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Blood-based cellular and molecular biomarkers in acute ischemic stroke and hemorrhagic stroke, volume II

Edited by
Robert G. Kowalski, Joachim Eduard Weber, Timo Uphaus, Gerrit M. Grosse and Ramona Schuppner

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Blood-based cellular and molecular biomarkers in acute ischemic stroke and hemorrhagic stroke, volume II

Topic editors

Robert G. Kowalski — University of Colorado Anschutz Medical Campus, United States

Joachim Eduard Weber — Charité University Medicine Berlin, Germany

Timo Uphaus — University Medical Centre, Johannes Gutenberg University Mainz, Germany

Gerrit M. Grosse — University Hospital of Basel, Switzerland

Ramona Schuppner — Hannover Medical School, Germany

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EDITED AND REVIEWED BY
Jean-Claude Baron,
University of Cambridge, United Kingdom

*CORRESPONDENCE
Gerrit M. Grosse
✉ grosse.gerrit@mh-hannover.de;
✉ gerrit.grosse@usb.ch

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Editorial: Blood-based cellular and molecular biomarkers in acute ischemic stroke and hemorrhagic stroke, volume II

Ramona Schuppner[1], Robert G. Kowalski[2,3], Joachim E. Weber[4], Timo Uphaus[5] and Gerrit M. Grosse[1,6]*

[1]Department of Neurology, Hannover Medical School, Hannover, Germany, [2]Department of Neurosurgery, Anschutz Medical Campus, University of Colorado, Aurora, CO, United States, [3]Department of Neurology, Anschutz Medical Campus, University of Colorado, Aurora, CO, United States, [4]Department of Neurology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany, [5]Department of Neurology, University Medical Centre, Johannes Gutenberg University Mainz, Mainz, Germany, [6]Department of Neurology, University Hospital Basel, Basel, Switzerland

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stroke, biomarkers, personalized medicine, prognosis, prediction

Editorial on the Research Topic
Blood-based cellular and molecular biomarkers in acute ischemic stroke and hemorrhagic stroke, volume II

Stroke remains a leading cause of death and disability worldwide, with acute ischemic and hemorrhagic strokes entailing specific diagnostic and therapeutic challenges. The search for robust biomarkers and a better understanding of disease mechanisms is crucial to improve risk prediction, acute treatment and long-term outcomes. This collection of articles in Frontiers in Neurology is the 2nd volume following the highly successful first edition and brings together original research and perspectives covering the spectrum from population-based risk assessment to molecular and genetic mechanisms underlying stroke subtypes, complications and prognosis.

A particular focus of the submitted contributions is on the differentiation of hemorrhagic and ischemic strokes, prognostication after stroke, and prediction of complications. The original papers and reviews cover inflammatory, metabolic and tissue damage markers that are promising for future clinical application.

Inflammatory biomarkers

Systemic inflammatory processes are crucial in the pathophysiology of ischemic stroke. The immune response following stroke, particularly in large-vessel occlusion (LVO), is explored by Ma et al.. Their work reveals that AIS due to LVO rapidly induces peripheral immune activation, characterized by increased neutrophil-to-lymphocyte ratio and shifts in T-cell subsets. High-throughput sequencing of T-cell receptors further uncovers unique repertoire changes, pointing to potential diagnostic biomarkers and new insights into stroke pathophysiology.

Xue et al. analyze the Systemic Immune-Inflammation Index (SII) in over 28,000 adults from the NHANES cohort, finding a strong positive association between SII and stroke risk, independent of traditional confounding risk factors. This underscores the value of composite inflammatory indices for population-level risk stratification.

At the intersection of inflammation and metabolism, Chen J. et al. investigated the Hemoglobin, Albumin, Lymphocyte, and Platelet (HALP) score as a predictor of hemorrhagic transformation after intravenous thrombolysis in acute ischemic stroke patients. Their results show that lower HALP scores are strongly associated with both the risk and severity of hemorrhagic transformation, suggesting that this composite biomarker could guide risk stratification and treatment monitoring.

Metabolic biomarkers

Prognostic biomarkers are essential for guiding clinical decision-making. Vasile et al. review the role of copeptin, a stable peptide derived from vasopressin, in acute ischemic stroke. Their systematic overview of the current evidence confirms that copeptin levels on admission predict both short- and long-term outcomes, as well as the efficacy of revascularization therapies, supporting its integration into prognostic models.

Recent advances in epidemiological and genetic research have identified novel risk factors and biomarker candidates for stroke. In a large-scale, 10-year prospective cohort study, Li et al. evaluated the Triglyceride-Glucose (TyG) index as a predictor of stroke incidence in a Chinese population. Their findings demonstrate that a higher TyG index, reflecting insulin resistance, is independently associated with increased risk of total and ischemic stroke, but not hemorrhagic stroke, underscoring the importance of metabolic health in stroke prevention.

Arginine derivatives are considered biomarkers of endothelial (dys-)function. Pihlasviita et al. describe plasma symmetric dimethylarginine (SDMA) as a metabolite biomarker that distinguishes severe acute ischemic stroke from hemorrhagic stroke within the first 90 min after symptom onset. Elevated SDMA levels were also linked to cardioembolic stroke and poor outcomes, highlighting its promise in diagnostic algorithms and tailored management.

Zhang et al. use Mendelian randomization to reveal that specific plasma lipids with different fatty acid side chains have causal relationships with intracerebral and subarachnoid hemorrhages. Lipids containing arachidonic acid chains therefore could be protective, while those with linoleic acid chains increase risk, offering new mechanistic insights and potential therapeutic targets.

Finally, Chen H. et al. employ a comprehensive genetic approach to explore the causality between lipidomic and immune cell profiles and ischemic stroke subtypes. Their analysis identifies genetic links between specific lipids, immune cell phenotypes, and large artery, small vessel, and cardioembolic stroke. Mediation analyses highlight the role of immune cells in the lipid–stroke pathway, suggesting new avenues for personalized prevention and intervention.

Tissue damage biomarkers

Quality assurance in acute stroke interventions is addressed by Lieschke and Foerch, who propose serum S100B as a surrogate marker for astroglial tissue damage after mechanical thrombectomy. The authors discuss how S100B levels measured post-intervention correlate with infarct size and functional outcome, offering a potential objective metric for benchmarking endovascular therapy success.

Similarly, Freitas et al. demonstrate that neuron-specific enolase (NSE) measured 48 h after reperfusion therapy is strongly associated with 90-day functional outcomes in ischemic stroke patients. Higher NSE levels correlate with worse neurological disability, suggesting its utility as a prognostic tool for patient stratification.

Distinguishing hemorrhagic from ischemic strokes is still not possible without imaging, but would have enormous relevance for faster process times in stroke therapy. Paul et al. show that serum glial fibrillary acidic protein (GFAP) is markedly elevated in intracerebral hemorrhage compared to ischemic stroke, even in the hyperacute phase. GFAP levels also reflect the extent of tissue injury and time from onset, supporting its use in early subtype differentiation and assessment of tissue damage.

Conclusion

Taken, the work collected in this Research Topic represents a significant step forward in the quest for reliable blood-based biomarkers in stroke. From GFAP and S100B to copeptin, NSE, and beyond, these investigations illuminate new possibilities for faster diagnosis, more accurate prognosis, and individualized patient care. As the field continues to advance, the integration of these biomarkers into clinical practice holds the promise of transforming stroke management and improving outcomes for patients worldwide. However, despite the exciting progress reflected in these studies, challenges remain. A considerable obstacle in biomarker studies often remains the limited statistical power and lack of external validation. Therefore, all efforts toward harmonization in cross-center projects are essential to advance the field.

We thank all contributing authors for their rigorous and innovative work and hope this Research Topic will inspire further research and translation into clinical practice.

Author contributions

RS: Validation, Writing – original draft, Project administration, Supervision, Conceptualization. RK: Project administration, Conceptualization, Writing – review & editing, Validation, Supervision. JW: Conceptualization, Project administration, Supervision, Validation, Writing – review & editing. TU: Methodology, Validation, Conceptualization, Project administration, Supervision, Writing – review & editing.

GG: Writing – original draft, Supervision, Conceptualization, Project administration, Validation, Writing – review & editing.

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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EDITED BY
Timo Uphaus,
Johannes Gutenberg University Mainz, Germany

REVIEWED BY
Marieta Peycheva,
Plovdiv Medical University, Bulgaria
Adel Hamed Elbaih,
Suez Canal University, Egypt

*CORRESPONDENCE
Tiago Esteves Freitas
esteves.freitas@sesaram.pt

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Neuron-specific enolase as a prognostic biomarker in acute ischemic stroke patients treated with reperfusion therapies

Tiago Esteves Freitas[1]*, Ana Isabel Costa[2], Leonor Neves[3], Carolina Barros[1], Mariana Martins[1], Pedro Freitas[1], Duarte Noronha[4], Patrício Freitas[1], Teresa Faria[2], Sofia Borges[5], Sónia Freitas[5], Eva Henriques[5] and Ana Célia Sousa[5]

[1]Stroke Centre, Hospital Dr. Nélio Mendonça, Funchal, Portugal, [2]Internal Medicine Department, Hospital Dr. Nélio Mendonça, Funchal, Portugal, [3]Internal Medicine Department II, Hospital Prof. Doutor Fernando Fonseca, Amadora, Portugal, [4]Neurology Department, Hospital Dr. Nélio Mendonça, Funchal, Portugal, [5]Centro de Investigação Clínica Dra. Maria Isabel Mendonça, Funchal, Portugal

Introduction: Ischemic stroke is a significant global health concern, with reperfusion therapies playing a vital role in patient management. Neuron-specific enolase (NSE) has been suggested as a potential biomarker for assessing stroke severity and prognosis, however, the role of NSE in predicting long-term outcomes in patients undergoing reperfusion therapies is still scarce.

Aim: To investigate the association between serum NSE levels at admission and 48 h after reperfusion therapies, and functional outcomes at 90 days in ischemic stroke patients.

Methods: This study conducted a prospective cross-sectional analysis on consecutive acute ischemic stroke patients undergoing intravenous fibrinolysis and/or endovascular thrombectomy. Functional outcomes were assessed using the modified Rankin Scale (mRS) at 90 days post-stroke and two groups were defined according to having unfavorable (mRS3-6) or favorable (mRS0-2) outcome. Demographic, clinical, radiological, and laboratory data were collected, including NSE levels at admission and 48 h. Spearman's coefficient evaluated the correlation between analyzed variables. Logistic regression analysis was performed to verify which variables were independently associated with unfavorable outcome. Two ROC curves determined the cut-off points for NSE at admission and 48 h, being compared by Delong test.

Results: Analysis of 79 patients undergoing reperfusion treatment following acute stroke revealed that patients with mRS 3–6 had higher NIHSS at admission ($p < 0.0001$), higher NIHSS at 24 h ($p < 0.0001$), and higher NSE levels at 48 h ($p = 0.008$) when compared to those with mRS 0–2. Optimal cut-off values for NSE_0 (>14.2 ng/mL) and NSE_{48h} (>26.3 ng/mL) were identified, showing associations with worse clinical outcomes. Adjusted analyses demonstrated that patients with $NSE_{48h} > 26.3$ ng/mL had a 13.5 times higher risk of unfavorable outcome, while each unit increase in $NIHSS_{24h}$ score was associated with a 22% increase in unfavorable outcome. Receiver operating characteristic analysis indicated similar predictive abilities of NSE levels at admission and 48 h ($p = 0.298$). Additionally, a strong positive correlation was observed between NSE_{48h} levels and mRS at 90 days ($r = 0.400$ and $p < 0.0001$), suggesting that higher NSE levels indicate worse neurological disability post-stroke.

