predictive models were reported according to the TRIPOD statement (Checklist in the Supplemental material) (11).

Predictors

According to the SAFIRE and SAHIT models, age, medical history of hypertension, aneurysm location, aneurysm size, World Federation of Neurological Surgeons (WFNS) grade on admission, Fisher grade on admission, and methods of treatment (clip, coil, or no treatment) were collected.

Aneurysm size and Fisher grade categories were defined separately as SAFIRE or SAHIT predictive tool. In the SAFIRE model, aneurysm sizes were categorized as <10 mm, 10–19.9 mm, or ≥ 20 mm, and Fisher grades were categorized into 1–3 or 4. In the SAHIT model, aneurysm sizes were categorized as ≤12 mm, 13–24 mm, or ≥ 25 mm, and original Fisher grades were enrolled. Similarly, age was treated as a continuous variable in the SAHIT model and a categorical variable in the SAFIRE model (≤50 y, 50–60 y, 60–70 y, or ≥ 70 y). Due to the limited data, locations of aneurysms were imputed as anterior or posterior circulation.

According to the current studies, we identified five markers to predict outcomes in aSAH patients, including the NAR, NLR, PLR, MLR, and SII. Their calculation methods were presented in Supplementary Figure S1. Considering the Practical clinical application, only laboratory examination results within 24 h were selected. Multicollinearity was assessed using the variance inflation factor (VIF). A VIF value >5 indicates severe collinearity (13).

Outcome

The outcome was defined as mortality at 3 months. All death records were extracted through the Household Registration System, which documents Chinese citizens' death dates. The system is based on self-reporting death by relatives and the Seventh National Census, with a missing registration rate of 5 per 10,000, which was reported by the National Bureau of Statistics (14). Therefore, this system has accurate death records and bind assessments (15).

Missing data

All data were complete except aneurysm size and Fisher grade. In the derivation cohort, missing aneurysm size and Fisher grade values were filled using multiple imputation (16) with a predictive mean matching method to generate 5 imputations. Complete case analysis was adopted in the validation cohort.

Comparison of inflammatory markers

Considering the colinearity of the five inflammatory markers, we compared their predictive abilities before adding to the predictive models using binary logistic regression and area under the receiver operator characteristics curve (AUC). The DeLong test was employed to distinguish the difference between AUCs (17).

Abbreviations: aSAH, aneurysmal subarachnoid hemorrhage; NAR, neutrophil-to-albumin ratio; SII, systemic immune-inflammation index; WFNS, World Federation of Neurological Surgeons; AUC, area under the receiver operator characteristics curve; IDI, integrated discrimination improvement; NRI, net reclassification improvement; DCA, decision-curve analysis; VIF, variance inflation factor.

Model development

Following the original SAFIRE predictive tool, binary logistic regression was adopted to establish the predictive model, and the same variables (age, aneurysm size, Fisher grade, and WFNS grade) were included. Similarly, we applied the same predictors (age, history of hypertension, aneurysm location, aneurysm size, Fisher grade, WFNS grade, and treatment) as the SAHIT model used to build a binary logistic regression model. The inflammation marker with the highest predictive value was added to these models for further development.

Model performance

The modified models were compared with the original models. Performance between models was evaluated from different perspectives by a variety of approaches. AUC values with 95% CIs and integrated discrimination improvement (IDI) were reported to represent the discrimination significance of the modified models. Categorical net reclassification improvement (NRI) was employed to illustrate the reclassification, and decision-curve analysis (DCA) was used to show the net benefit and visualize the clinical usefulness. According to the previous study (18), we defined risk ratio < 0.1 as low, 0.1–0.6 as moderate, and > 0.6 as high risk of long-term mortality.

The R^2 statistic was reported to identify the proportion of variance explained by the predictive models, and the contribution of each predictor to the predictive models was represented with the partial R^2 statistic (19).

Model calibration

The calibration plots (20) were used to evaluate the calibration of the prediction models, and the Brier score (21) was computed to measure the prediction accuracy. The Brier score ranges from 0 to 0.25. The closer the Brier score is to 0, the better the model calibration degree is. When the Brier score equals 0.25, the model has no prediction ability.

Model validation

Two parts of model validation were completed. A 400 times 10-fold cross-validation was adopted as the internal validation strategy, and mean AUC values and average error were reported.

We compared the derivation cohort (2009–2017) and the validation cohort (2018–2019) as a temporal validation, and the AUC, IDI, and NRI values of the temporal validation cohort were calculated.

Sample size

The sample size of this study was calculated using the formula developed by Riley et al. (22). The required minimum sample size was obtained for developing a new model using the SAFIRE predictive tool consisting of 323 patients. Using the SAHIT predictive tool, the minimum sample size was 458 patients.

Sensitivity analysis

For further sensitivity analysis, inflammatory markers were added as categorical variables using their cut-off values, and potentially meaningful interaction effects were reported.

All analyses were conducted using R software (version 4.2.1, R Foundation for Statistical Computing). Web-based nomograms were developed using the DynNom R package.

Results

Patient demographics and missing data

A total of 3,173 patients were included in this study (Figure 1 details patient inclusion flow chart). Patients were divided into the derivation cohort ($n = 2,525$) and the validation cohort ($n = 648$). The median age was 55 (interquartile range 47–63) in the derivation cohort and 55 (48–66) in the validation cohort. Most patients were female (65.3% in the derivation cohort and 63.6% in the validation cohort). Mortalities at 3 months were similar in the two cohorts (11.8% and 11.6, respectively). Details of the characteristics were summarized in Table 1.

In the derivation cohort, 23.3% of patients were missing aneurysm size values, and 27.4% were missing Fisher grade values. Any missing data in the derivation cohort were imputed. The validation cohort included 423 patients with complete data in the final computation.

Model development

In the derivation cohort, NAR performed the best predictive ability with the highest AUC of 0.707 (95% CI 0.673–0.740), and PLR was the weakest with the lowest AUC of 0.562 (95%CI 0.525–0.599), as shown in Figure 2. Similar results were represented in the validation cohort (NAR: AUC = 0.770, 95% CI 0.712–0.828; PLR: AUC = 0.453, 95% CI 0.381–0.524). Details of each marker's performance were reported in Supplementary Table S1. Considering the poor performance of PLR, we did not add it to the models furtherly.

Before adding to SAFIRE and SAHIT models, the collinearity of the original model predictors and the inflammatory markers was examined *via* VIF, as shown in Supplementary Tables S2, S3. There was no VIF value over 5.

In the original SAFIRE model, the strongest predictor was WFNS grade (partial $R^2 = 10.02\%$), followed by age (partial $R^2 = 0.93\%$), Fisher grade (partial $R^2 = 0.73\%$), and aneurysm size (partial $R^2 = 0.40\%$). In the original SAHIT model, the strongest predictor was WFNS grade (partial $R^2 = 7.71\%$), followed by treatment (partial $R^2 = 4.35\%$), Fisher grade (partial $R^2 = 1.24\%$), aneurysm size (partial $R^2 = 1.00\%$), age (partial $R^2 = 0.23\%$), history of hypertension (partial $R^2 = 0.05\%$), and aneurysm location (partial $R^2 = 0.03\%$).

As shown in Figure 3, NAR was the second significant predictor in the modified SAFIRE model (partial $R^2 = 2.01\%$) and the third in the modified SAHIT model (partial $R^2 = 2.04\%$). The significance of other markers after adding to the predictive models was illustrated in Supplementary Figure S2. Details of the logistic regression models were reported in Supplementary Tables S4, S5.

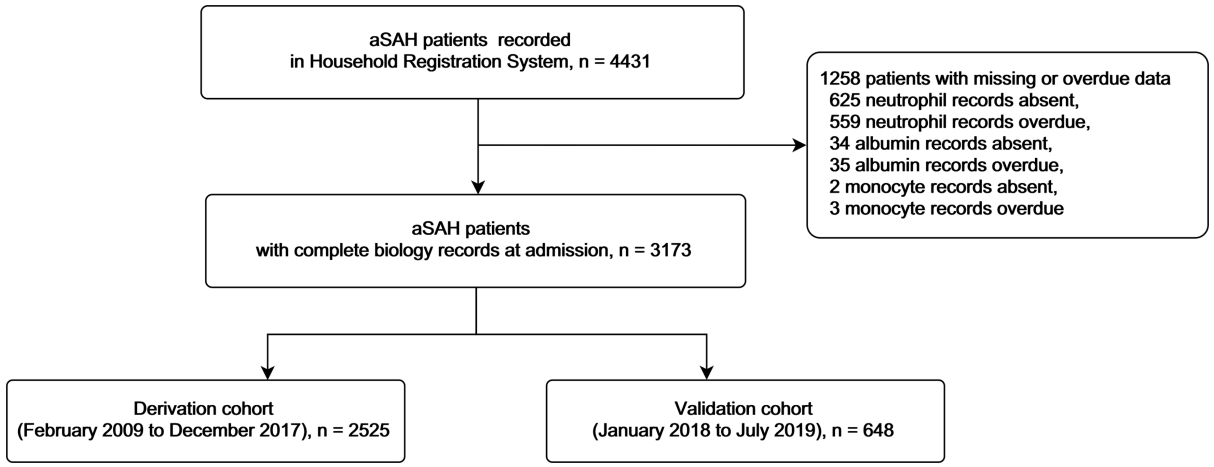

FIGURE 1
Flow chart for patients included in this study.

Model performance

SAFIRE + inflammatory markers

As shown in Table 2, inputting the same predictors as the SAFIRE predictive tool generated a comparable AUC value in the derivation cohort (AUC = 0.778, 95% CI 0.750–0.906). After adding inflammatory markers, all modified predictive models achieved a better discriminative ability in the derivation cohort. Among the four markers, adding NAR to the original model acquired the highest AUC development (SAFIRE+NAR vs. SAFIRE: ΔAUC = 0.016, $p = 0.012$) and the highest IDI (SAFIRE+NAR vs. SAFIRE: IDI = 0.025, $p < 0.001$).

Compared with the original SAFIRE model, the addition of NAR (NRI = 0.073, $p = 0.002$), MLR (NRI = 0.041, $p = 0.024$), and NLR (NRI = 0.045, $p = 0.020$) could improve the reclassification and the addition of NAR showed the most remarkable reclassification improvement among them. Supplementary Table S6 shows that including NAR as an additional predictor led to 22 (7.36%) extra deaths being classified into a higher risk category, although 2 (0.09%) extra survivors were reclassified into a higher risk category. As shown in Figure 4C, SAFIRE+NAR showed a higher net benefit than the original model.

SAHIT + inflammatory markers

As shown in Table 3, the AUC value of the SAHIT model in the derivation cohort (AUC = 0.819, 95% CI 0.793–0.845) was also comparable with that previously reported. The addition of inflammatory markers all improved the modified predictive models. Among the four markers, adding NAR to the SAFIRE and SAHIT models acquired the highest AUC development (SAHIT+NAR vs. SAHIT: ΔAUC = 0.012, $p = 0.016$) and the highest IDI (SAHIT+NAR vs. SAHIT: IDI = 0.023, $p < 0.001$).

Compared with the original SAHIT model, only the addition of NAR (NRI = 0.081, $p < 0.001$) and NLR (NRI = 0.055, $p = 0.011$) could improve the reclassification, and the NRI of addition of NAR was still the highest. As shown in Supplementary Table S7, SAHIT+NAR reclassified 24 (1.08%) survivors into a lower risk category and 21 (7.02%) deaths into a higher risk category, which meant both specificity and sensitivity was enhanced. As shown in Figure 4C, including NAR enhanced the net benefit compared with the original SAHIT model, which meant greater clinical usefulness.

Model validation

SAFIRE + inflammatory markers

In the internal validation, for all SAFIRE+NAR models using the derivation cohort data, the mean AUC was 0.785, and the average error was 11.8%. In the temporal validation, the performances of the developed models using the validation cohort data were comparable to those using the derivation cohort data. As shown in Table 2, only the addition of NAR improved the discrimination ability of the SAFIRE model (SAFIRE+NAR vs. SAFIRE: ΔAUC = 0.044, $p = 0.001$; IDI = 0.057, $p < 0.001$). Moreover, including NAR as an additional predictor improved the reclassification ability of the SAFIRE model (NRI = 0.101, $p = 0.044$), and details of the reclassification improvement were shown in Supplementary Table S8. Net benefit was also improved, as shown in Figure 4D.

SAHIT + inflammatory markers

In the internal validation, for all SAHIT+NAR models, the mean AUC was 0.820, and the average error was 10.7%. In the temporal validation, the performances of the developed models using the validation cohort data were comparable to those using the derivation cohort data. As shown in Table 3, only the addition of NAR improved the discrimination ability of the SAHIT model (SAHIT+NAR vs. SAHIT: ΔAUC = 0.032, $p = 0.004$; IDI = 0.053, $p < 0.001$). Moreover, including NAR as an additional predictor improved the reclassification ability of the SAHIT model (NRI = 0.151, $p = 0.015$), and details of the reclassification improvement were shown in Supplementary Table S9. According to the DCA in Figure 4D, SAHIT+NAR also showed higher clinical usefulness in the validation cohort.

TABLE 1 Data of patients included in the study.

Characteristics	Derivation cohort (*n*=2,525)	Validation cohort (*n*=648)	*p* value
Demographics			
Age, *n* (%)			
≤50 y	959 (38.0)	209 (32.3)	0.001
50–60 y	656 (26.0)	152 (23.5)	
60–70 y	645 (25.5)	194 (29.9)	
≥70 y	265 (10.5)	93 (14.4)	
Medical history, n (%)			
Hypertension	629 (24.9)	144 (22.2)	0.17
Aneurysm characteristics			
Posterior location, *n* (%)	360 (14.3)	241 (37.2)	<0.001
Size of the aneurysm, n (%)			
<10 mm	1512 (59.9)	443 (68.4)	<0.001
10–20 mm	317 (12.6)	46 (7.1)	
≥20 mm	107 (4.2)	13 (2.0)	
Missing	589 (23.3)	146 (22.5)	
Hemorrhagic characteristics, n (%)			
WFNS grade			
I	1465 (58.0)	377 (58.2)	<0.001
II	389 (15.4)	134 (20.7)	
III	75 (3.0)	4 (0.6)	
IV	290 (11.5)	35 (5.4)	
V	306 (12.1)	98 (15.1)	
Fisher grade			
I	116 (4.6)	22 (3.4)	0.04
II	406 (16.1)	81 (12.5)	
III	280 (11.1)	84 (13.0)	
IV	1,032 (40.9)	293 (45.2)	
Missing	691 (27.4)	168 (25.9)	
Treatment of aneurysms, n (%)			
Clip	1758 (69.6)	365 (56.3)	<0.001
Coil	350 (13.9)	45 (6.9)	
No treatment	417 (16.5)	238 (36.7)	
Biology, mean (SD)			
Neutrophil, 10^9/L	8.78 (4.40)	9.08 (4.62)	0.12
Platelet, 10^9/L	173.00 (70.40)	180.11 (70.80)	0.02
Lymphocyte, 10^9/L	1.21 (0.61)	1.15 (0.58)	0.02
Albumin, g/L	39.91 (5.14)	40.36 (5.32)	0.05
Monocyte, 10^9/L	0.53 (0.29)	0.56 (0.29)	0.06
NAR	0.22 (0.11)	0.23 (0.12)	0.28
NLR	9.79 (8.51)	10.67 (8.88)	0.02
PLR	174.41 (118.31)	193.52 (137.02)	<0.001
MLR	1.64 (1.62)	1.92 (2.06)	<0.001
SII	0.52 (0.37)	0.58 (0.42)	<0.001
Outcome at 3 months, n (%)			
Survivor	2226 (88.2)	573 (88.4)	0.90
Death	299 (11.8)	75 (11.6)	

NAR, neutrophil-to-albumin ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; SII, systemic immune-inflammation index.

Model calibration

We demonstrate the calibration plot in Supplementary Figures S3, S4, and there was no significant evidence of miscalibration. None of the original and developed models got a brier score over 0.25, where lower scores signify better calibration.

Sensitivity analysis

In the sensitivity analysis, as shown in Supplementary Tables S10, S11, adding markers as categorical variables reduced the improvement of the predictive ability. Meanwhile, the addition of the interaction terms, including NAR and age, WFNS grade, Fisher grade, or treatment, failed to develop the SAFIRE+NAR or SAHIT+NAR model (Supplementary Table S12).

Model presentation

To better use the developed SAFIRE and SAHIT model, we developed two web-based nomograms, accessible at https://sahit-nar.shinyapps.io/SAHIT-NAR/ and https://sahit-nar.shinyapps.io/SAFIRE-NAR/. The presentation of the webs was presented in Supplementary Figures S5, S6.

Discussion

This study demonstrated that inflammatory markers could improve the predictive effectiveness of existing models, including discrimination, reclassification, and clinical usefulness. The predictive ability of various inflammatory markers was compared through temporal validation, and it was found that NAR had the best predictive improvement ability. An online calculator was developed for the improved models to facilitate further validation and application.

The involvement of inflammatory response in the acute phase of aSAH may be the source of the ability of inflammatory indicators to predict short-term outcomes of aSAH (23). Studies have reported a peak of inflammatory cytokines within 48 h after the onset of aSAH (24). An excessive inflammatory response leads to a poor prognosis for aSAH (25). Due to the destruction of BBB, peripheral immune cells and their products will also affect the central nervous system. Neutrophils produce oxygen-free radicals and proteolytic enzymes that damage neurons and endothelial cells (26). In recent studies, albumin has been suggested to have a possible protective effect on

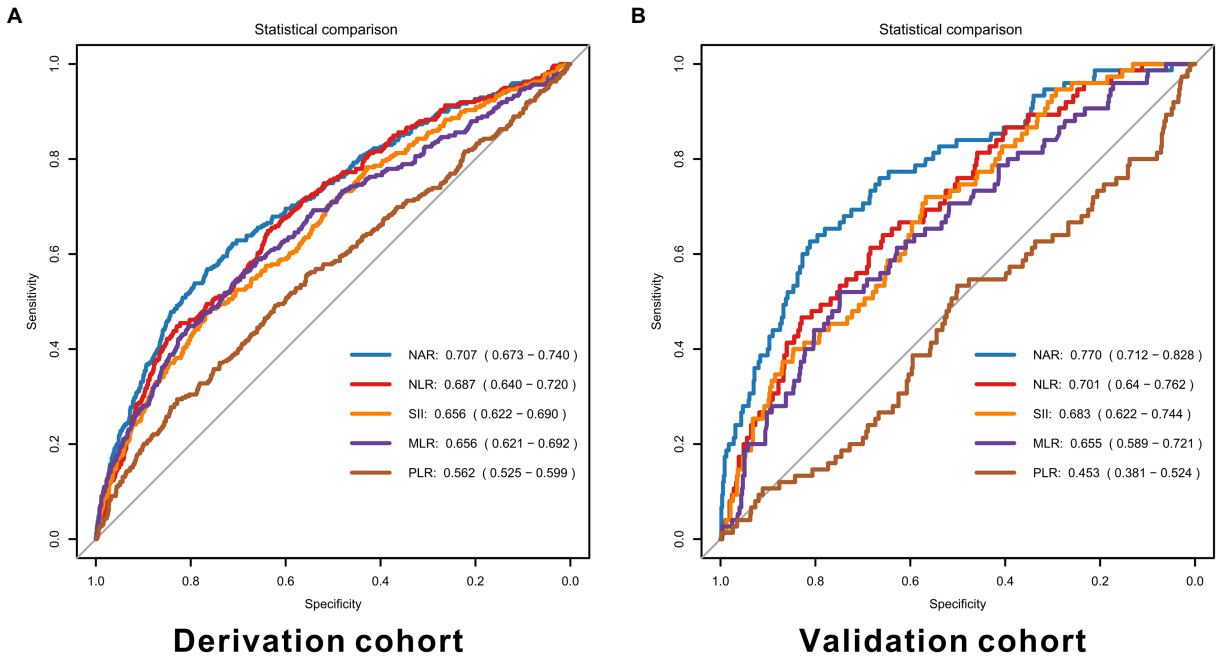

FIGURE 2
ROC curves for inflammatory markers in the derivation **(A)** and validation cohorts **(B)**. NAR, neutrophil-to-albumin ratio, NLR, neutrophil-to-lymphocyte ratio, PLR, platelet-to-lymphocyte ratio, MLR, monocyte-to-lymphocyte ratio, SII, systemic immune inflammation index.

TABLE 2 Performance of SAFIRE and SAFIRE + inflammatory biomarkers models.

Models	Discrimination				Reclassification		Calibration
	AUC (95% CI)	*p* value	IDI (95% CI)	*p* value	Categorical NRI (95% CI)	*p* value	Brier score
Derivation cohort							
SAFIRE	0.778 (0.750–0.806)	Ref.	Ref.		Ref.		0.090
SAFIRE+NAR	0.794 (0.766–0.821)	0.012	0.025 (0.015–0.035)	<0.001	0.073 (0.026–0.119)	0.002	0.087
SAFIRE+MLR	0.791 (0.764–0.818)	0.012	0.014 (0.005–0.022)	<0.001	0.041 (0.006–0.077)	0.024	0.088
SAFIRE+NLR	0.790 (0.763–0.817)	0.012	0.015 (0.006–0.024)	<0.001	0.045 (0.007–0.083)	0.020	0.088
SAFIRE+SII	0.787 (0.760–0.814)	0.037	0.013 (0.006–0.020)	<0.001	0.014 (−0.019–0.047)	0.404	0.088
Validation cohort							
SAFIRE	0.771 (0.709–0.833)	Ref.	Ref.		Ref.		0.108
SAFIRE+NAR	0.815 (0.757–0.873)	0.001	0.057 (0.030–0.085)	<0.001	0.101 (0.003–0.199)	0.044	0.099
SAFIRE+MLR	0.787 (0.728–0.846)	0.140	0.005 (−0.012–0.022)	0.571	−0.001 (−0.070–0.069)	0.992	0.109
SAFIRE+NLR	0.782 (0.721–0.844)	0.271	0.017 (−0.001–0.035)	0.062	−0.052 (−0.130–0.025)	0.185	0.106
SAFIRE+SII	0.780 (0.718–0.841)	0.385	0.022 (0.002–0.041)	0.033	−0.014 (−0.103–0.075)	0.759	0.106

NAR, neutrophil-to-albumin ratio; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; SII, systemic immune-inflammation index; AUC, the area under the receiver operator characteristics curve; IDI, integrated discrimination improvement; NRI, net reclassification improvement; CI, confidence interval.

BBB (27). Many clinical studies have also shown that high neutrophils are associated with prognosis in patients with aSAH (28), while hypoproteinemia is associated with infection during hospitalization (29, 30). This may be the NAR's mechanism for predicting the outcome of aSAH.

Many previous studies have compared the predictive effects of inflammatory factors alone instead of included in a complete model (31, 32). As shown in Figure 2, Tables 2, 3, we found that the predictive power of inflammatory markers alone was not parallel to their ability to improve the predictive models. In a prediction model proposed by Lai et al. (33), although NLR was included, it was not reported how the inclusion of NLR improved the prediction efficiency of the model. Similarly, in the TAPS model presented by Li et al. (34), the contribution of white blood cells (WBC) to the model was not reported. Moreover, the same predictors (NLR and WBC) presented different predictive

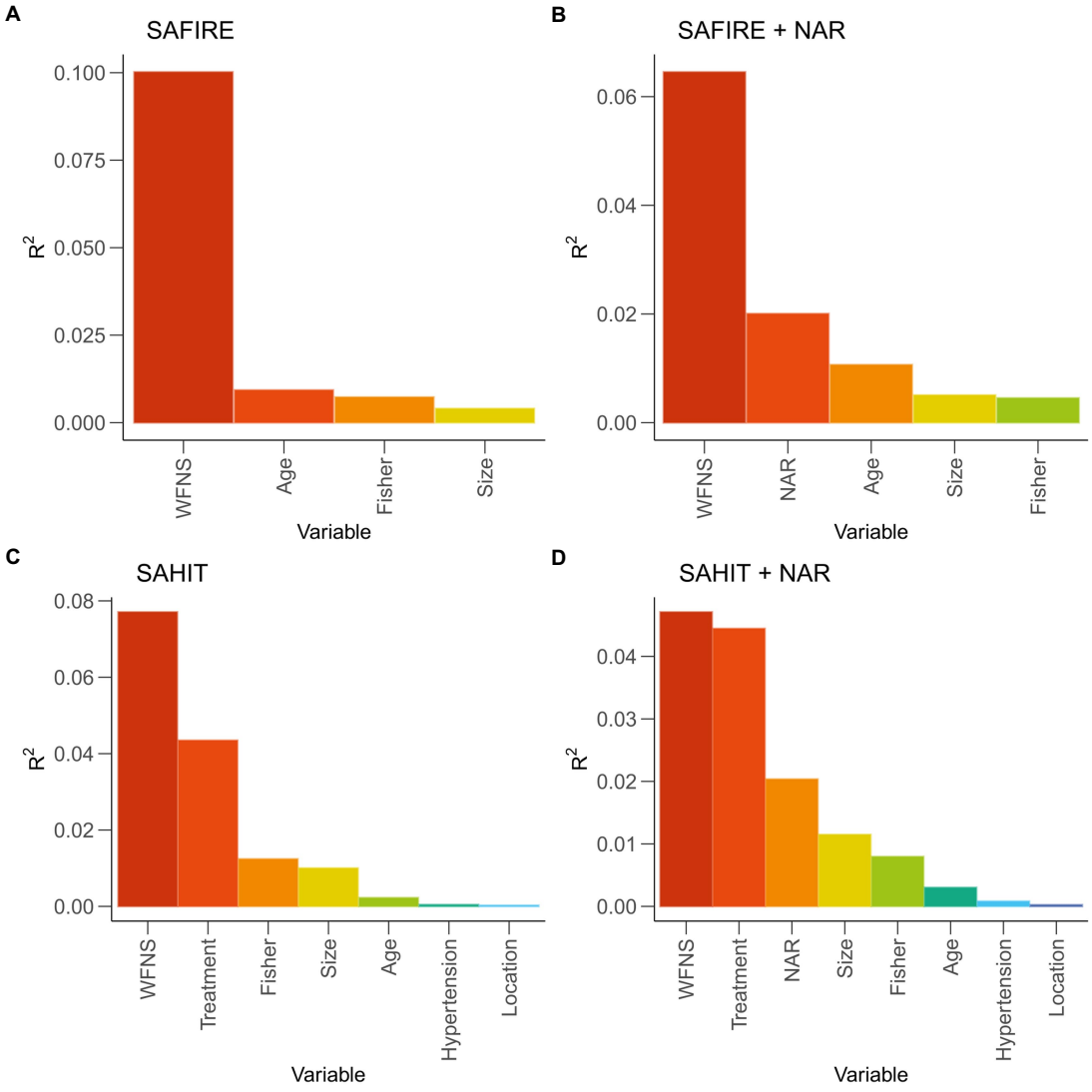

FIGURE 3
General dominance of predictors reported as partial R^2 statistic in original and modified models. SAHIT, original Subarachnoid Hemorrhage International Trialists' model; SAFIRE, the SAFIRE grading scale indicates size of the aneurysm, age, fisher grade, World Federation of Neurological Surgeons; WFNS, World Federation of Neurological Surgeons; NAR, neutrophil-to-albumin ratio.

performances in the two studies. When demographic information, imaging information, clinical status, laboratory examination, and other indicators of different dimensions are combined, it was unknown whether inflammatory factors could improve the prediction effect of the original model. Therefore, it was necessary to quantify the predictive value of inflammatory factors in the same basic model. Figure 3 showed that in the SAFIRE and SAHIT models, WFNS always had the highest predictive ability. In contrast, the contribution of variables including age, aneurysm location, and size to the model lagged far behind that of WFNS. As shown in Supplementary Figure S2, after adding inflammatory markers to the models and comparing their partial R^2, we found that the contribution of all inflammatory markers except SII ranked in the top three, and NAR had the highest contribution among them.