Conclusion: Serum NSE levels at 48 h post-reperfusion therapies are associated with functional outcomes in ischemic stroke patients, serving as potential tool for patient long-term prognosis.

KEYWORDS

ischemic stroke, biomarkers, neuron specific enolase, reperfusion therapies, functional outcome, prognosis, neurological disability

1 Introduction

Prognostication plays an essential role in decision making by offering patients and their families the necessary information to set realistic and achievable care goals. It helps determine eligibility for specific benefits and targets interventions to those who are most likely to benefit. Prognostication includes three main components: clinicians estimate the likelihood of a particular outcome over a certain period using their clinical judgment or other tools; this estimate is shared with the patient according to their preferred way of receiving information; and the patient or their surrogate uses this information to make informed clinical decisions (1).

Age, stroke severity, stoke mechanism, infarct location, comorbid conditions, clinical findings, and related complications influence stroke prognosis. Whether reperfusion therapies (fibrinolysis and/or thrombectomy) are conducted and the quality of stroke unit care given, including early start of physical rehabilitation and prompt introduction of secondary prevention measures, also influence the outcome of ischemic stroke (2).

Patient history, clinical examination and imaging technology have been the cornerstone in assessing stroke patients, guiding therapeutic decisions, and monitoring disease progression. Akin to the established gold standard in Cardiology, where biomarkers such as high sensitivity troponins have revolutionized the assessment of myocardial infarction (3), incorporating biomarkers into stroke management holds immense potential. These biomarkers offer a promising avenue to identify patients at heightened risk of severe disease, tailor treatment strategies, predict the response to reperfusion therapies and reasonably predict the overall prognosis and outcomes. While numerous proteins serve as markers of brain tissue damage, inflammation and coagulation/thrombosis, their utility is hindered by a lack of specificity to ischemic stroke, given that various other diseases processes can also damage the brain tissue (4).

For a biomarker to prove useful in stroke management, it should meet some basic criteria: be specific to the brain tissue, rise immediately within hours of a tissue insult, proportionally reflect the extent of brain damage, and ultimately serve as a reliable prognostic indicator for the event (5). Numerous biomarkers have been associated with short-and long-term clinical outcomes after stroke, but most of them have failed to improve the prediction capacities of conventional clinical variables. Biomarkers of ischemic brain injury include S100 calcium binding protein B (S-100B), neuron-specific enolase (NSE), myelin basic protein and glial fibrillary acid protein, among others (6).

The NSE is a dimeric intracellular neuronal glycolytic enzyme, primarily located in the cytoplasm of neurons, cells of the diffuse neuroendocrine system and erythrocytes. Elevated levels of NSE have been observed in response to sudden central nervous system events, such as cerebral infarction, subarachnoid hemorrhage, head injury, hypoxia, seizures, and cardiac arrest. These conditions are characterized by the disruption of the blood–brain barrier and subsequent damage of neuronal cells, leading to a leakage of NSE, which can be detected in cerebral spinal fluid but also in saliva or blood samples (7). Several studies propose NSE as a marker of brain damage following an ischemic event. There is also a correlation between NSE levels and acute ischemic stroke severity, infarction volume, extent of brain tissue damage [as clinically measured by the National Institutes of Health Stroke Scale (NIHSS) score], and poor functional outcomes (5, 8, 9).

NSE levels change dynamically following symptom onset, reflecting the necrosis of neuronal cells within the ischemic penumbra. Lower NSE levels are associated with clinical diffusion mismatch (7), serving as an alternative indicator for salvageable ischemic tissue that may be more responsive to interventions such as intravenous fibrinolysis and/or mechanical thrombectomy. However, high NSE levels are not exclusive to ischemic stroke and can also be found in other diseases such as neuroendocrine cell cancers like small cell lung cancers, neuroblastomas, melanomas and carcinoid tumors (10).

Since its approval by the European Stroke Organization in 2008, intravenous thrombolysis with alteplase has been a recognized systemic reperfusion treatment for patients with acute ischemic stroke (11). The pivotal MR CLEAN study published in 2015 further solidified the landscape by demonstrating the safety, efficacy and favorable impact on functional outcomes associated with intraarterial thrombectomy (12).

Building upon these advancements, our study aimed to evaluate the relationship between NSE levels at patient admission and 48 h post-reperfusion therapies, and functional outcome in ischemic stroke patients.

Abbreviations: AF, atrial fibrillation; CAD, coronary artery disease; CRP, C reactive protein; CT, computed tomography; CTA, computed tomography angiography; DAYLs, disability-adjusted life-years lost; HF, heart failure; Hs-CRP, high sensitivity C-reactive protein; HTN, hypertension; ICH, intracranial hemorrhage; mRS, modified rankin scale; NIHSS, national institutes of health stroke scale; NSE, neuron specific enolase; ROC, receiver operating characteristic; rtPA, recombinant tissue type plasminogen activator; TACI, total anterior circulation infarction.

2 Materials and methods

2.1 Participants

This was a prospective cross-sectional study targeting consecutive patients with acute ischemic stroke admitted do the Stroke Centre of

Hospital Dr. Nélio Mendonça between March 1st to October 31st of 2023 and treated with recombinant tissue type plasminogen activator (rtPA) within 4.5 h after symptom onset and/or endovascular treatment with thrombectomy up to 24 h after symptom onset. Endovascular treatment was indicated in patients with large vessel occlusion after discussion with the interventional neuroradiology team. Stroke onset was defined as the last time the patient was seen well, without any neurological deficit. Inclusion and exclusion criteria for intravenous rtPA were used in accordance with those used in the ECASS III (13). Patients eligible for thrombolysis received 0.9 mg of alteplase (Actilyse®, Boehringer Inglheim, Ingelheim am Rhein, Germany) per kilogram, administered intravenously (with an upper limit of 90 mg).

Patients eligible for thrombectomy were generally treated with a guide-catheter (Infinity Plus® by Stryker, Neuronmax® by Penumbra or Cerebase® by Cerenovus), an aspiration catheter (Catalyst® 5, 6 or 7 or Vecta 74® by Stryker, ACE68® or 4MAX® by Penumbra or Embovac® by Cerenovus) and a microcatheter (Trevo Trak® or AXS Offset® by Stryker, 3MAX® by Penumbra or Prowler® by Cerenovus), guided by a microwire (Synchro-14® by Stryker or Neuroscout® by Cerenovus); whenever deemed necessary by the interventionalist, a stent retriever was also used (Trevo NXT® by Stryker or Embotrap® by Cerenovus).

Informed consent was obtained from patients or their family members to participate in the study. The study protocol was approved by the local ethics committee.

2.2 Measures

2.2.1 Demographic and medical history

Upon arrival to the emergency department, all patients were evaluated by the on-duty stroke physician. Standard neurological examinations, electrocardiogram, chest radiography, bloodwork and computed tomography angiography (CTA) scans of the brain, cerebral arteries, cervical arteries, and aortic arch where performed. The following clinical data were collected: (1) patient age and gender; (2) degree of neurological deficit determined by the NIHSS score at hospital admission and 24 h after reperfusion therapies; (3) risk factors of stroke including history of hypertension (HTN), diabetes mellitus (DM), hyperlipidemia, current or former smoking (14), atrial fibrillation (AF) (15), heart failure (HF) (16), previous stroke (17), coronary artery disease (CAD) and previous myocardial infarction (18); and (4) prior medical treatments (anti-hypertensives, anti-diabetics, antiplatelets, direct oral anticoagulants, warfarin and statins).

2.2.2 Clinical assessment

Stroke severity was assessed by a NIHSS score (19) certified stroke physician, and was categorized as mild (0–4 points), moderate (5–15 points) and severe (16–42 points). Furthermore, stroke was classified according to the Bamford/Oxfordshire Community Stroke Project Classification criteria (20) in: total anterior circulation infarction (TACI), partial anterior circulation infarction (PACI), posterior circulation infarction (POCI) and lacunar infarction (LACI).

Clinical outcomes at 90 days were also assessed by certified investigators using the mRS (21) in a follow-up appointment. Outcomes were dichotomized as favorable (mRS score of 0–2 points) and unfavorable (mRS score of 3–6 points). All patients underwent a CT scan at 24 h post-reperfusion therapies or whenever neurological deterioration occurred to assess for the presence of ICH. CT scans were reviewed by an experienced neuroradiologist who was blinded to clinical details and laboratory data. Symptomatic intracranial hemorrhage (sICH) was defined as any extravascular blood within the brain or the cranium that was associated with clinical deterioration, defined by a rise of at least 4 points in the NIHSS score and/or leading to death, and that was considered to be the predominant cause for neurologic deterioration. Complications during the stroke unit stay, including pneumonia, urinary tract infections, sepsis, pulmonary embolism, deep venous thrombosis, and pressure sore ulcers, were recorded (22).

2.2.3 Radiological assessment

All patients underwent a CT and CTA scans of the brain, cerebral arteries, cervical arteries and aortic arch at admission and a brain CT scan at 24 h after reperfusion therapies.

2.2.4 Laboratory test

All patients had baseline blood samples drawn in the emergency room to determine: levels of NSE, glucose, HbA_1C, hemoglobin and CRP. NSE levels were measured again in every patient at 48 h post-reperfusion therapies. Blood samples obtained were collected in chemistry test tubes. After centrifugation, serum samples were separated, and kept frozen at −80°C until assayed. NSE analysis was performed using Cobas® e801 (Roche) and the reference values were 0–17 ng/mL. Glucose and CRP were assessed using Cobas® c702 (Roche). HbA_1C analysis was performed by a D-100® system (Biorad). Hemoglobin was assessed using a DxH 800® hematology analyzer (Beckman Coulter). Since NSE is also present in erythrocytes, hemolyzed samples were discarded. All laboratory analyses were carried out in the Clinical Pathology laboratory of the Hospital Dr. Nélio Mendonça with quality accreditation, from the national and official model of the Portuguese Ministry of Health, based on the Agencia de Calidad Sanitaria de Andalucia (ACSA) Model (international version).

2.2.5 Statistical analysis

Continuous variables were described as mean ± SD or median (minimum-maximum) and compared with Student t-test or Mann–Whitney U-test, as appropriate. The number of patients and percentages for categorical variables were given, and compared using a X^2 or Fisher exact test, as appropriate. The Spearman coefficient was applied to verify the correlation between examined variables. The relative risks of each variable for an unfavorable outcome were estimated as odds ratios (OR) in a logistic regression analysis.

A receiver-operating characteristic (ROC) curve in conjunction with the Youden's index were applied to determine the cut-off of NSE, at admission and at 48 h post-reperfusion therapies, that distinguished between favorable and unfavorable outcome, and compared by Delong test.

SPSS 25 software (IBM SPSS Statistic) was used to perform statistical analysis.

A level of $p < 0.05$ was accepted as statistically significant.

TABLE 1 Baseline characteristics of patients with and without unfavorable outcome (mRS 3–6) at 90 days.