At the same time, we compared the differentiation ability of inflammatory factors and, furtherly, their reclassification ability. NRI can reflect the degree to which the improved model differentiates patients at different risk levels. Although NRI has been used in previous studies (35, 36), these studies reported continuous NRI, which is less explanatory than categorical NRI. We found 7–8% NRI for including NAR in the derivation cohort and 10–15% NRI in the validation cohort. Further reclassification analysis found that NAR was better able to identify high-risk (>65%) aSAH patients, which can help to distinguish early and intervene early in clinical practice. In addition, we demonstrated that NAR is a stable and reliable predictor across periods.

Apart from the outstanding discriminative and reclassification ability, NAR also had a significant value in clinical application in other aspects. First, NAR is an inexpensive and easy-to-use inflammatory marker. Neutrophil and albumin levels are routine in-hospital tests for aSAH patients, and the calculation is simple. Moreover, neutrophil and albumin levels are easy targets for clinical intervention. Human albumin treatment can be used to treat hypoalbuminemia. The 1.25 g/kg/day

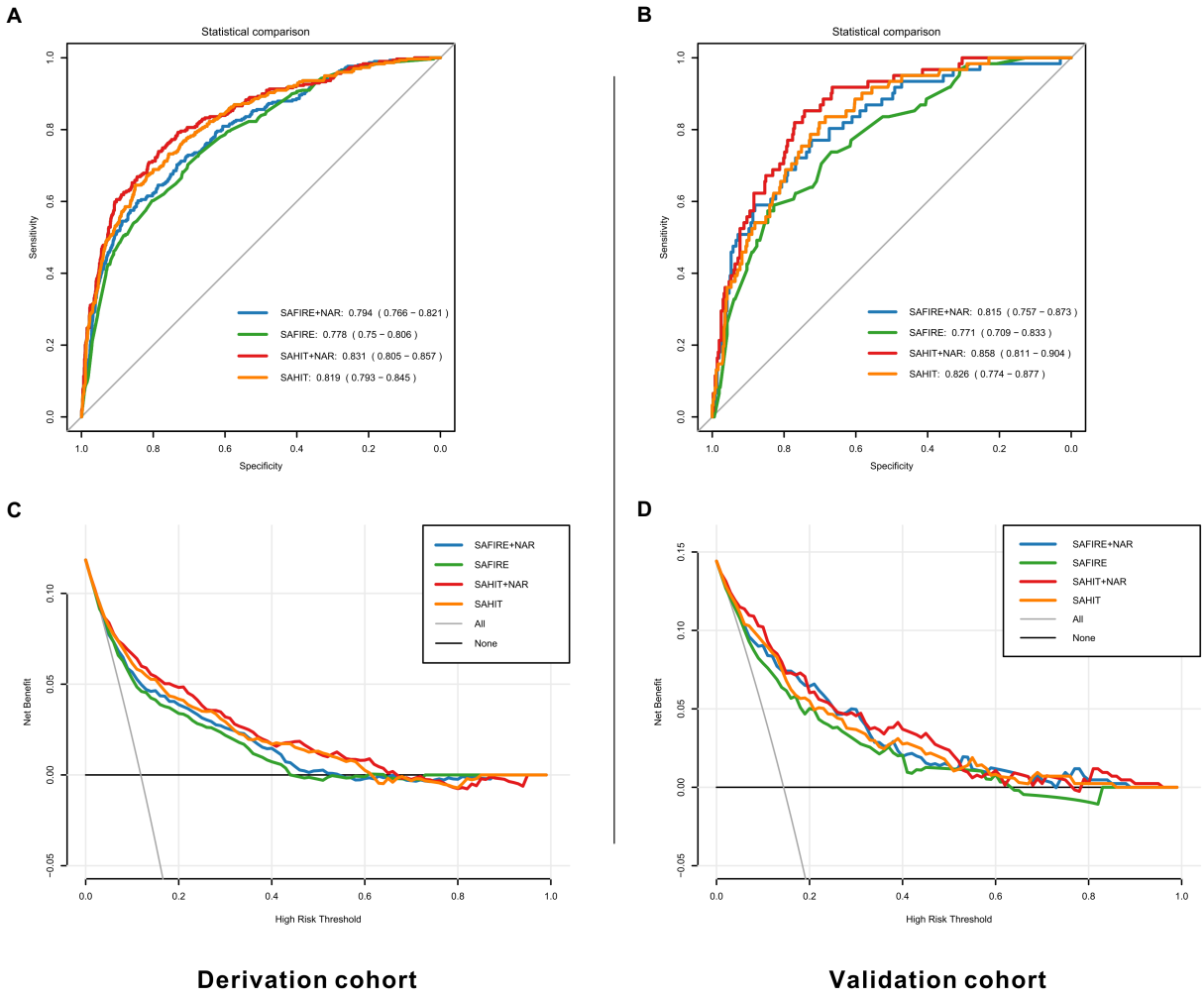

FIGURE 4
ROC curves and decision curves for original and modified models in the derivation **(A,C)** and validation cohorts **(B,D)**. SAHIT, original Subarachnoid Hemorrhage International Trialists' model; SAFIRE, the SAFIRE grading scale; NAR, neutrophil-to-albumin ratio.

TABLE 3 Performance of SAHIT and SAHIT + inflammatory biomarkers models.

Models	Discrimination				Reclassification		Calibration
	AUC (95% CI)	*p* value	IDI (95% CI)	*p* value	Categorical NRI (95% CI)	*p* value	Brier score
Derivation cohort							
SAHIT	0.819 (0.793–0.845)	Ref.	Ref.		Ref.		0.083
SAHIT+NAR	0.831 (0.805–0.857)	0.016	0.023 (0.013–0.032)	<0.001	0.0810 (0.038–0.124)	<0.001	0.081
SAHIT+MLR	0.828 (0.802–0.853)	0.015	0.010 (0.002–0.018)	0.012	0.0402 (−0.004–0.084)	0.072	0.082
SAHIT+NLR	0.827 (0.802–0.853)	0.015	0.011 (0.004–0.019)	0.003	0.0554 (0.013–0.098)	0.011	0.082
SAHIT+SII	0.824 (0.798–0.850)	0.103	0.009 (0.003–0.015)	0.004	0.0324 (−0.008–0.073)	0.113	0.082
Validation cohort							
SAHIT	0.826 (0.774–0.877)	Ref.	Ref.		Ref.		0.099
SAHIT+NAR	0.858 (0.811–0.904)	0.004	0.053 (0.027–0.079)	<0.001	0.1505 (0.029–0.272)	0.015	0.094
SAHIT+MLR	0.830 (0.778–0.882)	0.597	0.006 (−0.011–0.023)	0.478	0.0519 (−0.048–0.152)	0.308	0.100
SAHIT+NLR	0.829 (0.776–0.881)	0.660	0.018 (−0.001–0.036)	0.056	0.0328 (−0.050–0.115)	0.437	0.098
SAHIT+SII	0.828 (0.775–0.881)	0.719	0.019 (0.002–0.036)	0.031	0.0357 (−0.040–0.112)	0.356	0.098

NAR, neutrophil-to-albumin ratio; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; SII, systemic immune-inflammation index; AUC, the area under the receiver operator characteristics curve; IDI, integrated discrimination improvement; NRI, net reclassification improvement; CI, confidence interval.

albumin therapy for SAH patients was reported to be tolerable without major complications and might be neuroprotective (37). Furthermore, neutrophil depletion following SAH was suggested to increase memory *via* NMDA receptors (38). However, this biomarker ratio still could not be directly applied in clinical practice. It should be furtherly proven beneficial in the laboratory and then carefully verified in the clinical trials.

This study has numerous strengths. Our study was based on a 10 year large cohort study, supporting our temporal validation. In addition, we obtained the accurate survival status of the enrolled patients at 3 months through the household registration system. Third, we used various methods to quantify how inflammatory factors improved the prediction model. The web-based prognostic calculator can also improve the clinical application value of this study.

However, our study had some limitations. This study was a single-center retrospective study, which could not support us in conducting geographical validation. Further multi-center studies could overcome the limitations of single-center temporal validation. In addition, due to retrospective collection, part of the data was lost. Although multiple imputations were carried out, the feasibility of the research conclusion was reduced. Third, we did not obtain the functional outcome of patients, which prevented us from proving that inflammatory factors were equally good at predicting the functional outcome of aSAH patients.

This study suggested that more attention should be paid to inflammatory indicators when establishing aSAH prediction models. We also preliminarily demonstrated that the combined use of NAR improved the predictive performance of existing prediction models. NAR was an inexpensive and convenient early laboratory index that deserves further clinical validation in more prospective multicenter studies.

Conclusion

This study illustrated that among the inflammatory markers associated with aSAH prognosis, NAR could improve the SAFIRE and SAHIT models for the 3-month mortality of aSAH.

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 ethics committee of West China Hospital (No. 20211701). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

RZ, YZ, CY, LM, and FF: study concept and design. RZ, YZ, ZL, YP, YH, and JY: acquisition, analysis, or interpretation of data. RZ, YZ, and ZL: statistical analysis. RZ: drafting of the manuscript. RZ, YZ, ZL, YP, YH, JY, CY, LM, and FF: critical revision of the manuscript for important intellectual content. 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.2023.1078926/full#supplementary-material

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Predictive model of recurrent ischemic stroke: model development from real-world data

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Background: There are established correlations between risk factors and ischemic stroke (IS) recurrence; however, does the hazard of recurrent IS change over time? What is the predicted baseline hazard of recurrent IS if there is no influence of variable predictors? This study aimed to quantify the hazard of recurrent IS when the variable predictors were set to zero and quantify the secondary prevention influence on the hazard of recurrent ischemic stroke.

Methods: In the population cohort involved in this study, data were extracted from 7,697 patients with a history of first IS attack registered with the National Neurology Registry of Malaysia from 2009 to 2016. A time-to-recurrent IS model was developed using NONMEM version 7.5. Three baseline hazard models were fitted into the data. The best model was selected using maximum likelihood estimation, clinical plausibility, and visual predictive checks.

Results: Within the maximum 7.37 years of follow-up, 333 (4.32%) patients had at least one incident of recurrent IS. The data were well described by the Gompertz hazard model. Within the first 6 months after the index IS, the hazard of recurrent IS was predicted to be 0.238, and 6 months after the index attack, it reduced to 0.001. The presence of typical risk factors such as hyperlipidemia [HR, 2.22 (95%CI: 1.81–2.72)], hypertension [HR, 2.03 (95%CI: 1.52–2.71)], and ischemic heart disease [HR, 2.10 (95%CI: 1.64–2.69)] accelerated the hazard of recurrent IS, but receiving antiplatelets (APLTs) upon stroke decreased this hazard [HR, 0.59 (95%CI: 0.79–0.44)].

Conclusion: The hazard of recurrent IS magnitude differs during different time intervals based on the concomitant risk factors and secondary prevention.

KEYWORDS

recurrent, ischemic stroke, pharmacometrics, time to event model, NONMEM

Introduction

Stroke is the world's second leading cause of death and mortality (1–4). The risk of recurring strokes is much greater for survivors of acute ischemic stroke (IS). For survivors of acute ischemic stroke (IS), the risk of repeated strokes is significantly larger (5–7). In Malaysia, ~33% of the IS population had recurrent stroke (8). In recurrence stroke,

neurological damage is usually severe, harder to deal with, and has a higher mortality rate compared with the first stroke (9). Therefore, secondary prevention is crucial to reduce recurrent IS events (9).