Characteristics	Total (n = 79)	mRS (0–2) (n = 44)	mRS (3–6) (n = 35)	p-value
Age, years	69.2 ± 14.4	65.3 ± 15.0	74.0 ± 12.0	**0.006**
Female, n (%)	40 (50.6)	23 (52.3)	17 (48.6)	0.744
History of hypertension, n (%)	44 (55.7)	19 (43.2)	25 (71.4)	**0.012**
History of diabetes mellitus, n (%)	19 (24.1)	10 (22.7)	9 (25.7)	0.758
History of dyslipidemia, n (%)	36 (45.6)	16 (36.4)	20 (57.1)	0.065
Previous stroke, n (%)	18 (22.8)	12 (27.3)	6 (17.1)	0.286
Smokers, n (%)	20 (25.3)	10 (22.7)	10 (28.6)	0.553
History of atrial fibrillation, n (%)	7 (8.9)	3 (6.8)	4 (11.4)	0.693
History of heart failure, n (%)	8 (10.1)	1 (2.3)	7 (20.0)	**0.019**
History of coronary artery disease, n (%)	6 (7.6)	1 (2.3)	6 (14.3)	0.083
History of previous medical treatment, n (%)	53 (67.1)	25 (56.8)	28 (80.0)	**0.029**
$NIHSS_0$ score	10.0 (0.0–30.0)	7.0 (0.0–21.0)	17.0 (4.0–30.0)	**<0.0001**
$NIHSS_{24h}$ after reperfusion treatment	8.0 (0.0–32.0)	3.0 (0.0–21.0)	18.0 (1.0–32.0)	**<0.0001**
Time from symptom onset to hospital-door (min)	113.0 (10.0–720.0)	99.5 (10.0–720.0)	130.0 (15.0–720.0)	0.914
Time from symptom onset to treatment (min)	165.0 (35.0–900.0)	155.0 (35.0–900.0)	170.0 (45.0–860.0)	0.611
Oxfordshire classification – TACI, n (%)	45 (57.0)	15 (34.1)	30 (85.7)	**<0.0001**
Fibrinolysis or Thrombectomy, n (%)	59 (74.7)	35 (79.5)	24 (68.6)	0.265
Post stroke medical complications, n (%)	39 (49.4)	9 (20.5)	30 (85.7)	**<0.0001**
Intracranial hemorrhage (ICH), n (%)	14 (17.7)	6 (13.6)	8 (22.9)	0.286
Symptomatic intracranial hemorrhage (sICH), n (%)	6 (7.6)	0 (0.0)	6 (17.1)	**0.006**

Notes – values are presented as mean ± SD or median (minimum-maximum) for continuous variables, and number (percentages) for categorical variables. NIHSS, National Institutes of Health Stroke Scale; TACI, total anterior circulation infarct; ICH, intracranial hemorrhage; sICH, symptomatic intracranial hemorrhage; mRS, modified Rankin Scale. Statistical significance ($p < 0.05$) is represented by bold values.

3 Results

A total of 85 consecutive patients who fulfilled the established criteria for reperfusion treatment (either thrombolysis, thrombectomy or both) were included in the study. Of these patients six were excluded: two patients due to follow-up loss at 90 days post-stroke, three due to hemolyzed blood samples that invalidated the NSE level result, and one due to lung cancer history. Consequently, 79 patients (50.6% female; mean age 69.2 ± 14.4 years) were enrolled into the present study. The median time from symptom onset to hospital door was 113 min and the median time from symptom to reperfusion treatment as 165 min (when patients underwent both treatments, the time of rtPA bolus was chosen as the reperfusion treatment time, as it was the first treatment applied). The median NIHSS score was 10 points (range 0 to 30) before, and 8 points (range 0 to 32) 24 h after reperfusion therapies. The median NSE level at admission (NSE_0) was 16.3 ng/mL (4.7–45.1) and the median NSE level at 48 h post-reperfusion therapies (NSE_{48h}) was 17.5 ng/mL (4.7–117.0). At 90 days post-stroke, patients were assessed and distributed into two groups according to the mRS score at that time: 44 (55.7%) were placed in the favorable outcome group with a mRS score of 0–2 points, and 35 (44.3%) were in placed in the unfavorable outcome group with a mRS score of 3–6 points. Both groups were compared regarding baseline characteristics as shown in Table 1. There were no significant differences between the two groups in terms of sex, history of DM, hyperlipidemia, AF, previous stroke, smoking, CAD, time from symptom onset to hospital-door, time from symptom onset to treatment, fibrinolysis or thrombectomy and ICH. However, compared to favorable outcome group, patients in the unfavorable outcome group were more likely to be older ($p = 0.006$), have history of HTN ($p = 0.012$), HF ($p = 0.019$), and history of previous medical treatment ($p = 0.029$). They also presented a higher NIHSS score both at admission ($p < 0.0001$) and at 24 h ($p < 0.0001$) post-reperfusion therapies. Lastly, they were also more likely to have suffered a TACI type-stroke ($p < 0.0001$), post-stroke medical complications ($p < 0.0001$) and sICH ($p = 0.006$) (Table 1).

Of the 14 patients with ICH, 8 were asymptomatic, 6 had sICH and 1 died of sICH within 7 days after treatment. Among the 13 deceased patients, 4 died due to malignant infarct, 4 due to nosocomial pneumonia, 3 due to sepsis (urinary tract and endocarditis primary infections), 1 due to sICH and 1 due to hemorrhagic shock (gastric ulcer).

3.1 Biomarkers and clinical outcome

The biomarkers and hematologic parameters in the two groups are shown in Table 2. CRP median levels were significantly higher in the unfavorable outcome group (57.4 vs. 5.5; $p < 0.0001$), as were NSE levels at 48 h (NSE_{48h}) (21.0 vs. 15.5; $p = 0.008$). The NSE median levels at admission (NSE_0) were higher in the unfavorable outcome group (19.0 vs. 15.4), without statistical significance ($p = 0.059$).

TABLE 2 Biomarkers of patients with and without unfavorable outcome (mRS 3–6) at 90 days.

Biomarkers	Total (n = 79)	mRS (0–2) (n = 44)	mRS (3–6) (n = 35)	p-value
Hemoglobin	12.6 (8.7–17.0)	12.9 (8.7–17.0)	5.8 (4.9–8.0)	0.077
HbA1C (%)	5.7 (4.4–9.2)	5.6 (4.4–9.2)	11.9 (9.2–16.7)	0.098
Glycemia	129.0 (88.0–259.0)	130.0 (92–259)	125.0 (88.0–194.0)	0.564
CRP	13.2 (0.6–419.0)	5.5 (0.6–419.0)	57.4 (1.0–279.0)	**<0.0001**
NSE_0 (ng/mL)	16.3 (4.7–45.1)	15.4 (5.7–45.1)	19.0 (4.7–39.8)	0.059
NSE_{48h} (ng/mL)	17.5 (4.7–117.0)	15.5 (4.7–63.2)	21.0 (8.4–117.0)	**0.008**

Notes – values are presented as median (minimum-maximum). HbA1C, hemoglobin A1C; CRP, C reactive protein and NSE; neuron-specific enolase; mRS, modified Rankin Scale. Statistical significance ($p < 0.05$) is represented by bold values.

TABLE 3 Baseline characteristics and clinical outcome in patients classified according to NSE at admission (NSE_0) subgroups.

Characteristics	$NSE_0 \leq 14.2$ (n = 27)	$NSE_0 > 14.2$ (n = 52)	p-value
Age, years	67.7 ± 14.7	69.9 ± 14.3	0.529
Female, n (%)	13 (48.1)	27 (51.9)	0.750
History of hypertension, n (%)	12 (44.4)	32 (61.5)	0.161
History of diabetes mellitus, n (%)	5 (18.5)	14 (26.9)	0.407
History of hyperlipidemia, n (%)	10 (37.0)	26 (50.0)	0.273
History of previous stroke, n (%)	7 (25.9)	11 (21.2)	0.631
History of smoking, n (%)	11 (40.7)	9 (17.3)	**0.031**
History of atrial fibrillation, n (%)	1 (3.7)	6 (11.5)	0.412
History of heart failure, n (%)	2 (7.4)	6 (11.5)	0.564
History of coronary artery disease, n (%)	2 (7.4)	4 (7.7)	1.000
History of previous medical therapy, n (%)	14 (51.9)	39 (75.0)	**0.038**
$NIHSS_0$	13.0 (2.0–24.0)	10.0 (0.0–30.0)	0.860
$NIHSS_{24h}$	6.0 (0.0–31.0)	10.0 (0.0–32.0)	0.313
Oxfordshire classification – TACI, n (%)	13 (48.1)	32 (61.5)	0.254
Thrombolysis or Thrombectomy, n (%)	22 (81.5)	37 (71.2)	0.317
Unfavorable outcome 90d – mRS (3–6)	7 (25.9)	28 (53.8)	**0.018**
Post stroke medical complications, n (%)	11 (40.7)	28 (53.8)	0.269
Intracranial hemorrhage (ICH), n (%)	4 (14.8)	10 (19.2)	0.626
Symptomatic intracranial hemorrhage (sICH), n (%)	3 (11.1)	3 (5.8)	0.406
Mortality, n (%)	2 (7.4)	10 (19.2)	0.165
Hemoglobin (g/dL)	12.7 (8.7–16.7)	12.5 (8.8–17.0)	0.549
HbA1C (%)	5.6 (4.6–7.4)	5.7 (4.4–9.2)	0.149
CRP (mg/dL)	8.0 (0.6–419.0)	19.7 (0.6–302.0)	0.114

Notes – values are presented as mean ± SD or median (minimum-maximum) for continuous variables, and number (percentages) for categorical variables. NIHSS, National Institutes of Health Stroke; TACI, total anterior circulation infarction; mRS, modified Rankin Scale; HbA1C, hemoglobin A1C; CRP, C reactive protein and NSE, neuron-specific enolase. Statistical significance ($p < 0.05$) is represented by bold values.

3.2 Comparison of baseline characteristics, biomarker NSE_0 and NSE_{48h} and clinical outcome

The optimal cut-off value to distinguish unfavorable from favorable outcomes was calculated for both NSE_0 and NSE_{48h}, using a receiver operating characteristics (ROC) curve in conjunction with the Youden's index: NSE_0 was 14.2 ng/mL, with sensitivity of 80% and specificity of 45.5%; and NSE_{48h} was 26.3 ng/mL with sensitivity of 43.3% and specificity of 97.7%. To further estimate the clinical importance of the NSE level, all patients were divided into two subgroups according to the cut-off value of NSE_0 and NSE_{48}. Low and high NSE_0 levels were defined as: ≤14.2 ng/mL and > 14.2 ng/mL, respectively; and NSE_{48h} levels as: ≤ 26.3 ng/mL and > 26.3 ng/mL, respectively. Baseline characteristics and clinical outcomes classified according to NSE_0 and NSE_{48h} subgroups are shown in Tables 3, 4.

Regarding the NSE_0 group, there were no significant differences in terms of sex, history of HTN, DM, hyperlipidemia, previous stroke, HF, AF, $NIHSS_0$ and $NIHSS_{24}$. Patients in the high NSE_0

TABLE 4 Baseline characteristics and clinical outcome in patients classified according to NSE at 48 h (NSE_{48h}) subgroups.