The prognosis of recurrent IS has been widely studied. The probability of recurrent IS after the index attack was predicted to vary over time, i.e., it was predicted to range from 11.2% to 30% within the first 24 months (10, 11) and be 9.5% within 5 years after the IS attack (12). In contrast, the most recent study reported that the rate of recurrent IS was 1.2% in the first 30 days, 3.4% within 90 days, 7.4% within 1 year, and 19.4% within 5 years (13). Moreover, the reported risk factors of recurrent stroke vary (14–16), in which hypertension (HTN), atrial fibrillation (AF), diabetes mellitus (DM), hyperlipidemia (HPLD), ischemic heart disease (IHD), and smoking were the most common reported predictors of recurrent stroke (17, 18). Despite improvements in recurrent IS risk classification and prevention measures in the past decades, IS remains a devastating disease. Currently, most of the methods of secondary prevention of IS are focused on reducing and controlling the risk factors that lead to recurrent IS. Nevertheless, does the hazard of recurrent IS change over time?

The Essen Stroke Risk Score (ESRS) is a score that is used to predict stroke recurrence in a hospital-based follow-up study. It includes 9 points depending on risk factors: 2 point for age >75, but only 1 point for 65–75, HTN, DM, previous myocardial infarction, other cardiovascular diseases, peripheral arterial disease, smoking history, and previous TIA or IS (19). The Recurrence Risk Estimator at 90 days (RRE-90) is a web-based prognostic scoring tool designed to calculate 90-day recurrent stroke risk by including risk factors of stroke, such as the history of a mini-stroke or transient ischemic attack (TIA), age, and the type of first stroke the person experienced (20). These conventional recurrent IS prediction scores did not incorporate time to follow the longitudinal natural changes of recurrent IS.

The majority of the previous prognosis studies of recurrent stroke used the most common semi-parametric survival analysis method, i.e., the Cox regression analysis. The Cox model incorporates the effect of covariates on the hazard without quantifying the shape or form of the recurrent stroke hazard rate at baseline. The hazard of the event at baseline is defined as the hazard of having an interest event when all the predictor variables were set to zero or their reference level was set for categorical variables. Thus, in addition to quantifying the effect of predictor variables on the occurrence of the event (e.g., recurrent IS), defining specific shape or distribution of the event hazard at baseline (e.g., just after the index stroke) may allow better prediction of the event of interest, taking into account the natural effect of the disease itself. Studies using this approach on recurrent strokes are still lacking (21, 22).

Abbreviations: APLT, antiplatelet; DM, diabetes mellitus; HPLD, hyperlipidemia; HTN, hypertension; IHD, ischemic heart disease; IS, ischemic stroke; MOH, ministry of health, Malaysia; NNEUR, national neurology registry; OFV, objective function value; RTTE, repeated time to event; RSE, relative standard error; TTE, time to event; SIR, sampling importance resampling; VPC, visual predictive check.

In this study, we performed a non-conventional way of developing a predictive model for recurrent IS using the parametric approach of the time-to-event analysis. We quantified the specific trend of recurrent IS after the index IS when all the predictor variables were set to zero. This permits more time-dependent prognostic information that better reflects the disease's expected "natural effect." Moreover, the validated prognostic models of recurrent IS are limited. This study used real-world population-based data of the IS population and aimed to quantify the hazard of recurrent IS when the variable predictors were set to zero, to quantify the hazard of the recurrent IS at different time points after the index IS, and to quantify the secondary prevention influence on the hazard of recurrent ischemic stroke.

Method

Patients and data acquisition

This population cohort study used the secondary analysis of data from the National Neurology Registry (NNEUR) of Malaysia. Data of all Malaysian patients with a history of index IS from August 2009 to December 2016 were extracted from the NNEUR of Malaysia. The details on the National Stroke Registry of Malaysia were published previously (23–25). The stroke was diagnosed according to the World Health Organization's criteria (26). All diagnoses were confirmed using brain computed tomography or magnetic resonance imaging. Index IS was defined as the first stroke registered in the NNEUR for patients from 2009 to 2016. Recurrent IS was defined as any IS event recorded by involving hospitals after the index IS for a specific patient in the NNEUR database. Malaysian adults aged above 18 years with a history of IS and registered with NNEUR were included. Non-Malaysian citizens and those with diagnoses other than IS were excluded from the study. The minimum events needed to develop this prognostic model were calculated as 228. Sample size—Survival analysis|Sample Size Calculators (sample-size.net).

Stroke registry in Malaysia

The NNEUR in Malaysia was established in 2009. It has recorded data from multiethnic stroke cases from 13 states in the country. The NNEUR aims to provide comprehensive epidemiological data on the country's stroke statistics, trends, and management, representing a multicenter, hospital-based registry. The registry development is funded by the Ministry of Health, Malaysia (MOH). A comprehensive explanation of the NNEUR has been previously published (27).

Ethics approval

Ethical approval for this study was obtained from the Medical Research and Ethics Committee (MREC), Ministry of Health, Malaysia (Research ID: NMRR-08-1631-3189).

Collected variables

Based on demographic data and concomitant diseases, including DM, HTN, HPLD, IHD, and hyperuricemia, medications used for secondary prevention were tested. They were defined either by physician diagnosis, by patients' electronic records, or from the medication history, and the medications were prescribed during discharge.

Analysis

The time to the recurrent events of IS and factors predicting the recurrence of IS were quantified and determined using NONMEM version 7.5 software and Perl-speaks-NONMEM (PsN) version 4.1.0. After the index IS, the event was described as having recurrent IS events. All event times were treated as exact time models, in which the event was assumed to occur at the time of observation. For the baseline hazard model, three models, namely, exponential, Gompertz, and Weibull, were investigated.

Model development

The model was developed in the following two steps: (i) a base model without any explanatory factors and (ii) an exploration of covariates.

Development of the base model

A parametric survival function based on Equation 1 was used to describe the time to the recurrent IS.

$$S(t) = e^{-\int_0^t h(t)dt}, \tag{1}$$

where $S(t)$ is the survivor function calculated from the integral of hazard concerning time. The hazard is $h(t)$, and the survival $S(t)$ is a function of the cumulative hazard within the time interval between the time zero and the time t, describing the probability of not experiencing any recurrent IS within this interval.

The base model was developed by exploring different functions for the hazard $h(t)$, starting from a simple time-independent constant hazard and then gradually progressing to more complex functions, including Gompertz and Weibull, according to Equations (2), (3), and (4), respectively (28).

$$h = h_0 \times e^0 \tag{2}$$

$$h(t) = h_0 \times e^{\beta t} \tag{3}$$

$$h(t) = h_0 \times e^{\beta \ln(t)} \tag{4}$$

The hazard of recurrent IS at baseline or baseline hazard function at different time points after the index was quantified based on Equation 5. Equation 5 shows an example of changes in the baseline hazard $h_0(t)$ based on different time t intervals.

$$h_0(t) = \begin{cases} \theta_1, & if\ 0 < t < t\ 1 \\ \theta_2, & if\ t1 < t \leq t2 \\ \cdots\cdots\cdots\cdots \\ \theta_{n\,t}, & if\ t(n-1) < t \leq tn \end{cases} \tag{5}$$

Between-subject variability around the hazard was estimated, assuming an exponential distribution for the random effect.

Development of the covariate model

Possible explanatory variables that may influence or predict the changes in hazard were explored by including each explanatory variable in the hazard function. A parameter, β_n, for each of the n explanatory variables, X_n, was estimated using the following equation.

$$h\,(t) = h_0\ (t) * \exp^{\beta 1X1 + \beta 2X2 \ldots . + \beta nXn},$$

where h_0 is the baseline hazard and β_n is the coefficient for the explanatory variable, Xn, which describes how the hazard varies with the explanatory variable. Exponentiation of the explanatory variable coefficient provides the hazard ratio (HR), which reflects the influence of the explanatory variables relative to the hazard when the explanatory variable is not present.

Initially, the covariates were tested in a univariate manner, i.e., each covariate relationship was evaluated on the base hazard individually. Then, based on the results, covariate relationships were identified for a systematic covariate search by applying a stepwise analysis approach, i.e., with stepwise forward inclusion followed by backward elimination (29).

In the forward inclusion, the statistical significance level was set at a P-value of <0.05, which corresponds to a reduction of the OFV of at least 3.84, for one degree of freedom (addition of one covariate parameter). While in the backward deletion, the significant value was set to a P-value of <0.01, corresponding to an increase in the OFV of at least 6.64 to be kept in the model for one degree of freedom.

Model evaluation

Parameters were estimated using the LAPLACE method (ADVAN = 6 TOL = 9 NSIG = 3) in NONMEM to obtain maximum likelihood estimates of time-to-event parameters. The parametric time-to-event (TTE) analysis was performed using NONMEM version 7.5 and Perl-speaks-NONMEM (PsN) version 4.1.0.7. Model selection was based on comparing the OFV between models, bootstrap confidence intervals for parameter estimates, and biological plausibility. The improvement in the fit was measured by

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a decrease (30) in the OFV generated by NONMEM. The difference in OFV between the two hierarchical models is approximately X^2 distributed and can be tested for significance with $X_{1,0.052} = 3.84$.

To evaluate the predictive performance of the model throughout the model building, Kaplan-Meier visual predictive checks (VPCs) for internal and temporal validation and Xpose4 (version 4.7.1) function (31, 32) in the RStudio software (version 1.1.456, RStudio, Inc., Boston, MA, http://www.rstudio.com/) were utilized. The plots were based on simulations of 1,000 simulated datasets. To enable simulations for time points where no clinical observations had been made, extra dummy time points were added to the dataset for all individuals until 7.37 years for the VPC simulation. The parameter certainty was evaluated through relative standard error (RSE) produced from the sampling importance resampling (SIR) method (33).

Results

Out of 7,697 subjects, 333 patients (4.32%) developed recurrent IS within the maximum follow-up period of 7.37 years. The median time to the first recurrent IS was 1.2 years. The study population included all age groups, from young to elderly, with a median age of 63.47 years at the time of index IS. As shown in Table 1, most of the patients were women (4,289, 55.72%). The percentage of smokers in this study population was 48%. Of 7,697 subjects, 3,493 (45.38%) subjects had diabetes before index IS, while the number of patients with HTN before index IS was 5,506 (71.5%). The number of subjects with HPLD before index IS was 2,028 (26.34%), of patients who had IHD before index IS was 879 (11.4%), and of patients who had AF before index IS were 3.4%. Among patients who had recurrent IS, the percentage of patients who received antiplatelets (APLT), antihyperlipidemics, angiotensin-converting enzyme inhibitors (ACEI), beta-blockers (BB), calcium channel blockers (CCB), diuretics (DIU), and antidiabetics (ADM) for concurrent disease control and secondary prevention were 85.58%, 86.18%, 29.42%, 11.71%, 24.02%, 8.70%, and 39.63%, respectively.

TABLE 1 Characteristic of patients with recurrent IS during different time intervals that included into the study ($N = 333$).

Variable	Patients with recurrent IS $N = 333$ (%)	Patients with no recurrent IS $N = 7,364$ (%)
Recurrent IS < 6 months	108 (31.43)	–
Age group		
<60	150 (45.04)	2,924 (39.70)
≥60	183 (54.95)	4,440 (60.29)
Female	186 (55.85)	4,103 (55.71)
2nd recurrent stroke	36 (0.108)	–
Ethnicity		
Malay	155 (46.54)	1,479 (20.08)
Chinese	7 (2.10)	206 (2.79)
Indian	3 (0.9)	80 (1.08)
Others	167 (50.15)	5,601 (76.05)
Smoker	202 (60.66)	3,547 (48.166)
DM	195 (58.55)	3,298 (44.78)
Duration of diabetes (years)		
<1	9 (2.70)	340 (4.61)
1–5	97 (29.12)	1,675 (22.74)
6–10	42 (12.61)	520 (7.06)
>10	43 (12.91)	763 (10.36)
Unknown	4 (1.2)	–
Family history of stroke	27 (8.10)	339 (4.60)
HTN	288 (86.48)	5,218 (70.85)
HTN duration (years)		
≤5	163 (48.94)	2,167 (29.42)
>5	125 (37.53)	2,051 (27.85)
IHD	77 (23.12)	802 (10.89)
HPLD	159 (47.74)	1,869 (25.38)
AF	4 (1.2)	263 (3.57)
HU	16 (4.8)	218 (2.96)
NIHSS		
Minor	145 (43.54%)	3,407 (46.26%)
Moderate/severe	188 (56.45%)	3,957 (53.73%)
Received medications for concurrent disease control and/or secondary prevention		
APLT	285 (85.58%)	6,613 (89.80%)
Antihyperlipidemic	287 (86.18%)	6,607 (89.72%)
ACEI	98 (29.42%)	2,298 (31.20%)
BB	39 (11.71%)	776 (10.53%)
CCB	80 (24.02%)	1,520 (20.64%)
DIU	29 (8.70%)	425 (5.77%)
ADM	132 (39.63)	2,306 (31.31%)

ACEI, angiotensin converting enzyme inhibitors; ADM, antidiabetics; AF, atrial fibrillation; APLT, antiplatelet; BB, beta blockers; CCB, calcium channel blockers; DIU, diuretics; DM, diabetes mellitus; FHOS, family history of stroke; IHD, ischemic heart disease; HTN, hypertension; HPLD, hyperlipidemia; HU, hyperuricemia; NIHSS, national institute of health stroke scale; N, number of patients.