Characteristics	$NSE_{48h} \leq 26.3$ ($n = 59$)	$NSE_{48h} > 26.3$ ($n = 14$)	*p*-value
Age, years	68.1 ± 14.5	74.1 ± 15.1	0.172
Female, *n* (%)	30 (50.8)	6 (42.9)	0.591
History of hypertension, *n* (%)	32 (54.2)	10 (71.4)	0.242
History of diabetes mellitus, *n* (%)	15 (25.4)	3 (21.4)	0.755
History of hyperlipidemia, *n* (%)	25 (42.4)	8 (57.1)	0.318
History of previous stroke, *n* (%)	16 (27.1)	1 (7.1)	0.112
History of smoking, *n* (%)	15 (25.4)	3 (21.4)	0.755
History of atrial fibrillation, *n* (%)	5 (8.5)	0 (0.0)	0.576
History of heart failure, *n* (%)	3 (5.1)	4 (28.6)	**0.007**
History of coronary artery disease, *n* (%)	2 (3.4)	4 (28.6)	**0.011**
History of previous medical therapy, *n* (%)	39 (66.1)	11 (78.6)	0.367
$NIHSS_0$	9.0 (0.0–24.0)	17.5 (6.0–30.0)	**0.004**
$NIHSS_{24h}$	6.0 (0.0–30.0)	19.5 (3.0–32.0)	**<0.0001**
Oxfordshire classification – TACI, *n* (%)	28 (47.5)	13 (92.9)	**0.002**
Thrombolysis or Thrombectomy, *n* (%)	46 (78.0)	8 (57.1)	0.110
Unfavorable outcome 90d – mRS (3–6)	17 (28.8)	13 (92.9)	**<0.0001**
Post stroke medical complications, *n* (%)	21 (35.6)	13 (92.9)	**<0.0001**
Intracranial hemorrhage (ICH), *n* (%)	10 (16.9)	4 (28.6)	0.321
Symptomatic intracranial hemorrhage (sICH), *n* (%)	3 (5.1)	3 (21.4)	0.080
Mortality, *n* (%)	4 (6.8)	6 (42.9)	**<0.0001**
Hemoglobin (g/dL)	12.8 (8.7–12.8)	11.4 (8.8–14.4)	0.053
HbA1C (%)	5.6 (4.4–9.2)	5.8 (4.7–6.4)	0.661
CRP (mg/dL)	7.4 (0.6–419.0)	116.5 (3.4–302.0)	**<0.0001**

Notes – values are presented as mean ± SD or median (minimum-maximum) for continuous variables, and number (percentages) for categorical variables. NIHSS, National Institutes of Health Stroke; TACI, total anterior circulation infarction; mRS, modified Rankin Scale; HbA1C, hemoglobin A1C; CRP, C reactive protein and NSE, neuron-specific enolase. Statistical significance ($p < 0.05$) is represented by bold values.

TABLE 5 Relative risks for unfavorable outcome (mRS 3–6) at 90 days in patients with reperfusion treatment.

Variables	B	S.E.	Wald	df	OR 95% CI	*p*-value
$NIHSS_{24h}$	0.200	0.056	12.616	1	1.221 (1.094–1.363)	**<0.0001**
$NSE_{48h} > 26.3$ ng/mL	2.599	1.231	4.461	1	13.456 (1.206–150.145)	**0.035**

Notes – variables that did not remain in the equation: hyperlipidemia, heart failure, hypertension, NIHSS at admission, TACI type stroke, hemoglobin, hemoglobin A1C, post stroke medical complications, NSE > 14.2 ng/mL. B – Beta coefficient; S.E. – Standard error; df – degrees of freedom; OR – Odds ratio; CI, confidence interval; NIHSS – National Institutes of Health Stroke; NSE – Neuron-specific enolase; statistical significance ($p < 0.05$) is represented by bold values.

subgroup had a higher percentage of non-smokers ($p = 0.031$) and history of previous medication intake ($p = 0.038$). They also had more frequent unfavorable outcomes ($p = 0.018$) than those in the low NSE_0 subgroup (Table 3).

Concerning the NSE_{48h} group, there were no significant differences in terms of sex, history of HTN, DM, hyperlipidemia, previous stroke, smoking and previous medication intake. Patients in the high NSE_{48h} group were more likely to have HF ($p = 0.007$) and history of CAD ($p = 0.011$). High NSE_{48h} subgroup was associated with higher NIHSS at admission ($NIHSS_0$) and NIHSS at 24 h ($NIHSS_{24h}$) scores ($p = 0.004$ and $p < 0.0001$). They also had higher percentage of unfavorable outcomes ($p < 0.0001$), TACI type-strokes ($p = 0.002$), post-stroke medical complications ($p < 0.0001$), and higher CRP median levels ($p < 0.0001$) than those in the low NSE_{48h} subgroup. Mortality was also more frequent among the high NSE_{48} subgroup ($p < 0.0001$) (Table 4).

3.3 Unfavorable outcome at 90 days in reperfused patients

After multivariate analysis, the variables that were independently and significantly associated with unfavorable outcome were $NIHSS_{24h}$ score and NSE_{48h}.

Patients with $NSE_{48h} > 26.3$ ng/mL have a 13.5 higher risk of having an unfavorable outcome (95% CI 1.2–150.1, $p = 0.035$). As the $NIHSS_{24h}$ increases, the risk of having an unfavorable outcome increases by 22% (95% CI 1.1–1.4, $p < 0.0001$) (Table 5).

3.4 Receiver operating characteristic analysis of NSE at the different sampling times

By comparing the two ROC curves of NSE_0 and NSE_{48h} through the Delong test, there were no significant differences between the two curves ($p = 0.298$) regarding unfavorable outcome (Figure 1). The area under curve at admission and 48 h are similar (0.597 and 0.683, respectively).

3.5 Correlation between NSE_{48h} and mRS at 90 days

We found highly significant positive correlation between NSE_{48h} levels and mRS at 90 days ($r = 0.400$ and $p < 0.0001$) (Figure 2). The graph demonstrates that patients with an adverse neurological disability (higher mRS) had a significant higher release of the marker.

4 Discussion

This study evaluated the predictive value of neuron-specific enolase (NSE) levels in determining stroke outcomes for patients undergoing reperfusion therapies. It was found that older age, history of hypertension (HTN), heart failure (HF), and previous medication use were significantly associated with unfavorable outcomes (mRS score of 3–6). Higher NIHSS scores at baseline and 24 h post-reperfusion were linked to worse outcomes and increased mortality. Notably, the study revealed that higher NSE levels at 48 h post-stroke were significantly associated with more severe outcomes and greater neurological disability, while NSE levels at admission showed a non-significant trend towards worse outcomes.

Clinicians are often expected to predict outcome after stroke, whether by patient, family, or other healthcare workers. There are a wide variety of factors that influence stroke prognosis, including age, stroke severity, stroke mechanism, infarct location, reperfusion treatments, comorbid conditions, neurological disability, and stroke complications (23, 24).

The modified Rankin Scale has been used as a measure of stroke-related handicap and is frequently used as a global measure of the functional impact of stroke. In addition, the mRS at 90 days after intravenous thrombolysis or endovascular interventions for acute ischemic stroke is the proposed "core metric" of most interventional trials and worldwide comprehensive stroke centers (25). The mRS score shows moderate correlation with the volume of cerebral infarction (26).

NSE has predictive value for determining severity and early neurobehavioral outcomes after stroke (9). However, the role of NSE in predicting long-term outcomes is still an evolving area, and evidence regarding its role in ischemic stroke patients undergoing reperfusion therapies is still scarce.

In this study we demonstrated that risk factors such as older age, a history of HTN, HF and previous medication were associated with unfavorable outcomes (mRS score of 3–6 points). Previous studies (27–29) have shown a clear associated between age, history of HTN and HF with stroke prognosis. In our study, hyperlipidemia and CAD showed a non-significant trend to be more prevalent among patients with unfavorable outcomes. Recent study by Chang et al. (30) showed a poorer outcome in stroke patients with CAD. A study from Menet et al. (31), mention that hyperlipidemia exacerbate vascular damage and is importantly associated to an increased risk of mortality in stroke patients.

According to Muir et al. (32), the NIHSS score offers the best specificity, sensitivity, and accuracy for predicting prognosis, with high scores correlating proportionally with higher mortality rates (33). As was demonstrated by Chalos study et al. (34), our study showed that higher $NIHSS_0$ and $NIHSS_{24}$ post-reperfusion therapies are associated with unfavorable outcome and higher mortality rates.

In Yang's study (35), TACI type-strokes exhibited worse 3-month clinical outcomes and higher mortality rates. Similarly, in our study, patients with ischemic stroke undergoing reperfusion therapies, we found that the TACI type-stroke was associated with stroke severity, unfavorable outcomes (85.7%) and mortality (100%) when compared to non-TACI type-strokes (PACI, LACI and POCI).

Bustamante et al. (36) reported that post-stroke complications significantly impact stroke outcomes. In our study, stroke complications were statistically significantly associated with unfavorable outcomes ($p < 0.0001$). Thirty-nine patients (49.4%) experienced post-stroke complications, with pneumonia and urinary tract infection being the most frequent. Although infections commonly occur after stroke and are strongly associated with an unfavorable outcome in these patients, effective management strategies for post-stroke infection remain scarce, presenting a significant challenge for stroke care (37).

Our study demonstrated that the median values of the biomarker CRP at admission were significantly higher among patients with unfavorable outcomes. Two studies (38, 39) indicate a strong association of poor outcomes with high sensitivity (hs)-CRP measurements at 24–48 h and 7 days post-stroke, reflecting impairment of the recovery process due to prolonged inflammation after ischemic stroke. Similar associations were also observed with CRP values at admission (40).

Since 2005 (41), we have known that NSE levels are higher in stroke patients than in controls. However, it was after Zaheer et al. (42) that a correlation was made between NSE levels on day 1, infarct volume and functional outcomes at 30 days post-stroke. Consistent with this, previous studies focusing on stroke patients not-submitted to reperfusion treatments suggested that the initial NSE level positively associated with the degree of neurological deficit (41). One single study (43) reported that NSE values did not differ between patients who underwent intravenous thrombolysis alone versus conservative medical treatment.