Baseline hazard model of recurrent IS

The Gompertz model fits the data well in terms of OFV, clinical plausibility, and the Kaplan–Meier plots. The baseline hazard of recurrent IS was quantified at two different time points, as shown in Table 2. As shown in Table 3, the hazard of recurrent IS when the predictor variables were set to zero was 0.238 in the first 6 months after the index IS, and the hazard remained non-zero afterward (Figure 1). After incorporating the factor of time and established risk factor, the exponential increase in the hazard of recurrent IS was observed in the first 3 years after the index IS and then exponentially reduced afterward (Figure 3).

Factors influencing the risk of having recurrent IS after index IS

In our model, the presence of established cardiovascular risk factors prior to index IS determine the risk of recurrent IS.

TABLE 2 Objective function value differences between different models.

Number of parameters	Variable	Model	OFV	ΔOFV	*p*-value
1	Constant	$h(t) = \theta_1$	3,803.971	0	–
2	Gompertz	$h(t) = \theta_1 \times e^{(\theta_2)t}$	3,777.083	−26.888	<0.0001*
2	Weibull	$h(t) = \theta_1 \times e^{(\theta_2)\ln(t)}$	3,788.58	−15.391	0.003955*
After inserting different time intervals					
4	Gompertz	$h(t) = \theta_x \times e^{(\theta_y)t}$	2,808.68	−959.291	<0.0001*
4	Weibull	$h(t) = \theta_x \times e^{(\theta_y)\ln(t)}$	3,185.810	−623.161	<0.0001*

OFV, objective function value; h, hazard; t, time; θ_x equals, θ_1 if time < 0.5 year; θ_3 if time ≥ 0.5, θ_y equals; θ_2 if time < 3 years, θ_4 if time ≥ 3 years. *Significance; p-value < 0.05.

TABLE 3 Parameters of the final developed model for recurrent IS after index IS.

Parameter		Description	Typical value	Half-life (Ln2/α)	aHR 95%CI	RSE%
θ_1 (<6 months)	θ_1	Baseline hazard	0.238	–		19.92%
θ_3 (≥6 months)	θ_3		0.0016			21.62%
α (<3)	θ_2	Shape parameter in the first 3 years after index IS	1.63	0.42 (5.06 months)		4.81%
α (≥3)	θ_4	Shape parameter after 3 years of index IS	0.23	3.008 years		20.19%
HPLD (covariate)	θ_5	Effect of baseline HPLD on hazard	0.799	–	2.22 (1.81–2.72)	12.89%
IHD (covariate)	θ_6	Effect of baseline IHD on hazard	0.745	–	2.10 (1.64–2.69)	16.85%
HTN (covariate)	θ_7	Effect of baseline HTN on hazard	0.711	–	2.03 (1.52–2.71)	20.62%
APLT	θ_8	Effect of receiving APLT on hazard	−0.514		0.59 (0.79–0.44)	28.41%

APLT, antiplatelet; h, baseline hazard; RSE, relative standard error; 95% CI, 95% confidence interval; α, shape parameter; aHR, adjusted hazard ratio; HPLD, hyperlipidaemia; HTN, hypertension; IHD, ischemic heart disease; NIHSS, national institute of heath stroke scale; The RSE (%) were obtained from sampling importance resampling (SIR) method (33).

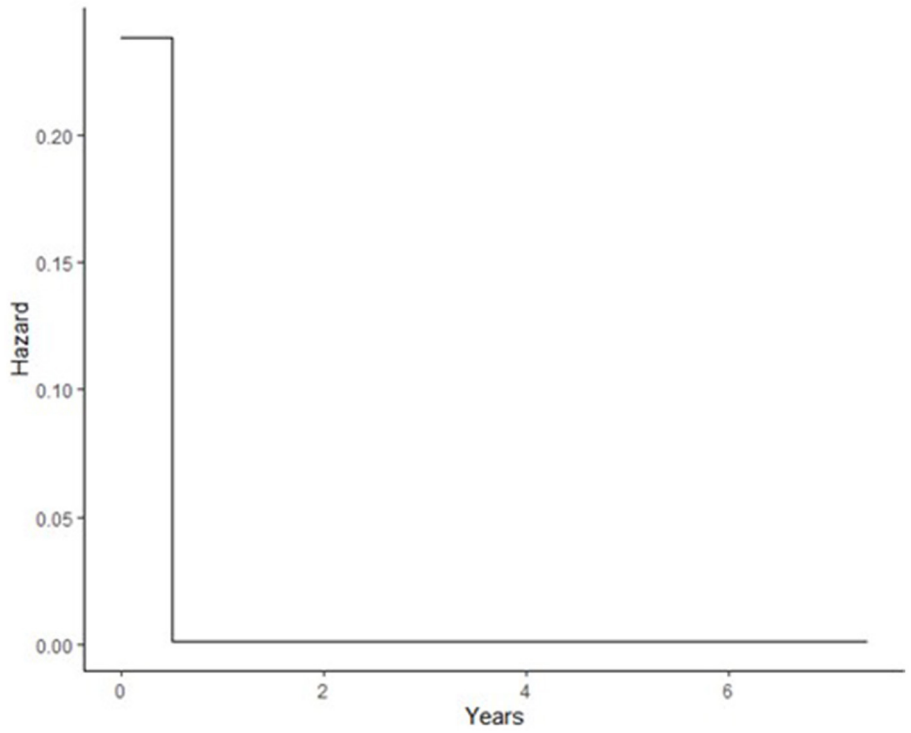

FIGURE 1
Baseline hazard during different time intervals after index IS; during first 6 months after index IS, after 6 months.

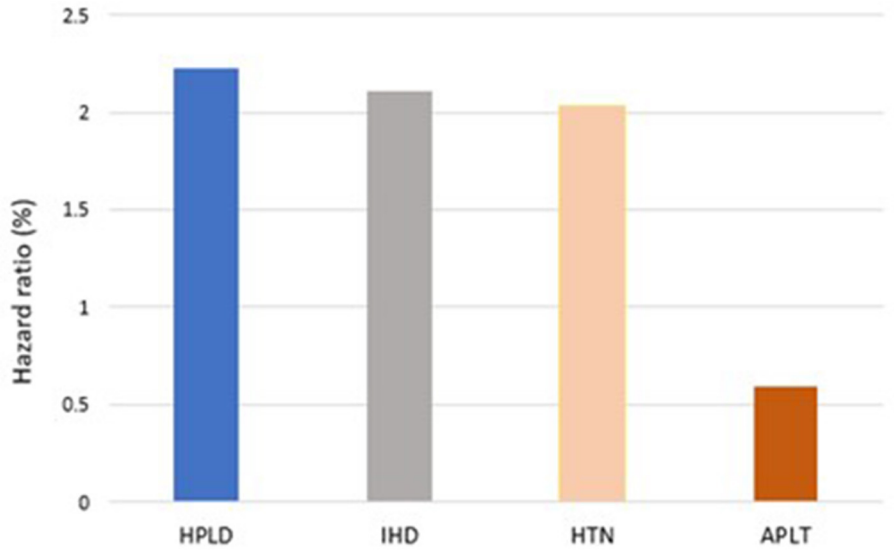

FIGURE 2
Effect of covariates on hazard of recurrent IS after index IS.

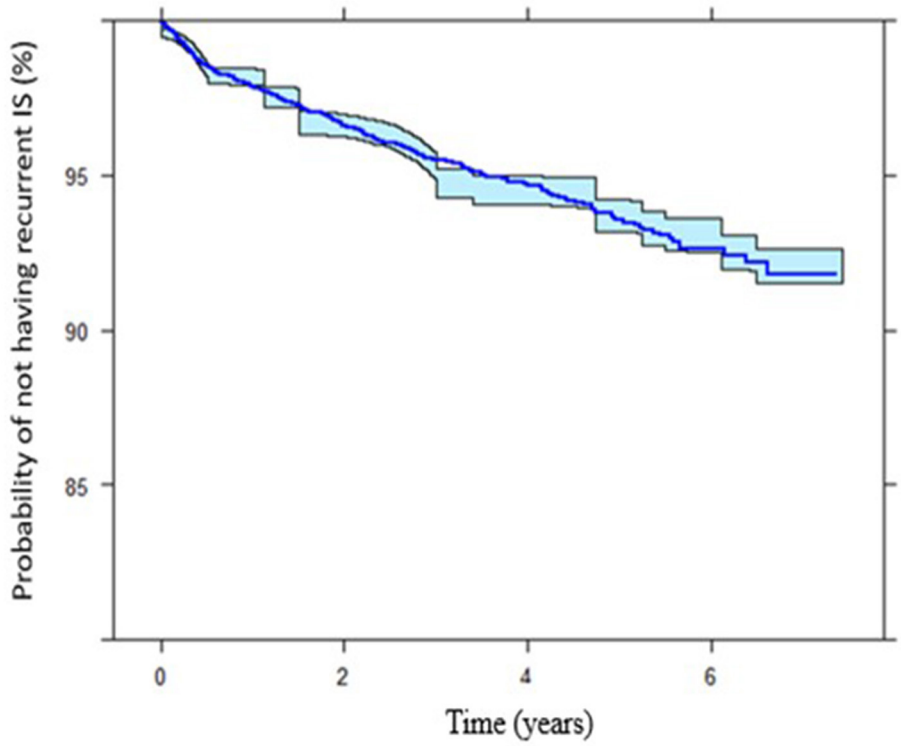

FIGURE 3
Kaplan-Meier plots showing the IS survivor function (probability of not having recurrent ischemic stroke) throughout different time intervals. The final time-to-event model of the internal data.

Prior to index IS, diagnosis of HPLD, HTN, and IHD increases the risk of recurrent IS with [HR, 2.22 (95%CI: 1.81–2.72)], [HR, 2.03 (95%CI: 1.52–2.71)], and [HR, 2.10 (95%CI: 1.64–2.69)], respectively, while receiving APLT for secondary prevention decreased this hazard [HR, 0.59 (95%CI: 0.79–0.44)] (Figure 2). The Kaplan-Meier VPCs for recurrent IS after index IS showed good predictions (Figure 3).

Figure 4 shows the survival (probability of not having recurrent IS) among patients who received APLT vs. patients who did not receive APLT for secondary prevention.

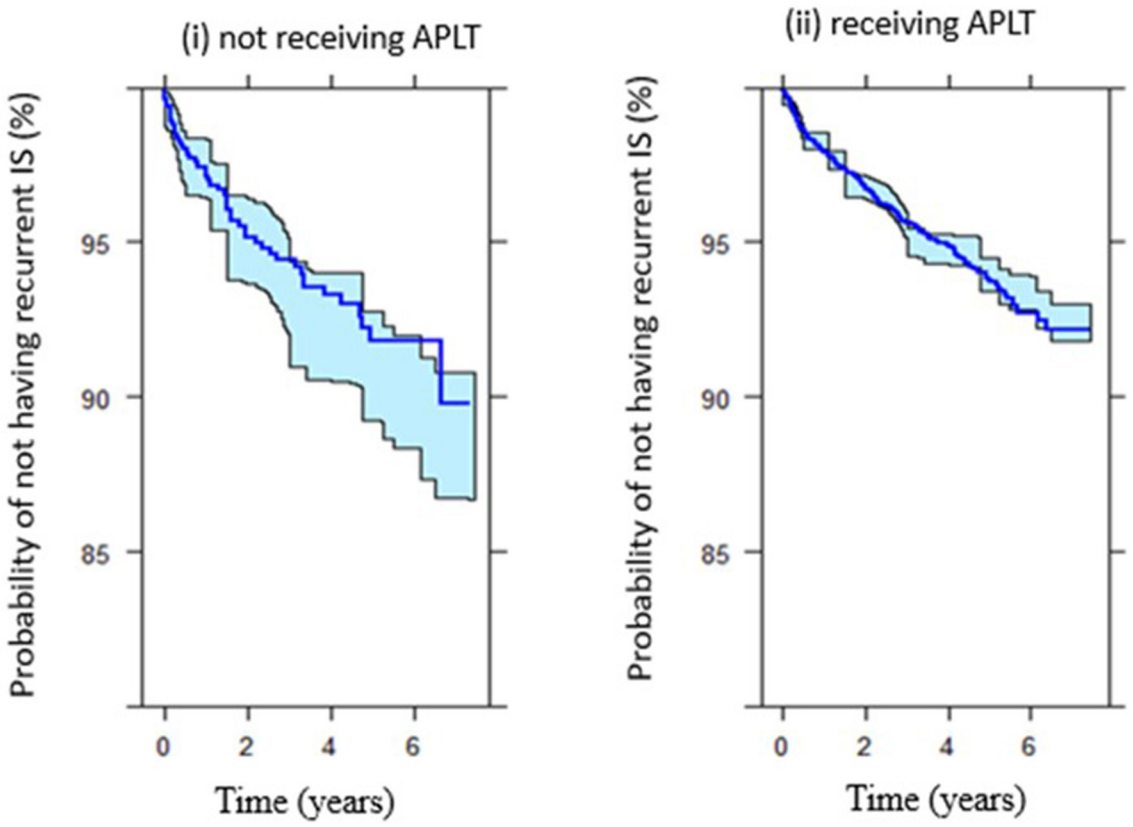

FIGURE 4
Survival (probability of not having recurrent IS after index IS) among **(i)** patients did not receive APLT vs. **(ii)** patients received APLT.