In our study, the NSE_{48h} levels were statistically significantly associated with an unfavorable outcome and the NSE_0 levels were non-significantly higher in the unfavorable outcome group. Wunderlich et al. (44) reported that the release pattern of NSE rises 2–3 h after stroke onset, then decreases until 12 h, followed by a secondary increase until the last measurement on day 5 after stroke onset, probably reflecting secondary mechanism of brain damage, ongoing neuronal cell death or persistent disturbance of the blood–brain barrier. NSE levels have been shown to change dynamically after an ischemic stroke. A pathological neurovascular status on admission resulted in a significantly higher release of NSE from 48 h onward, although NSE concentrations from 18 h onward were highly correlated with the severity of the corresponding neurological deficit

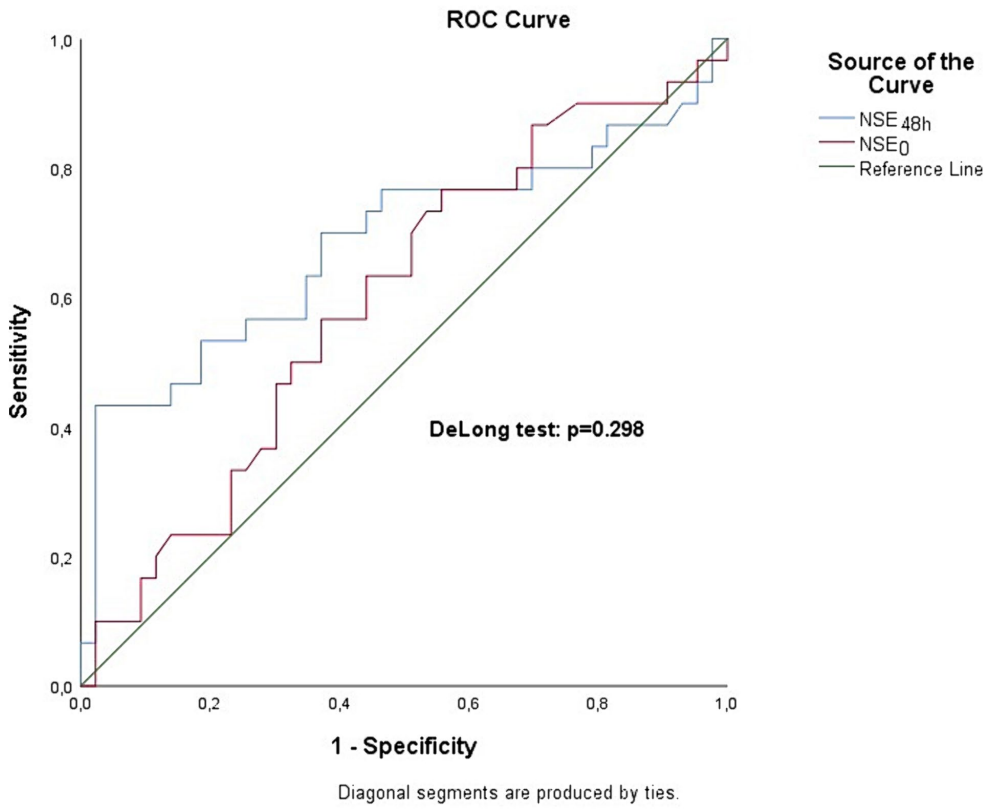

FIGURE 1
Receiver operating characteristics (ROC) analysis of NSE_0 and NSE_{48h}. The area under the curve (AUC) is 0.597 and 0.683 at admission and 48 h, respectively. There are no significant differences between AUCs, $p = 0.298$.

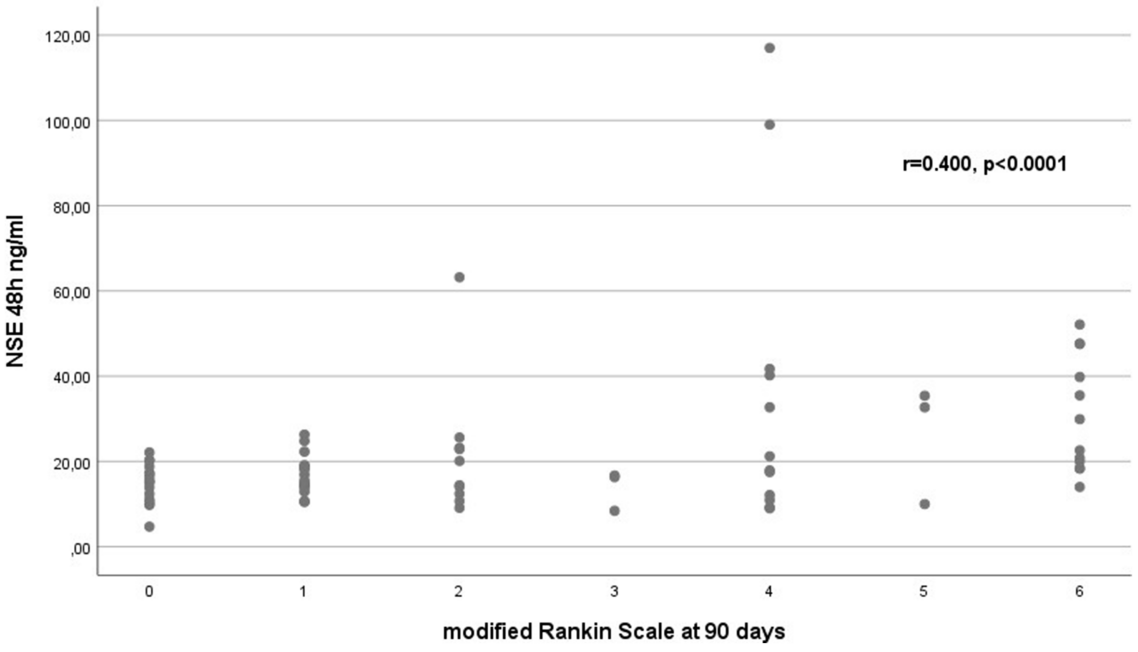

FIGURE 2
Spearman correlation between NSE_{48h} and modified Rankin Scale at 90 days. R coefficient is positive and significant ($r = 0.400$; $p < 0.0001$).

as quantified by the NIHSS. Considering that reperfusion therapies may increase the odds of vessel recanalization rates, possibly interfering with NSE levels, we measured the NSE level at admission (immediately before reperfusion therapies), and at 48 h post-reperfusion therapies.

A previous study (45) showed that the NSE level at 24 h post-intravenous fibrinolysis highly correlated with the severity of the corresponding neurological deficit as quantified by the NIHSS score at 24 h post-fibrinolysis.

There are several possible explanations for this finding, but the explanation that garners the most consensus is related with the penumbra area involved in the ischemic stroke. Cells in the ischemic penumbra, as they suffer necrosis, release NSE that passes from the cerebral spinal fluid to the peripheral blood through an impaired blood–brain barrier (46). Patients submitted to thrombolysis show decreased NIHSS scores as the occluded artery recanalizes and the ischemic penumbra area reperfuses, leading to a decrease in NSE levels. However, no studies have been conducted with ischemic stroke patients submitted to thrombectomy. On the other hand, patients who poorly respond to thrombolysis (considered as no or subpar recanalization) have larger core and smaller penumbra areas, leading to a higher NSE release from disrupted neuronal cells and both higher serum NSE levels and NIHSS scores.

In our study, higher NSE_0 levels showed a non-significant trend toward an unfavorable outcome ($p = 0.059$), reflecting the above correlation between NSE and the penumbra area (42). By the other hand, higher concentration of NSE_{48h} were statistically significantly associated with an unfavorable outcome ($p = 0.008$), reflecting a larger area of necrosis and infarct core and consequently higher neurological disability.

Two previous studies (42–45) showed a similar NSE level threshold for poor functional outcomes. As mentioned before, low NSE levels might be associated with clinical-diffusion mismatch, which has been a surrogate for brain tissue at risk of infarct.

By comparing the two ROC curves of NSE_0 and NSE_{48h} through the DeLong test, we found no significant differences between the two curves, leading us to conclude that the NSE_0 and NSE_{48h} have identical predictive value for unfavorable outcome.

Our study shown that NSE_{48h} and $NIHSS_{24h}$ were independently and significantly associated with unfavorable outcome, after multivariate analysis adjusted for other risk factors for unfavorable outcome.

A previous study (9) showed that the NSE levels had high predictive value for the degree of short-term disability (measured with NIHSS at day 7). In the current study the NSE_{48h} levels positively correlated with the severity of neurological long-term disability (mRS at 90d), reflecting the NSE utility as a marker for destructive process in the central nervous system, a marker of neuron loss and consequently associated with higher disability.

This study has some limitations that must be considered. First, our data result from hyperacute ischemic stroke treated with reperfusion therapies, without a control group. Whether reperfusion treatments interfere with the blood–brain barrier, increasing the serum NSE concentration is still unknown. Thus, the association between NSE, especially at 48 h, and stroke severity estimated by the NIHSS score may have been overestimated. Second, a small sample size might have confounded the results of factors predicting the severity and outcome of stroke and resulted in wider confidence intervals. The authors are still recruiting more patients to increase the sample size and further ascertain this hypothesis. We also note that the titration of NSE levels is time consuming and vulnerable to the collecting methods, resulting in hemolysis and making the interpretation of hemolytic samples impossible. Eventually, in the future, a saliva NSE sample test could be more feasible, safe, and economic (47).

In conclusion, our results show that NSE levels at 48 h are associated with patient's deficits assessed by the mRS. Like NIHSS score, NSE can be used, on a large scale in clinical practice, as a valuable tool in clinical management and prognostic estimation in ischemic stroke patients undergoing reperfusion therapies.

In the future, NSE levels might also be used in combination with CT perfusion of brain to study the core and penumbra area, maximizing the sensitivity of both.

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 humans were approved by Hospital Ethics Committee, Hospital Dr. Nélio Mendonça. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

TiF: Writing – original draft, Writing – review & editing. AC: Writing – review & editing. LN: Writing – review & editing. CB: Writing – review & editing. MM: Writing – review & editing. PeF: Writing – review & editing. DN: Writing – review & editing. PaF: Visualization, Writing – review & editing. TeF: Writing – review & editing, Visualization. SB: Writing – review & editing. SF: Writing – review & editing, Data curation, Methodology. EH: Writing – review & editing. AS: Writing – review & editing, Writing – original draft.

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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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EDITED BY
Timo Uphaus,
Johannes Gutenberg University Mainz, Germany

REVIEWED BY
Silke Zimmermann,
Leipzig University, Germany
Lingli Sun,
Peking University, China

*CORRESPONDENCE
Qinghai Shi
✉ shiqinghai@aliyun.com

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The plasma lipids with different fatty acid chains are associated with the risk of hemorrhagic stroke: a Mendelian randomization study

Xingkai Zhang[1,2], Xiaoyu Zhu[2,3] and Qinghai Shi[2]*

[1]Department of Graduate School, Xinjiang Medical University, Urumqi, China, [2]Department of Clinical Laboratory Diagnostic Center, General Hospital of Xinjiang Military Command, Urumqi, China, [3]School of Medicine, Shihezi University, Shihezi, China

Background and objective: Hemorrhagic stroke, characterized by acute bleeding due to cerebrovascular lesions, is associated with plasma lipids and endothelial damage. The causal relationship between genetic plasma lipid levels and hemorrhagic stroke remains unclear. This study employs a two-sample Mendelian randomization (MR) analysis to explore the causal relationship between plasma lipid profiles with different fatty acid chains and the risk of intracerebral and subarachnoid hemorrhage, the two main subtypes of hemorrhagic stroke.

Methods: The datasets for exposure and outcome summary statistics were obtained from publicly available sources such as the GWAS Catalog, IEU OpenGWAS project, and FinnGen. The two-sample MR analysis was employed to initially assess the causal relationship between 179 plasma lipid species and the risk of intracerebral and subarachnoid hemorrhage in the Finnish population, leading to the identification of candidate lipids. The same methods were applied to reanalyze data from European populations and conduct a meta-analysis of the candidate lipids. The Inverse Variance Weighting (IVW) method served as the primary analysis for causal inference, with additional methods used for complementary analyses. Sensitivity analysis was conducted to clarify causal relationships and reduce biases.

Results: Two analyses using Mendelian randomization were performed, followed by meta-analyses of the results. A causal relationship was established between 11 specific lipid species and the occurrence of intracerebral hemorrhage within the European population. Additionally, 5 distinct lipid species were associated with subarachnoid hemorrhage. Predominantly, lipids with linoleic acid and arachidonic acid side chains were identified. Notably, lipids containing arachidonic acid chains (C20:4) such as PC 18:1;0_20:4;0 consistently showed a decreased risk of both intracerebral hemorrhage [$p < 0.001$; OR(95% CI) = 0.892(0.835–0.954)] and subarachnoid hemorrhage [$p = 0.002$; OR(95% CI) = 0.794(0.689–0.916)]. Conversely, lipids with linoleic acid chains (C18:2) were associated with an increased risk of intracerebral hemorrhage.

Conclusion: This study identifies a potential causal relationship between lipids with different fatty acid side chains and the risk of intracerebral and subarachnoid hemorrhagic stroke, improving the understanding of the mechanisms behind the onset and progression of hemorrhagic stroke.

KEYWORDS

hemorrhagic stroke, plasma lipids, Mendelian randomization, intracerebral hemorrhage, subarachnoid hemorrhage, PUFA

1 Introduction

Hemorrhagic stroke, marked by acute intracranial bleeding, is clinically significant despite being less common than ischemic stroke. It has a higher mortality rate and more severe clinical outcomes, posing a serious threat to health and quality of life (1). Hemorrhagic stroke encompasses subtypes such as intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH). These subtypes are classified based on bleeding location and have different etiologies (2). Non-traumatic ICH is mainly caused by small vessel diseases like hypertension and arteriosclerosis, leading to ruptured arterioles or microaneurysms (3). In contrast, SAH is typically due to the rupture of large arterial aneurysms or arteriovenous malformations (4). Both ICH and SAH involve endothelial damage and vascular remodeling (2).

Plasma lipids are closely associated with endothelial damage. Dyslipidemia can contribute to endothelial injury and vascular remodeling through various mechanisms, accelerating vascular diseases like atherosclerosis (5). Previous studies have focused on conventional lipid components such as high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and total cholesterol (TC). Mendelian randomization has been used to examine the impact of these conventional lipids on the incidence of hemorrhagic stroke (6–8).

Modern high-throughput lipidomics technologies have greatly expanded our understanding of the diversity and complexity of circulating lipids. Additionally, genome-wide association studies (GWAS) have transformed our understanding of the genetic variations influencing lipid levels (9, 10). Research shows that while diet affects circulating lipids, plasma levels of these components are heritable, highlighting a significant role of endogenous regulation in lipid metabolism (11). Notably, genetic mechanisms do not uniformly regulate all lipid species within categories.

Exploring the relationship between comprehensive plasma lipid profiles and hemorrhagic stroke development offers insights into early screening and prevention while increasing our understanding of the physiological mechanisms involved. However, a prospective study directly linking plasma lipid profiles to hemorrhagic stroke is still lacking, leaving the causal relationship unclear. Traditional observational studies often have flaws, as data are typically collected post-stroke. Additionally, patients may change their lifestyle or medication after cardiovascular events, altering plasma lipid profiles and further obscuring the causal relationship.

Mendelian randomization (MR) has become increasingly popular for studying disease etiology. In the absence of randomized controlled trials, MR effectively identifies potential disease-causing factors and provides a reliable strategy for investigating causal relationships between exposures and outcomes (12). It determines the causal influence of an exposure on outcomes by using genetic variants associated with the exposure as instrumental variables (IVs), typically derived from single nucleotide polymorphisms (SNPs) (13). Since SNPs are randomly allocated to offspring during conception, confounding factors are significantly reduced, allowing MR to approximate randomized controlled trials to some extent (14). Moreover, MR studies have been employed in stroke risk research to identify various pathogenic factors (15, 16).

There is a significant association between plasma lipid levels and endothelial damage. However, the causal relationship between comprehensive plasma lipid profiles and hemorrhagic stroke remains uncertain. To investigate this, this study will utilize a two-sample MR analysis to evaluate the causal connection between various plasma lipid profiles containing distinct fatty acid side chains and the risk of ICH and SAH. The objective is to provide new strategies and insights for predicting and preventing hemorrhagic stroke.

2 Materials and methods

2.1 Study design

Mendelian randomization (MR) employs genetic variations as proxies for risk factors, with effective IVs needing to satisfy three primary assumptions for causal inference (17): (1) Genetic instruments must be directly and strongly associated with the exposure; (2) Genetic instruments should be independent of potential confounding factors; (3) Genetic instruments must be unrelated to the outcome and only influence the outcome through the exposure. The main MR analyses in this study were performed using R software (version 4.2.3) with the Two Sample MR and MR_PRESSO packages. This study's design was based on the MR study by Yun et al. (18). An overview of the study is presented in Figure 1.

2.2 Exposure data sources

The plasma lipids data were sourced from a GWAS conducted by Ottensmann et al., which examined the genetic characteristics of plasma lipidomics (19). This study analyzed genetic variations in 179 lipid species across 13 lipid categories in 7,174 Finnish individuals from the GeneRISK cohort. The aim was to identify genetic variants associated with plasma lipidomic features and assess their impact on certain diseases. The 13 lipid classes included cholesterol (Chol), cholesterol esters (CE), ceramides (CER), diacylglycerols (DAG), lysophosphatidylcholines (LPC), phosphatidylcholines (PC), ether-linked phosphatidylcholines (PCO), phosphatidylethanolamines (PE), lysophosphatidylethanolamines (LPE), ether-linked phosphatidylethanolamines (PEO), sphingomyelins (SM), and triacylglycerols (TAG). These classes follow the naming conventions from Ottensmann et al.'s study. The GWAS data for these 179 lipid

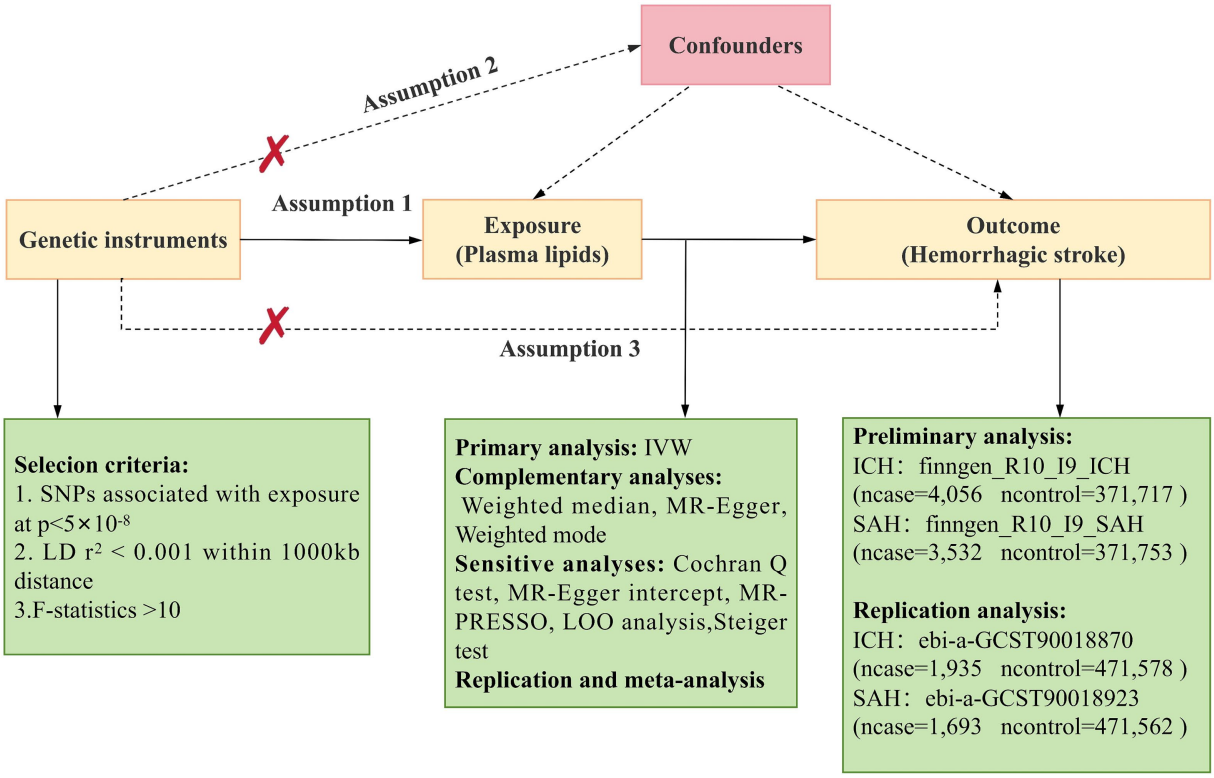

FIGURE 1
Overview of Mendelian Randomization (MR) analysis. Assumption 1: Genetic instruments are directly and strongly associated with the exposure. Assumption 2: Genetic instruments are independent of potential confounding factors. Assumption 3: Genetic instruments are unrelated to the outcome and only affect the outcome through the exposure. SNPs, single nucleotide polymorphisms; IVW, inverse variance weighted; LD, linkage disequilibrium; LOO analysis, leave-one-out analysis; MR-PRESSO, MR-Pleiotropy RESidual sum and outlier; ICH, Intracerebral hemorrhage; SAH, Subarachnoid Hemorrhage.

species were retrieved from the GWAS Catalog.[1] Supplementary Table 1 provides detailed information on lipid types, identifiers,[2] abbreviations, and GWAS numbers.

2.3 Outcome data sources

The data for two types of hemorrhagic stroke were initially analyzed using the tenth release of FinnGen[3] in the Finnish population. FinnGen is a public-private collaboration that integrates genotype data from Finnish biobanks with digital health records from the Finnish National Institute for Health and Welfare. We used the phenotypes labeled "I9_ICH" for ICH, which includes 4,056 cases and 371,717 controls, and "I9_SAH" for SAH, which includes 3,532 cases and 371,753 controls.

For replication analysis, cross-population meta-analysis data on intracerebral and subarachnoid hemorrhages (categorized by ICD-10 codes and phecodes) from Saori's study were employed, utilizing UK Biobank (UKB) and FinnGen version 3 data (20). The original GWAS data for ICH (ebi-a-GCST90018870, including 1,935 cases and 471,578 controls) and SAH (ebi-a-GCST90018923, including 1,693 cases and 471,562 controls) were obtained from the IEU OpenGWAS project.[4] Additional information on the GWAS data is available in the study by Saori et al. (20).

2.4 Selection of IVs

To meet assumption (1), we refined the criteria for selecting IVs to ensure an accurate and effective assessment of the causal relationship between plasma lipids and disease risk. Initially, only single nucleotide polymorphisms (SNPs) with highly significant associations, specifically those with *p*-values less than 5e-08, were included as IVs for both exposure and outcome. Additionally, to reduce bias from linkage disequilibrium, which can cause a non-random distribution of alleles across multiple genetic loci, we applied stringent screening criteria. These criteria involved selecting SNPs under the conditions of $r^2 = 0.001$ and a clumping window of 10,000 kb, ensuring the independence of the IVs and reducing potential biases in subsequent analyses. Only SNPs meeting both the stringent *p*-value threshold and effectively countering the

1 https://www.ebi.ac.uk/gwas/
2 http://www.swisslipids.org
3 https://www.finngen.fi/en/access_results
4 https://gwas.mrcieu.ac.uk/

effects of linkage disequilibrium were included in the exposure analysis. Furthermore, to avoid bias from weak instruments, the F statistic was used to assess the correlation strength between each SNP and the exposure. IVs with an F statistic >10 were deemed strong instruments, whereas those with $F<10$ were considered to have weaker correlations between SNP and exposure. R^2 and F statistics were calculated as follows (21):

$$R^2 = \frac{2\times\beta^2\times MAF\times(1-MAF)}{2\times\beta^2\times MAF\times(1-MAF)+2\times(se(\beta))^2\times N\times MAF\times(1-MAF)}$$

$$F = \frac{R^2}{1-R^2}\times\frac{N-k-1}{k}$$

Where β represents the effect size of the target genetic variant, MAF denotes the minor allele frequency of the SNP, and se(β) denotes the standard error of the effect size. R^2 signifies the proportion of exposure explained by the IVs, or the determination coefficient of the regression equation. N represents the sample size of the exposure, and k indicates the number of SNPs (IVs). To meet assumption (3), SNPs associated with the outcome ($p<5e-05$) were excluded. Lastly, further MR analysis was conducted on lipids with two or more SNPs.