Discussion

To the best of our knowledge, this is the first study predicting the recurrence of IS in our population using real-world data of IS population as well as defining the baseline hazard of recurrent IS. A previous study (34) reported a constant hazard of having recurrent IS over time. In our population, the hazard of recurrent IS was reported to change over time after the first IS attack. Unlike the conventional model development (e.g., the Cox model), defining the specific shape or distribution of the event hazard at baseline (e.g., just after the index stroke) may allow for better prediction of the event of interest, taking into account the "natural effect" of the disease itself.

Recurrent stroke is associated with increased disability and mortality rates compared to index stroke (35). Even with appropriate secondary prevention, the risk of recurrence after IS is high, especially in the early phase after stroke (36). It has been reported that, within the first year after the initial stroke, the risk of stroke recurrence is higher (between 6 and 14%) as opposed to the risk in subsequent years (4% annually) (37–39). A more recent study showed that the incidence of stroke recurrence was the highest during the first year after index stroke at 12.8% with a declining annual rate, 6.3% during the second year, and 5.1% (95% CI, 4.0–6.5) during the third year after the index stroke (15).

In our population, we demonstrated the predicted hazard of recurrent IS at certain time points and change over time. Those with ≥2 concomitant diseases predicted a higher likelihood (>3.5%) of recurrent IS as compared to those who had at least one or no risk factor. This indicates that early and extensive secondary IS prophylaxis, especially in the first 3 years after the index IS as well as for those with the three risk factors, is paramount to prevent recurrent IS. The follow-up schedule after the index IS should be personalized depending on the risk factors. Those with more risk factors may require frequent follow-up after the stroke as well as different therapy goals for controlling the concomitant diseases.

The time course of recurrent IS hazards may represent the infarct involved during the stroke attack. There may be a relatively rapid increase in infarction cells after the initial diagnosis of stroke, which may increase the hazard of recurrent IS during the stage. However, the incidence of recurrent IS observed in the surviving population may decrease with time. This could be explained by the fact that the secondary prophylaxis therapy received may show a delay in obvious benefit in reducing the recurrent IS at this stage but with greater benefit later.

In this study, IHD, HPLD, and HTN were identified as independent predictors for recurrent IS. These findings are consistent with data reported in a previous study (22–25). The presence of HPLD, IHD, or HTN was found to increase the hazard of developing recurrent IS by 2.22, 2.10, and 2.03, respectively. In contrast, receiving APLT was found to decrease the hazard of recurrent IS by ∼40%.

HPLD findings could be explained through the angiopathy resulting from atherosclerotic plaque (40). For IHD, it was reported

that IHD and IS share similar pathophysiology, mainly because atherosclerosis is manifested in both conditions (22). Patients who have atherosclerosis are at risk for acute stroke. In both cases, a sudden change in circulation arises, and as a result, the blood supply decreases to some parts of the brain or heart (22). In agreement with these findings, receiving APLT was found to decrease the hazard of recurrent IS among the whole population with index IS. Effective management of these comorbidities is necessary to reduce the risk of recurrent IS. Although we reported the established and well-known risk factors of recurrent IS, our model allows the prediction and quantification of the recurrent IS hazard at different time points after the index IS. Moreover, the hazard is quantified according to the risk groups, which allows the future study to incorporate the time-varying effect of secondary prophylaxis therapies on the progression and hazard of recurrent IS.

Limitations

This was a retrospective study based on the available data from the National Stroke Registry of Malaysia. Therefore, the first stroke captured by the NNEUR from 2009 to 2016 was assumed to be the first stroke experienced by the patient. Any data on the prior TIA or stroke before the NNEUR establishment were not available and not considered in the current study. Due to the nature of the data captured from the registry database, the comorbidities were analyzed independently. Nevertheless, this study was a population-based study and large samples representing various ethnic groups across the country. This model may provide insights into the importance of frequent follow-up, especially in the early days (examples within the first 6 months to 1 year), and thus perhaps may make a positive shift in the Malaysian population regarding follow-up schedules during the management to prevent recurrent IS. This model is expected to be the basic model for future studies incorporating the time-varying effects of drugs, e.g., dosing changes and pharmacokinetic and pharmacodynamic characteristics.

Conclusion

Incorporating time in predicting the risk of recurrent IS may attribute positively to predicting the prognosis of recurrent IS. The hazard of recurrent IS changes over time after the index IS. In addition to concomitant diseases, secondary prevention time also plays a vital role in predicting the risk of recurrent IS population. These results may add to the knowledge related to patient follow-up schedules during the management of IS to prevent IS recurrence.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, upon request.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Research and Ethics Committee (MREC), Ministry of Health, Malaysia (Research ID: NMRR-08-1631-3189). 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

Conception and design: ME, SNH, SMSG, and OA. Data acquisition: IL, NNS, and ZAA. Drafting the manuscript: ME and SNH. Critically revising and reviewing the submitted version of the manuscript: All authors. 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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Supplementary material

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Qualitative electroencephalogram and its predictors in the diagnosis of stroke

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Introduction: Stroke is a typical medical emergency that carries significant disability and morbidity. The diagnosis of stroke relies predominantly on the use of neuroimaging. Accurate diagnosis is pertinent for management decisions of thrombolysis and/or thrombectomy. Early identification of stroke using electroencephalogram (EEG) in the clinical assessment of stroke has been underutilized. This study was conducted to determine the relevance of EEG and its predictors with the clinical and stroke features.

Methods: A cross-sectional study was carried out where routine EEG assessment was performed in 206 consecutive acute stroke patients without seizures. The demographic data and clinical stroke assessment were collated using the National Institutes of Health Stroke Scale (NIHSS) score with neuroimaging. Associations between EEG abnormalities and clinical features, stroke characteristics, and NIHSS scores were evaluated.

Results: The mean age of the study population was 64.32±12 years old, with 57.28% consisting of men. The median NIHSS score on admission was 6 (IQR 3-13). EEG was abnormal in more than half of the patients (106, 51.5%), which consisted of focal slowing (58, 28.2%) followed by generalized slowing (39, 18.9%) and epileptiform changes (9, 4.4%). NIHSS score was significantly associated with focal slowing (13 vs. 5, $p<0.05$). Type of stroke and imaging characteristics were significantly associated with EEG abnormalities ($p<0.05$). For every increment in NIHSS score, there are 1.08 times likely for focal slowing (OR 1.089; 95% CI 1.033, 1.147, $p=0.002$). Anterior circulation stroke has 3.6 times more likely to have abnormal EEG (OR 3.628; 95% CI 1.615, 8.150, $p=0.002$) and 4.55 times higher to exhibit focal slowing (OR 4.554; 95% CI 1.922, 10.789, $p=0.01$).

Conclusion: The type of stroke and imaging characteristics are associated with EEG abnormalities. Predictors of focal EEG slowing are NIHSS score and anterior circulation stroke. The study emphasized that EEG is a simple yet feasible investigational tool, and further plans for advancing stroke evaluation should consider the inclusion of this functional modality.

KEYWORDS

qualitative, diagnosis, stroke, predictors, electroencephalography

1. Introduction

Stroke remains a significant health burden worldwide. Worldwide, stroke is the second most common cause of death and the third most common cause of disability (1). The worldwide stroke prevalence was 80.1 million in 2016 (1). It carries a tremendous psycho-social burden and a significant impact on health resources. The global stroke burden is expected to rise, especially in developing countries, despite recent advances in stroke prevention, treatment, and rehabilitation (2). In Malaysia, stroke is the third leading cause of mortality after ischemic heart disease and pneumonia (3, 4).

Accurate stroke identification mainly depends on imaging such as computed tomography, perfusion imaging, and magnetic resonance imaging. These investigations provide clinicians with an objective and urgent assessment to select the appropriate patients for specific emergency treatment, particularly intravenous thrombolysis (5) and mechanical thrombectomy (6). Additional angiography (computed tomogram angiography or magnetic resonance angiography) is required to confirm large vessel occlusion for mechanical thrombectomy.

The role of EEG in acute ischemic stroke is somewhat limited as the diagnostic and therapeutic evaluation has been largely dominated by neuroimaging. EEG in stroke may be helpful in post-stroke epilepsy and exclusion of stroke mimics such as seizures and cerebral ischemia (7). Quantitative EEG in stroke showed the power of abnormal, slow activity relative to faster activity and interhemispheric voltage asymmetry (8). Qualitative EEG changes following stroke include focal slowing, generalized slowing, frontal intermittent rhythmic delta activity (FIRDA), and periodic lateralized epileptiform discharges (PLEDs) (9, 10). It has a role in acute and subacute stroke settings as a biomarker for predicting outcomes (11).

It is unclear whether electroencephalographic markers of acute vascular injury severity are independently associated with stroke findings. There needs to be more data on the use and clinical relevance of EEG in acute stroke patients. We embark on this study to evaluate the value of qualitative EEG and its associations with clinical and stroke features.

2. Manuscript

2.1. Materials and methods

This cross-sectional study was conducted in Hospital Canselor Tuanku Muhriz, the National University of Malaysia, from April 2021 to December 2022 with approval by the local Ethics and Research Board (FF-2021-135). Funding was obtained from the National University of Malaysia. Patients who were admitted with the diagnosis of acute ischemic stroke were recruited using purposive sampling. Patients with debilitating neurological disease, those with severe agitation that prevented a proper EEG recording, stroke mimics (such as patients with underlying seizures, epilepsy, hyperglycemia, metabolic, infection, and venous sinus thrombosis), traumatic brain injury, previous neurosurgery, and tumor were excluded from the study. The patients were diagnosed with acute ischemic stroke following history, clinical examination, and brain imaging. The data on clinical history, type of stroke, demographics, stroke risk factors, and the National Institute of Health Stroke Scale (NIHSS) score were tabulated on admission. The stroke severity was divided according to NIHSS score into mild (1–4), moderate (5–15), moderate to severe (16–20) and severe (21–42). The Oxfordshire Community Stroke Project (OCSP)was used to categorize the type of stroke syndromes which are divided into four subtypes: total anterior circulation infarcts (TACI), partial anterior circulation infarcts (PACI), lacunar infarcts (LACI), and posterior circulation infarcts (POCI) (12).

The EEG was performed as inpatient for all recruited stroke patients and recorded on the Nicolet One Extension (V32 Amplifier) using 24 reusable gold electrodes affixed to the scalp according to the international 10–20 system. The abbreviations on the EEG are as follows: Fp- frontopolar, C- central, F- frontal, T-temporal, P-parietal, O-occipital. Bipolar longitudinal and average referential montages were used for evaluation. The duration of each recording was half an hour. The EEG filter configuration was as follows: 50 Hz filter; low-frequency filter: 0.5 Hz; high-frequency filter: 70 Hz. EEG was evaluated by two trained neurologists blinded to the clinical and radiological findings. Each gave an individual report of the EEG based on the findings. Any discrepancies in the reports were then discussed and the EEG was re-examined and a final joint report was submitted for classification.

Abnormal EEG was defined as a generalized slowing (GS), focal slowing (FS), or presence of epileptiform patterns (spikes, sharp waves, rhythmic and periodic pattern). GS was defined as the dominant rhythm within the theta (4–8 Hz) or delta (< 4 Hz) frequency bands, occurring over all regions of the head. Focal slowing (FS) was defined as slow activity (theta or delta) occurring in a part limited in the area of the head.

Data were explored and analyzed using SPSS software version 21.0. Numerical variables were presented using mean and standard deviation for normally distributed data. The median and interquartile ranges were used for data that were not normally distributed. Categorical variables were presented as frequency and percentage. Distributions of continuous variables were compared using Student's *t*-tests; Pearson's chi-square tests or Fisher's exact tests were used for allocations of categorical variables. A p-value less than 0.05 defined statistical significance. Binary and multiple logistic regression was used to determine the risk factors. All odd ratios (ORs) are presented with a 95% confidence interval (CI).

2.2. Results

Table 1 shows the demographic data of the study population with a total of 206 participants with a mean age of 64.32 ± 12 years old (range 28–92). Most patients were men (118; 57.28%). The median NIHSS score on admission was 6 (IQR 3-13). According to stroke severity, majority of patients were minor stroke (85, 41.26%), followed by moderate (79, 38.35%) then severe (25, 12.13%), and moderate to severe (17, 8.25%). The most common type of stroke pattern was LACI (103, 50%), then PACI (79, 38.35%), followed by both TACI (12, 5.83%) and POCI (12, 5.83%). By imaging characteristics, most strokes were lacunar stroke (103, 50%), followed by MCA (77; 37.38%,) then PCA (10; 4.85%), infratentorial stroke (9;4.37%), ACA (6; 2.91%) and only one patient with brainstem stroke (1; 0.49%).

The EEG was performed within an average of 3.4 days (±3.5 standard deviation). EEG was abnormal in more than half of the patients (106, 51.5%), which constitutes focal slowing (58, 28.2%),

TABLE 1 Demographic of the study population.