2.5 Statistical analysis

Mendelian randomization (MR) is a technique that employs genetic instruments to investigate causal relationships between modifiable exposures and outcomes. In this study, the Inverse Variance Weighted (IVW) method is used to assess the potential causal effects of plasma lipid profiles on ICH and SAH. The IVW method, preferred for its efficiency with multiple genetic variations as instrumental variables (IVs), calculates the weighted average of the causal effects by integrating the estimates of each genetic variation's impact on exposure and outcomes (22). Additionally, to reduce the risk of false discoveries from multiple hypothesis testing, the Benjamini and Hochberg false discovery rate (FDR) correction is applied, setting a significance threshold of an FDR-adjusted *p*-value below 0.05 (23). In the IVW analysis, while certain causal associations exhibit *p*-values under 0.05, they do not meet the stricter criterion of an FDR-adjusted *p*-value below 0.05 and are thus regarded as only potentially causal (24).

In this study, in addition to the Inverse Variance Weighted (IVW) method, we utilized three other techniques: Weighted Mode, MR-Egger, and Weighted Median. The Weighted Mode approach addresses correlations between genetic instruments in scenarios similar to those considered by the IVW, which is crucial when using a conservative set of genetic instruments (25). The MR-Egger method conducts weighted regression analysis to estimate the ratios of genetic variation and assesses the average pleiotropic effects through a regression line. It assumes that all genetic variations may exhibit pleiotropic effects, provided these effects are uncorrelated with the genetic variation exposure (26). The Weighted Median technique, by calculating the median of ratio estimates from genetic variations, demonstrates significant resilience against outliers (27).

Sensitivity analyses were conducted to evaluate the presence of horizontal pleiotropy and heterogeneity, which could compromise MR assumptions. Heterogeneity was assessed using Cochran's Q method, with a $p<0.05$ indicating significant heterogeneity in the results (28). The MR-Egger intercept test was employed to evaluate horizontal pleiotropy in SNPs used as instrumental variables, with the intercept term suggesting horizontal pleiotropy at $p<0.05$ (29). Additionally, MR-Pleiotropy RESidual sum and outlier (MR-PRESSO) was utilized to detect horizontally pleiotropic SNPs (30). To ensure the robustness of the results, a leave-one-out (LOO) analysis was performed by sequentially excluding each SNP to determine if any individual SNP significantly influenced the results (31). The Steiger test was also conducted to eliminate biases from reverse causation (25). Detailed characteristics of the relevant analytical methods are provided in Supplementary Table 2.

To confirm the robustness of candidate lipids identified in the initial analysis, a repeat analysis was carried out using an alternative set of GWAS data, detailed in Supplementary Table 3. The meta-analysis was conducted based on a random effects model using Review Manager 5.4.1 software.

3 Results

3.1 Preliminary analysis of plasma lipids and ICH

Our research findings indicate that by employing the IVW method with a significance threshold of $p<0.05$, we initially identified 16 lipids with potential causal relationships to ICH in the Finnish population (Figure 2). Of the 13 lipid classes analyzed, 6 lipid classes were significantly associated with ICH, with PC showing the strongest relationship. However, after applying FDR correction, none of the results met the significance threshold, implying that the associated lipids may have potential causal relationships. To refine our selection of candidate lipids, we focused on those with significant estimates in IVW ($p<0.05$) and assessed their consistency in both direction and magnitude across IVW, Weighted Mode, MR-Egger, and Weighted Median methods (Table 1; Supplementary Figure 1). We further ensured that the selected lipids exhibited no heterogeneity via the Cochran Q test ($p>0.05$) and confirmed the absence of horizontal pleiotropy using the MR-Egger intercept test ($p>0.05$) and MR-PRESSO results ($p>0.05$). The Steiger test was then conducted to validate the causal relationships between these lipids and ICH (Table 1). LOO analysis verified that individual SNPs in candidate lipids did not bias the Mendelian Randomization analysis (Supplementary Figure 2). Based on comprehensive sensitivity analyses, we ultimately identified 11 lipids as candidates associated with ICH: CE 20:4;0 [$p=0.047$; OR(95% CI) = 0.94 (0.88–0.999)], LPC 20:4;0 [$p=0.034$; OR(95% CI) = 0.91 (0.83–0.99)], PC 16:0;0_20:4;0 [$p=0.040$; OR(95% CI) = 0.93 (0.86–0.996)], PC 17:0;0_18:2;0 [$p=0.049$; OR(95% CI) = 1.16 (1.001–1.35)], PC 18:0;0_18:2;0 [$p=0.024$; OR(95% CI) = 1.17 (1.02–1.34)], PC 18:0;0_20:4;0 [$p=0.016$; OR(95% CI) = 0.92 (0.87–0.99)], PC 18:1;0_20:4;0 [$p=0.017$; OR(95% CI) = 0.91 (0.84–0.98)], PE 16:0;0_18:2;0 [$p=0.002$; OR(95% CI) = 1.14 (1.05–1.24)], PE 16:0;0_20:4;0 [$p=0.03$; OR(95% CI) = 1.10 (1.01–1.19)], SM 36:2;2 [$p=0.003$; OR(95%

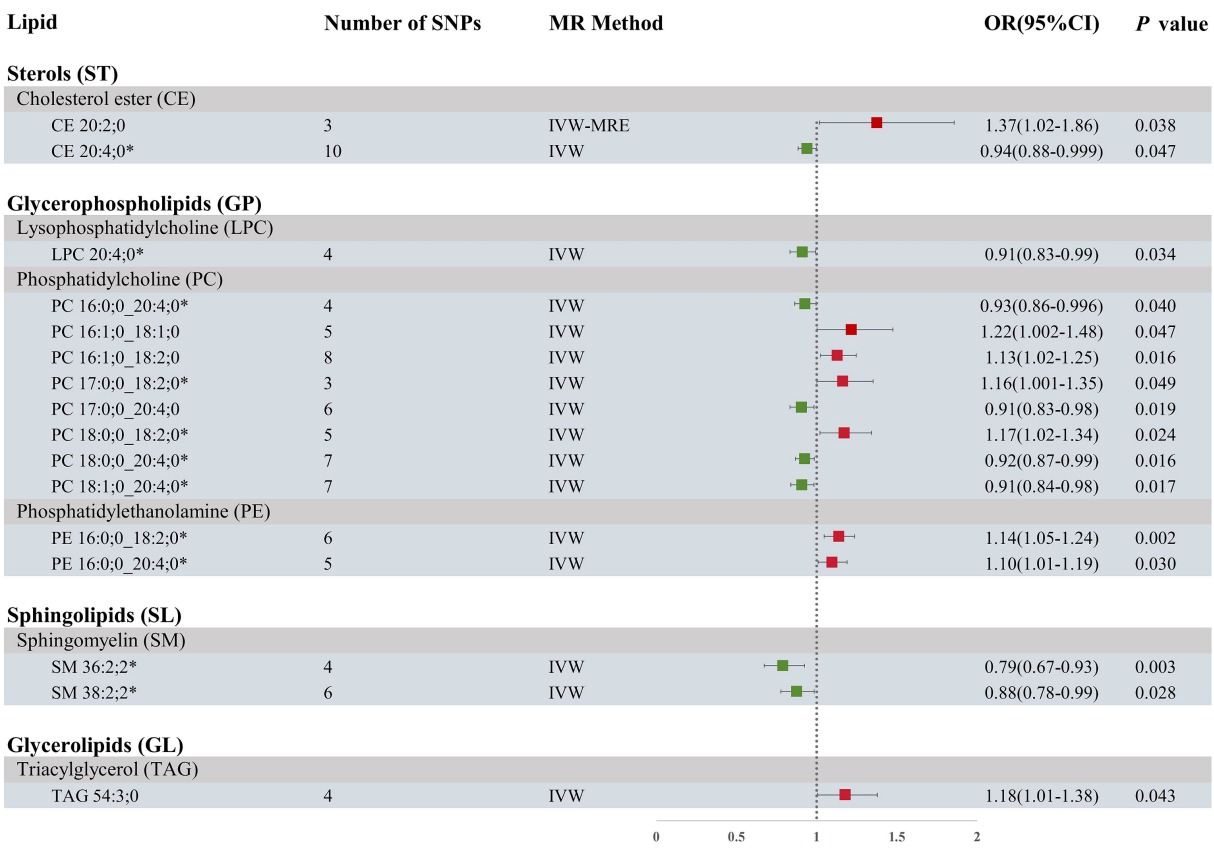

FIGURE 2
Forest plot of MR results for ICH in the Finnish population, depicting plasma lipids with different fatty acid chains, based on Inverse Variance Weighted (IVW) analysis. SNPs, single nucleotide polymorphisms; IVW, Inverse variance weighted; IVW-MRE, Inverse variance weighted (multiplicative random effects); OR, odds ratio; CI, confidence interval. *represents candidate lipids identified in preliminary analysis.

CI) = 0.79 (0.67–0.93)] and SM 38:2;2 [$p = 0.028$; OR(95% CI) = 0.88 (0.78–0.99)].

3.2 Preliminary analysis of plasma lipids and SAH

Our research findings indicate that, using the IVW method with a significance threshold of $p < 0.05$, eight lipids demonstrate potential causal relationships with SAH in the Finnish population (Figure 3). Among the 13 lipid categories analyzed, only three showed significant associations with SAH, with CE demonstrating the strongest causal relationship. However, after applying FDR correction, no lipids met the significance threshold, suggesting potential causal relationships. Consistent with the selection process for candidate lipids in the ICH group, we ensured the consistency of candidate lipid estimates across IVW, Weighted Mode, MR-Egger, and Weighted Median methods (Table 2; Supplementary Figure 3). The MR-PRESSO, Cochran Q test, and MR-Egger intercept test confirmed the absence of heterogeneity and horizontal pleiotropy, while the Steiger test validated the causal relationships between candidate lipids and SAH onset (Table 2). LOO analysis results also indicated no bias (Supplementary Figure 4). Comprehensive sensitivity analyses identified six lipids as potential biomarkers for SAH: CE 16:1;0 [$p = 0.02$; OR (95% CI) = 0.86 (0.76–0.98)], CE 18:1;0 [$p = 0.049$; OR (95% CI) = 0.80 (0.64–0.999)], CE 18:3;0 [$p = 0.025$; OR (95% CI) = 0.82 (0.69–0.98)], CE 20:3;0 [$p = 0.0002$; OR (95% CI) = 0.77 (0.66–0.88)], PC 18:1;0_20:4;0 [$p = 0.047$; OR (95% CI) = 0.82 (0.68–0.997)], and PE 18:0;0_20:4;0 [$p = 0.017$; OR (95% CI) = 0.90 (0.82–0.98)].