Baseline characteristics	Results (n=206)
Mean age ± SD (range) (years)	64.32 ± 12 (28–92)
Male	118 (57.28%)
Female	88 (42.72%)
Smoking	59 (28.64%)
Comorbidities	
Hypertension	156 (75.73%)
Diabetes mellitus	98 (47.57%)
Dyslipidemia	78 (37.86%)
Atrial fibrillation	23 (11.17%)
Coronary artery disease	19 (9.22%)
NIHSS score	
Median NIHSS score (1. and 3. quartile) (range)	6 (3, 13) (1–35)
Stroke severity	
Mild (NIHSS 1–4)	85 (41.26%)
Moderate (NIHSS 5–15)	79 (38.35%)
Moderate to severe (NIHSS 16–20)	17 (8.25%)
Severe (NIHSS 21–42)	25 (12.13%)
Type of stroke	
TACI	12 (5.83%)
PACI	79 (38.35%)
POCI	12 (5.83%)
LACI	103 (50%)
Imaging characteristics	
MCA	77 (37.38%)
ACA	6 (2.91%)
PCA	10 (4.85%)
Infratentorial	9 (4.37%)
Brainstem	1 (0.49%)
Lacunar	103 (50%)
EEG	
Abnormal EEG	106 (51.5%)
Generalized slowing	39 (18.9%)
Focal slowing	58 (28.2%)
Epileptiform	9 (4.4%)

NIHSS, National Institute of Health Stroke Scale; TACI, total anterior circulation infarct; PACI, partial anterior circulation infarct; POCI, posterior circulation infarct; LACI, lacunar cerebral infarct; MCA, middle cerebral artery; ACA, anterior cerebral artery; PCA, posterior cerebral artery.
*Data expressed as frequency and percentage n (%) unless specified.

followed by generalized slowing (39, 18.9%) and epileptiform (9, 4.4%).

Table 2 shows EEG abnormalities and demographic data of the study population. Patient age significantly affects EEG abnormalities (p = 0.002). Similarly, age is associated considerably with generalized slowing (p = 0.001). Gender and smoking did not have any association with EEG abnormalities. Among the comorbidities, only hypertension and diabetes mellitus significantly correlate with EEG abnormalities (p = 0.012, p = 0.035), respectively.

Table 3 shows the stroke characteristics and EEG abnormalities. The median NIHSS score was 10 (4, 19), higher in abnormal EEG compared to 4 (2, 7) in normal EEG (p < 0.05). NIHSS score was significantly associated with focal slowing (13 vs. 5, p < 0.05). Type of stroke and imaging characteristics were significantly associated with EEG abnormalities (p < 0.05). Similarly, focal slowing was also associated with stroke types and imaging characteristics (p < 0.05).

Table 4 shows the multivariate analysis using a logistic regression of EEG findings with patients' characteristics and stroke features. Older patients have higher odds of getting abnormal EEG (OR = 1.033; 95% CI 1.003, 1.063, p = 0.032). Among EEG abnormalities, older patients have higher odds of generalized slowing (OR = 1.067; 95% CI 1.024, 1.111, p = 0.002). The male gender is 3.396 times more likely to get generalized slowing on EEG (OR = 3.396; 95% CI 1.350, 8.540, p = 0.009).

Patients with diabetes mellitus appeared 2.17 times more likely to have abnormal EEG (OR = 2.177; 95% CI 1.048, 4.524, p = 0.037). In looking at the type of EEG abnormalities, patients with coronary artery disease have higher odds of focal slowing (OR = 3.467; 95% CI 1.068, 11.258, p = 0.039).

For every increase of 1 point in NIHSS score, there are 1.09 times more likely for abnormal EEG (OR 1.090; 95% CI 1.029, 1.154, p = 0.003). Similarly, for every increment in NIHSS score, there are 1.08 times likely focal slowing (OR 1.089; 95% CI 1.033, 1.147, p = 0.002). Anterior circulation stroke appeared 3.6 times more likely to have abnormal EEG (OR 3.628; 95% CI 1.615, 8.150, p = 0.002). In addition, anterior circulation strokes have 4.55 times higher to have focal slowing (OR 4.554; 95% CI 1.922, 10.789, p = 0.01).

2.3. Discussion

2.3.1. Incidence of EEG abnormality

In this study, about 51.5% of our patients had an EEG abnormality. In older studies, EEG abnormalities in acute ischemic stroke were reported from 48 to 86% (7, 13). Similarly, a more recent study by Wolf et al. (14) reported 58% of acute ischemic stroke patients had EEG abnormalities. Several studies on post-stroke epilepsy that compared patients with and without seizures quoted 4% (15), 46.2% (16), and 92.4% (17). A significant variation in the findings may be attributed to the type of study, population, and outcome. Several reports (7, 13, 14) on post-stroke epilepsy recruited patients 2–98 months from the onset of stroke (15, 16). EEG abnormalities were also used as predictors of stroke outcomes such as functional status (18–20), post-stroke seizures (16, 17, 21), cognitive decline (22), and mortality (23). Continuous EEG has been performed in 570 consecutive patients in an intensive care unit setting, where 37% were stroke-related, comprised of subarachnoid hemorrhage (19%), intracerebral hemorrhage (7.9%), and ischemic stroke (9.8%) (24). In a subgroup of stroke patients, 26.7% had EEG abnormalities comprising seizures, non-convulsive seizures, and non-convulsive status epilepticus (24).

2.3.2. Types of EEG abnormality

Among the EEG abnormalities in this study, focal slowing was found in 58/106 (54.71%) (Table 1). This figure was lower compared

TABLE 2 Demographic data and electroencephalographic abnormalities.

	EEG		*p*-value	Generalized Slowing		*p*-value	Focal slowing		*p*-value	Epileptiform		*p*-value
	Normal	Abnormal		No	Yes		No	Yes		No	Yes	
Number of patients	100 (48.5%)	106 (51.5%)	–	167 (81.1%)	39 (18.9%)	–	148 (71.8%)	58 (28.2%)	–	197 (95.6%)	9 (4.4%)	–
Mean Age ± SD (years)	61.5 ± 12	66.97 ± 12	**0.002**[c,*]	62.96 ± 11	70.13 ± 11	**0.001**[c,*]	64.36 ± 13	64.21 ± 13	0.938[c]	64.01 ± 13	71.11 ± 10	0.098[c]
Male	60 (29.1%)	58 (28.2%)	0.444[a]	92 (44.7%)	26 (12.6%)	0.188[a]	87 (42.2%)	31 (15%)	0.486[b]	117 (56.8%)	1 (0.5%)	0.005[b]
Female	40 (19.4%)	48 (23.3%)		75 (36.4%)	13 (6.3%)		61 (29.6%)	27 (13.1%)		80 (38.8%)	8 (3.9%)	
Smoking												
Yes	35 (17%)	24 (11.7%)	0.050[a]	50 (24.3%)	9 (4.4%)	0.393[a]	45 (21.8%)	14 (6.8%)	0.397[a]	58 (28.2%)	1 (0.5%)	0.451[b]
No	65 (31.6%)	82 (39.8%)		117 (56.8%)	30 (14.6%)		103 (50%)	44 (21.4%)		139 (67.5%)	8 (3.9%)	
Comorbidities												
Hypertension												
Yes	68 (33%)	88 (42.7%)	**0.012**[a,*]	123 (59.7%)	33 (16%)	0.15[a]	109 (52.9%)	47 (22.8%)	0.266[a]	148 (71.8%)	8 (3.9%)	0.691[b]
No	32 (15.5%)	18 (8.7%)		44 (21.4%)	6 (2.9%)		39 (18.9%)	11 (5.3%)		49 (23.8%)	1 (0.5%)	
Diabetes mellitus												
Yes	40 (19.4%)	58 (28.2%)	**0.035**[a,*]	74 (35.9%)	24 (11.7%)	0.052[a]	67 (32.5%)	31 (15%)	0.290[a]	95 (46.1%)	3 (1.5%)	0.382[b]
No	60 (29.1%)	48 (23.3%)		93 (45.1%)	15 (7.3%)		81 (39.3%)	27 (13.1%)		102 (49.5%)	6 (2.9%)	
Dyslipidemia												
Yes	42 (20.4%)	36 (17.5%)	0.235[a]	64 (31.1%)	14 (16.8%)	0.779[a]	59 (28.6%)	19 (9.2%)	0.344[a]	75 (36.4%)	3 (1.5%)	1.000[b]
No	58 (28.2%)	70 (34%)		103 (50%)	25 (12.1%)		89 (43.2%)	39 (18.9%)		122 (59.2%)	6 (2.9%)	
Atrial fibrillation												
Yes	7 (3.4%)	16 (7.8%)	0.065[a]	16 (7.8%)	7 (3.4%)	0.135[a]	15 (7.3%)	8 (3.9%)	0.453[a]	22 (10.7%)	1 (0.5%)	1.000[b]
No	93 (45.1%)	90 (43.7%)		151 (73.3%)	32 (15.5%)		133 (64.6%)	50 (24.3%)		175 (85%)	8 (3.9%)	
Coronary artery disease												
Yes	9 (4.4%)	10 (4.9%)	0.914[a]	18 (8.7%)	1 (0.5%)	0.134[b]	11 (5.3%)	8 (3.9%)	0.182[a]	18 (8.7%)	1 (0.5%)	0.589[b]
No	91 (44.2%)	96 (46.6%)		149 (72.3%)	38 (18.4%)		137 (66.5%)	50 (24.3%)		179 (86.9%)	8 (3.9%)	

[a]Pearson's chi-square test.
[b]Fisher's exact test.
[c]Independent *t*-test.
[d]Mann Whitney test.
[*]Significant, $p<0.05$.

TABLE 3 Stroke characteristics and electroencephalographic abnormalities.

	EEG		*p*-value	Generalized Slowing		*p*-value	Focal slowing		*p*-value	Epileptiform		*p*-value
	Normal	Abnormal		No	Yes		No	Yes		No	Yes	
Number of patients	100 (48.5%)	106 (51.5%)	–	167 (81.1%)	39 (18.9%)	–	148 (71.8%)	58 (28.2%)	–	197 (95.6%)	9 (4.4%)	–
NIHSS												
Median NIHSS (1. and 3. quartile) (range)	4 (2; 7) (0–25)	10 (4; 19) (0–35)	**<0.05**[d,*]	6 (3; 13) (0–35)	8 (3; 16) (0–31)	0.324[d]	5 (2; 8.75) (0–31)	13 (5.75; 23) (0–35)	**<0.05**[d,*]	6 (3; 13) (0–35)	8 (1; 14.5) (0–25)	0.895[d]
Stroke type												
TACI	0 (0%)	12 (5.8%)	**<0.05**[a,*]	11 (5.3%)	1 (0.5%)	0.472[a]	2 (1%)	10 (4.9%)	**<0.05**[a,*]	11 (5.3%)	1 (0.5%)	0.522[a]
PACI	24 (11.7%)	55 (26.7%)		64 (31.1%)	15 (7.3%)		44 (21.4%)	35 (17%)		74 (35.9%)	5 (2.4%)	
POCI	7 (3.4%)	5 (2.4%)		8 (3.9%)	4 (1.9%)		11 (5.3%)	1 (0.5%)		12 (5.8%)	0 (0%)	
LACI	69 (33.5%)	34 (16.5%)		84 (40.8%)	19 (9.2%)		91 (44.2%)	12 (5.8%)		100 (48.5%)	3 (1.5%)	
Imaging characteristic												
MCA	18 (8.7%)	59 (28.6%)	**<0.05**[a,*]	66 (32%)	11 (5.3%)	0.103[a]	35 (17%)	42 (20.4%)	**<0.05**[a,*]	71 (34.5%)	6 (2.9%)	0.568[a]
ACA	2 (1%)	4 (1.9%)		4 (1.9%)	2 (1%)		4 (1.9%)	2 (1%)		6 (2.9%)	0 (0%)	
PCA	5 (2.4%)	5 (2.4%)		6 (2.9%)	4 (1.9%)		9 (4.4%)	1 (0.5%)		10 (4.9%)	0 (0%)	
Infratentorial	6 (2.9%)	3 (1.5%)		7 (3.4%)	2 (1%)		8 (3.9%)	1 (0.5%)		9 (4.4%)	0 (0%)	
Brainstem	0 (0%)	1 (0.5%)		0 (0%)	1 (0.5%)		1 (0.5%)	0 (0%)		1 (0.5%)	0 (0%)	
Lacunar	69 (33.5%)	34 (16.5%)		84 (40.8%)	19 (9.2%)		91 (44.2%)	12 (5.8%)		100 (48.5%)	3 (1.5%)	

[a]Pearson's chi-square test.
[b]Fisher's exact test.
[c]Independent *t*-test.
[d]Mann Whitney test.
[*]Significant, $p<0.05$.

TABLE 4 The risk factors of electroencephalographic abnormalities in stroke patients.

	Abnormal EEG				Generalized slowing				Focal slowing				Epileptiform			
	Odds ratio	CI 95%		*p*-value	Odds ratio	CI 95%		*p*-value	Odds ratio	CI 95%		*p*-value	Odds ratio	CI 95%		*p*-value
		Lower limit	Upper limit			lower limit	Upper limit			Lower limit	Upper limit			Lower limit	Upper limit	
Age	1.033	1.003	1.063	**0.032***	1.067	1.024	1.111	**0.002***	0.977	0.944	1.011	0.175	1.034	0.963	1.111	0.357
Gender																
Male	0.836	0.386	1.812	0.651	3.396	1.350	8.540	**0.009***	1.285	0.547	3.018	0.565	0.046	0.002	1.217	0.065
Female (reference)																
Comorbidities																
Hypertension	1.503	0.645	3.505	0.345	1.128	0.372	3.424	0.831	1.414	0.515	3.880	0.501	3.966	0.339	46.337	0.272
Diabetes mellitus	2.177	1.048	4.524	**0.037***	2.242	0.958	5.248	0.063	1.719	0.783	3.773	0.177	0.455	0.093	2.214	0.329
Dyslipidemia	0.59	0.283	1.228	0.158	0.668	0.293	1.521	0.337	0.746	0.325	1.709	0.488	0.583	0.105	3.232	0.537
Atrial fibrillation	1.748	0.545	5.600	0.347	1.769	0.555	5.641	0.335	1.214	0.389	3.788	0.739	0.599	0.059	6.062	0.664
Coronary artery disease	1.046	0.332	3.293	0.938	0.136	0.015	1.231	0.076	3.467	1.068	11.258	**0.039***	1.187	0.103	13.642	0.891
Smoking	0.623	0.267	1.454	0.274	0.641	0.237	1.735	0.382	0.778	0.297	2.039	0.610	2.914	0.091	92.789	0.545
NIHSS on presentation	1.090	1.029	1.154	**0.003***	1.013	0.959	1.070	0.640	1.089	1.033	1.147	**0.002***	0.929	0.823	1.048	0.23
Stroke type																
Anterior circulation	3.628	1.615	8.150	**0.002***	0.692	0.256	1.871	0.468	4.554	1.922	10.789	**0.001***	4.192	0.724	24.282	0.11
Posterior circulation	0.777	0.241	2.500	0.672	2.009	0.582	6.939	0.27	0.389	0.066	2.279	0.295	0	0	0	0.998
Lacunar (reference)																

*Significant, $p < 0.05$.

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to previous studies, between 75 and 88.6% (13, 14, 18). This could be attributed to the selection of patients as we included different stroke types, from lacunar to territorial stroke. In contrast, other studies recruited mainly territorial stroke consisting of MCA infarct (14, 18). Lacunar infarcts comprised 50% of the stroke subtypes, almost twice higher as a previous study from Malaysia (27.4%) (4). Normal EEG was primarily associated with lacunar infarction in this study.

The second most common abnormality detected was generalized slowing (39/106, 36.79%). Similarly, Holmes et al. (17) reported that 31.6% of acute stroke patients with generalized slowing. On the contrary, a lower percentage of patients with generalized slowing was reported by Wolf et al. (14) (10.2%). The generalized slowing was likely to be found in larger strokes (13, 14). Background activity slowing was significantly associated with poor functional outcomes (18). Thus, the presence of generalized slowing can be determined by the size of the stroke and its prognosis.

Epileptic abnormalities in stroke were infrequently found in a previous study (14). In comparison, we found 4.4% of epileptic potentials in our stroke patients without seizures. Claassen et al. (24) reported 8.9% of acute ischemic stroke with epileptiform discharges. Several studies found epileptiform abnormalities in post-stroke seizures (17, 21, 25). Bentes et al. (18) reported higher epileptiform abnormalities in post-stroke patients with a modified Rankin Scale (mRS) of more than 3. Epileptiform discharges were also observed in critically ill hemorrhagic stroke patients (24).

2.3.3. Associated risk factors for EEG abnormalities

2.3.3.1. Age

EEG abnormalities have been found in the average elderly population comprising generalized slowing, focal slowing, and epileptic discharges (26). The factors associated with EEG abnormalities in a cohort were known epilepsy/seizure and structural brain lesion (27). Our study has a similar observation that age was a risk factor for the occurrence of EEG abnormalities post-stroke. Wolf et al. (14) identified older stroke patients with a significantly higher proportion of abnormal EEG. EEG abnormalities and age were independent predictors of functional outcome (18) and dementia (22). This study determined that age was also a risk factor for generalized slowing. Contrary to our finding, generalized slowing was not associated with age in a study by Wolf et al. (14). The presence of alpha/theta coma and generalized suppression were associated with worse clinical outcomes of stroke (28).

2.3.3.2. Male gender

In a systemic review, the incidence of stroke in males was 33% higher, and stroke prevalence was 41% higher than the females (29). Similar changes were also observed in a previous study in the Malaysian stroke population that showed 55% were males (4), comparable to our study (57%). Despite a higher proportion of male compared to female patients, previous studies in stroke did not reveal any significant gender differences in EEG changes (14, 18). However, our study showed males had a 3.39 higher odds ratio to have generalized slowing. This may be attributed to our larger sample population of 206 patients to achieve a significant result. Furthermore, 75% of the patients had hypertension. Appelros et al. (29) highlighted the incidence rates of brain infarction were higher among men, although stroke severity was higher in women. An experimental study on male hypertensive rats showed profound brain atrophy at 12 weeks post-stroke, possibly attributed to increased apoptosis (30). Brain atrophy has been linked with EEG changes in dementia (31). Further evaluation of the underlying mechanisms may yield gender disparities in EEG alterations.

2.3.3.3. Diabetes mellitus

Diabetes mellitus contributed to 47.57% of our study population, similar to the incidence of diabetes in the Malaysian stroke population in an earlier report (45%) (4). Both traditional risk factors, such as hypertension and diabetes mellitus, have been identified in Asian and Western countries (32). The rationale of studying the association of EEG findings in stroke patients with diabetes mellitus is largely attributed to the large population of diabetes in our cohort. Stroke patients with diabetes mellitus were found to have a significant association with EEG abnormalities. In the logistic regression analysis, stroke patients with diabetes mellitus are 2.17 times more likely to have abnormal EEG. Diabetes mellitus contributes to stroke as it increases the risk of cerebral ischemia and atherosclerotic changes in the cerebral circulation. There is an increased risk of different subtypes of ischemic stroke including lacunar, large artery occlusion and thromboembolic strokes (33). Mechanisms for these causative factors were identified as reduced cortical functional connection and hyperglycemia-induced changes (34). Brain dysfunction in diabetes mellitus has been attributed to glucose homeostasis impairment which is responsible for elevated oxidative damage (35). However, the underlying pathology of EEG abnormalities in diabetic stroke patients needs to be further ascertained.

2.3.4. NIHSS and EEG abnormalities

The stroke severity in terms of a higher NIHSS score is more likely to have abnormal EEG. In most cases, the NIHSS score corresponds to stroke size similar to our study. This was in line with previous studies of EEG changes found in larger ischemic lesions (7, 36). EEG changes correlate with the severity of initial clinical findings (14, 36) and also clinical deterioration of stroke (14). NIHSS score and abnormal EEG have also been independently associated with functional outcomes (18). Prediction of acute stroke evolution can be monitored using sensitive quantitative EEG data (11). The functional outcome of a stroke at 6 months can be indicated by a higher NIHSS score and quantitative EEG analysis such as derived Brains Symmetry Index (dBSI) and (delta + theta)/(alpha + beta) ratio (DTABR) (23).

Our study on qualitative EEG demonstrated that an increment in NIHSS score had increased the risk of developing focal slowing. Such finding was also reported in acute stroke settings where a higher NIHSS score was associated with focal slowing (14). However, in the same study, stroke deterioration was associated with abnormal EEG and generalized slowing (14). To date, only a few studies have observed an association between NIHSS score and focal slowing. Furthermore, quantitative EEG and stroke studies showed a significant correlation between NIHSS score at 30 days with acute delta change index (aDCI) (11) and delta alpha ratio (DAR) (37). The neurophysiological alterations post mono hemispheric stroke has been studied using spectral exponent (SE). Spectral exponent which is part of quantitative EEG, reflects EEG slowing and quantifies the power-law decay of the EEG Power Spectral Density (PSD). The study showed that stroke patients had significantly more negative SE values in over the affected hemisphere than healthy control and SE renormalization significantly correlated with NIHSS improvement (38).

2.3.5. Anterior circulation and EEG abnormalities

Focal abnormalities on EEG following cortical infarct depend on the location of the vascular territory. Anterior circulation stroke in our patients appeared to have 3.63 higher odds of having an abnormal EEG. Comparing the other vascular regions, anterior circulation stroke has 4.55 times to develop focal slowing. A previous study showed that 44.9% of patients with anterior circulation stroke had abnormal EEG (14). Lateralized EEG abnormalities were observed in 80% of cortical middle cerebral artery territory infarct and 86% of cortical watershed infarct (13). Focal slowing has a role in determining the functional outcome, as indicated by 92.9% of MCA stroke patients with mRS of more than three compared to 80.8% of patients with mRS of less than 3 ($p = 0.025$) (18). Quantitative EEG has been used in studies to determine larger infarcts and diagnosis of stroke and large vessel occlusion (39). Larger infarct size was associated with higher aDCI (11) and lower beta power (40). The presence of delta band power and alpha/delta frequency band ratio significantly distinguished patients with large acute ischemic stroke from all other suspected stroke cases (41). Although neuroimaging techniques have surpassed EEG for the diagnosis of stroke, there is still a role for EEG in resource limited settings where brain imaging is not readily available. Detection of focal slowing on the EEG may use to predict the location of the vascular territory and hence, the management of stroke may be tailored accordingly.

2.3.6. Type of EEG monitoring

This study used the standard EEG montage of 24 electrodes and the recordings take approximately 15–20 min to complete the data acquisition. The EEG were performed at the average of 3.4 days on the stroke patients. However, in acute stroke settings which are time-dependant, this procedure may not be feasible. A more viable approach can be carried out using a wearable portable EEG device (42). One study found that the Muse headband that uses seven electrodes reported increased brain asymmetry in stroke patients compared to the healthy controls (43). Additionally, moderate and severe strokes showed increased delta to alpha ratio (DAR) and increased (delta + theta)/(alpha + beta) ratio, (DTABR) (44). The application of quantitative EEG using pairwise-derived Brain Symmetry Index (pdBSI) and measures of slowing can be implemented in a prehospital setting with automated interpretation.

2.4. Conclusion

The stroke assessment largely depends on clinical examination and neuroimaging techniques to identify the ischemic core and salvageable penumbra. The role of EEG in providing functional monitoring for stroke can be considered. EEG can be viewed as a complementary investigative tool as part of the workup for diagnosing stroke. These EEG predictors allow the clinician to determine the vascular territory and the severity of the stroke especially in the resource-limited settings.

2.5. Limitation of study

The following reasons limited this study: The sample population was only recruited from a single center, and the timing of the EEG was variable and not standardized among the patients. Continuous EEG was not performed due to limitations in time and laboratory personnel. There was a limited follow-up for these patients for further complications such as hemorrhagic transformation or seizures. The standard EEG uses 24 electrodes that may require additional time for the procedure and thus, may not be practical in acute stroke patients.

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 Faculty of Medicine, National University of Malaysia. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MoA and HT: conceptualization, methodology, investigation, formal analysis, and writing-original draft. MoA: investigation, formal analysis, and writing-original draft. WW: methodology, investigation, formal analysis, and writing-review and editing. WY: methodology, investigation, and writing-review and editing. CK: data acquisition, formal analysis, and writing-review and editing. MuA: data acquisition and writing-review and editing. RH and HT: methodology and writing-review and editing. 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

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Glossary

NIHSS	National Institutes of Health Stroke Scale
EEG	Electroencephalography
FIRDA	Frontal intermittent rhythmic delta activity
PLEDs	Periodic lateralized epileptiform discharges
OCSP	Oxfordshire Community Stroke Project
TACI	Total anterior circulation infarct
PACI	Partial anterior circulation infarct
LACI	Lacunar infarct
POCI	Posterior circulation infarct
GS	Generalized slowing
FS	Focal slowing
ACA	Anterior cerebral artery
MCA	Middle cerebral artery
PCA	Posterior cerebral artery
OR	Odds ratio
CI	Confidence interval
aDCI	Acute delta change index
DAR	Delta alpha ratio
mRS	Modified Rankin Scale
dBSI	Derived Brains Symmetry Index
DTABR	(Delta + theta)/(alpha + beta) ratio

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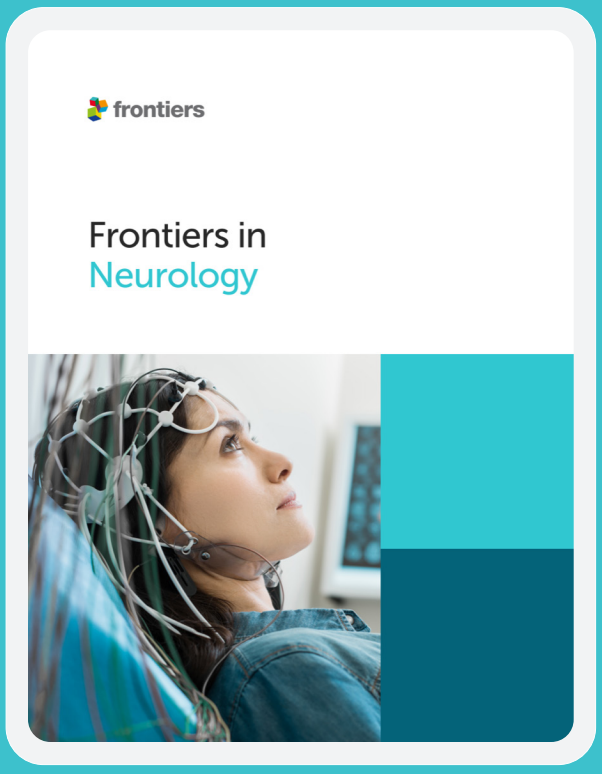

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