3.3 Replication and meta-analysis

To improve the reliability of our findings, we replicated the Mendelian Randomization (MR) analysis using GWAS data on ICH and SAH from Saori et al.'s study, which included 179 lipids. The repeated analysis, using the IVW method with a statistical significance threshold of $p < 0.05$, identified causal relationships between 20 lipids and ICH in the European population, and 13 lipids and SAH (Supplementary Figure 5; Supplementary Table 4). From the candidate lipids identified in the initial analysis (Sections 3.1 and 3.2), we performed a meta-analysis to compare the results of the repeated analysis with the initial analysis, further investigating the role of lipids in hemorrhagic stroke in the European population. The meta-analysis results showed that the risk associations of 11 candidate lipids in the ICH group remained consistent (Figure 4). Specifically, elevated levels of CE 20:4;0 [$p = 0.006$; OR(95% CI) = 0.929 (0.881–0.979)], LPC 20:4;0 [$p = 0.001$; OR(95% CI) = 0.904 (0.850–0.961)], PC 16:0;0_20:4;0 [$p = 0.007$; OR(95% CI) = 0.916 (0.858–0.977)], PC 18:0;0_20:4;0

TABLE 1 Supplementary and sensitivity analysis of causality between plasma lipids and ICH in preliminary analysis.

Lipid		N	MR analysis			Heterogeneity		Pleiotropy		MR-PRESSO		Steiger test	
			Meth	OR (95% CI)	*p*	Q	*p*	Int	*p*	RSS	*p*	*p*	D
Cholesterol ester (CE)													
	CE 20:2;0	3	ME	0.70(0.12–4.12)	0.76	7.21	0.03	0.148	0.59	NA	NA	9.44E-48	T
			WME	1.32(1.09–1.61)	0.01								
			WMO	1.31(1.05–1.65)	0.14								
	CE 20:4;0	10	ME	0.92(0.82–1.02)	0.16	9.06	0.43	0.01	0.6	9.29	0.63	0	T
			WME	0.94(0.88–1.01)	0.08								
			WMO	0.95(0.89–1.01)	0.13								
Lysophosphatidylcholine (LPC)													
	LPC 20:4;0	4	ME	0.99(0.86–1.14)	0.91	3.64	0.30	−0.034	0.28	24.83	0.48	2.37E-227	T
			WME	0.93(0.86–1.01)	0.07								
			WMO	0.93(0.86–1.01)	0.17								
Phosphatidylcholine (PC)													
	PC 16:0;0_20:4;0	4	ME	0.98(0.85–1.14)	0.85	2.93	0.40	−0.028	0.45	11.26	0.53	0	T
			WME	0.93(0.86–1.01)	0.07								
			WMO	0.94(0.87–1.01)	0.21								
	PC 16:1;0_18:1;0	5	ME	0.78(0.49–1.23)	0.36	5.45	0.24	0.071	0.13	7.50	0.34	1.52E-53	T
			WME	1.26(1.03–1.54)	0.03								
			WMO	1.28(1.02–1.62)	0.10								
	PC 16:1;0_18:2;0	8	ME	0.98(0.82–1.18)	0.87	5.21	0.63	0.031	0.13	7.78	0.63	5.00E-159	T
			WME	1.10(0.98–1.23)	0.11								
			WMO	1.09(0.97–1.23)	0.18								
	PC 17:0;0_18:2;0	3	ME	1.03(0.65–1.62)	0.92	0.86	0.65	0.026	0.68	NA	NA	1.31E-67	T
			WME	1.15(0.98–1.34)	0.09								
			WMO	1.14(0.96–1.35)	0.27								
	PC 17:0;0_20:4;0	6	ME	1.02(0.88–1.18)	0.82	5.88	0.32	−0.048	0.14	23.14	0.45	1.76E-276	T
			WME	0.91(0.84–0.99)	0.02								
			WMO	0.93(0.85–1.02)	0.18								
	PC 18:0;0_18:2;0	5	ME	1.21(0.79–1.85)	0.44	1.08	0.9	−0.006	0.88	1.63	0.91	1.86E-82	T
			WME	1.16(0.99–1.37)	0.07								
			WMO	1.20(0.99–1.44)	0.13								

(Continued)

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TABLE 1 (Continued)

Lipid		N	MR analysis			Heterogeneity		Pleiotropy		MR-PRESSO		Steiger test	
			Meth	OR (95% CI)	*p*	Q	*p*	Int	*p*	RSS	*p*	*p*	D
	PC 18:0;0_20:4;0	7	ME	0.95(0.86–1.06)	0.43	6.4	0.38	−0.014	0.51	22.98	0.45	0	T
			WME	0.94(0.88–1.01)	0.07								
			WMO	0.94(0.88–1.01)	0.14								
	PC 18:1;0_20:4;0	7	ME	0.99(0.85–1.15)	0.90	4.58	0.6	−0.032	0.24	9.72	0.56	4.72E-258	T
			WME	0.92(0.84–1.003)	0.06								
			WMO	0.93(0.85–1.02)	0.16								
Phosphatidylethanolamine (PE)													
	PE 16:0;0/18:2;0	6	ME	1.06(0.82–1.39)	0.67	1.43	0.92	0.017	0.63	1.63	0.95	3.05E-183	T
			WME	1.13(1.02–1.25)	0.01								
			WMO	1.13(1.01–1.26)	0.08								
	PE 16:0;0/20:4;0	5	ME	1.19(0.98–1.43)	0.18	1.32	0.86	−0.025	0.43	1.52	0.91	5.67E-227	T
			WME	1.11(1.01–1.21)	0.03								
			WMO	1.11(1.01–1.21)	0.09								
Sphingomyelin (SM)													
	SM 36:2;2	4	ME	0.86(0.49–1.51)	0.65	0.11	0.99	−0.014	0.8	0.16	0.99	1.07E-59	T
			WME	0.79(0.66–0.95)	0.01								
			WMO	0.79(0.63–0.98)	0.13								
	SM 38:2;2	6	ME	0.98(0.68–1.41)	0.92	2.56	0.77	−0.021	0.56	3.75	0.77	6.28E-109	T
			WME	0.88(0.76–1.02)	0.08								
			WMO	0.89(0.75–1.06)	0.24								
Triacylglycerol (TAG)													
	TAG 54:3;0	4	ME	0.86(0.57–1.30)	0.55	2.69	0.44	0.054	0.25	7.56	0.43	9.88E-63	T
			WME	1.24(1.03–1.49)	0.02								
			WMO	1.04(0.84–1.28)	0.77								

N, number of single nucleotide polymorphisms; Meth, methods; ME, MR-Egger; WME, Weighted median; WMO, Weighted mode; CI, confidence interval; OR, odds ratio; Int, MR-Egger intercept; MR-PRESSO, MR-Pleiotropy RESidual sum and outlier; RSS, RSSobs; D, Direction;T, True; NA, Not available.

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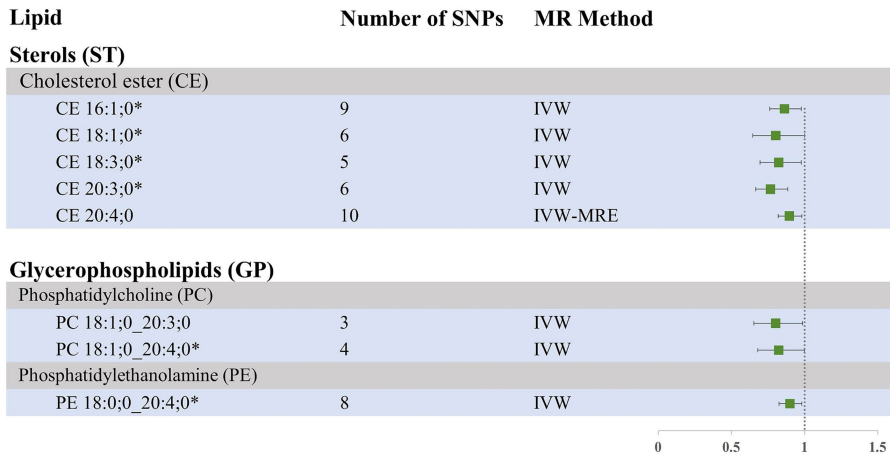

FIGURE 3
Forest plot of MR results for SAH in the Finnish population, depicting plasma lipids with different fatty acid chains, based on Inverse Variance Weighted (IVW) analysis. SNPs, single nucleotide polymorphisms; IVW, Inverse variance weighted; IVW-MRE, Inverse variance weighted (multiplicative random effects); OR, odds ratio; CI, confidence interval. *represents candidate lipids identified in preliminary analysis.

[$p < 0.001$; OR(95% CI) = 0.914 (0.866–0.964)], PC 18:1;0_20:4;0 [$p < 0.001$; OR(95% CI) = 0.892 (0.835–0.954)], SM 36:2;2 [$p < 0.001$; OR(95% CI) = 0.766 (0.676–0.868)] and SM 38:2;2 [$p = 0.004$; OR(95% CI) = 0.859 (0.774–0.954)] were associated with a reduced risk of ICH. Conversely, high levels of PC 17:0;0_18:2;0 [$p < 0.001$; OR(95% CI) = 1.232 (1.092–1.391)], PC 18:0;0_18:2;0 [$p = 0.002$; OR(95% CI) = 1.179 (1.065–1.306)], PE 16:0;0_18:2;0 [$p < 0.001$; OR(95% CI) = 1.147 (1.079–1.219)] and PE 16:0;0_20:4;0 [$p = 0.006$; OR(95% CI) = 1.088 (1.024–1.155)] were associated with an increased risk of ICH. In the SAH group, five candidate lipids exhibited consistent risk associations (Figure 5). Elevated levels of CE 16:1;0 [$p = 0.015$; OR(95% CI) = 0.878 (0.792–0.975)], CE 18:3;0 [$p = 0.035$; OR(95% CI) = 0.848 (0.727–0.989)], CE 20:3;0 [$p < 0.001$; OR(95% CI) = 0.786 (0.704–0.877)], PC 18:1;0_20:4;0 [$p = 0.002$; OR(95% CI) = 0.794 (0.689–0.916)] and PE 18:0;0_20:4;0 [$p = 0.003$; OR(95% CI) = 0.905 (0.846–0.967)] were associated with a reduced risk of SAH. However, in the meta-analysis, CE 18:1;0 [$p = 0.775$; OR (95% CI) = 0.951 (0.674–1.342)] did not reach significance in the SAH group ($p < 0.05$). The most significant lipids identified in the meta-analysis were plasma lipids containing arachidonic acid (C20:4), such as PC 18:1;0_20:4;0, which generally had a risk-reducing effect on ICH and SAH, except for PE 16:0;0_20:4;0. Lipids containing linoleic acid (C18:2) were most prevalent in ICH and showed an increased risk of onset.

4 Discussion

Utilizing extensive publicly available genetic data, our study explored the causal relationships between 179 plasma lipid species and two subtypes of hemorrhagic stroke within the European population. Previous MR studies on plasma lipid species have typically provided a broad overview of associated phenotypes without delving into the specific impact of plasma lipid species on hemorrhagic stroke subtypes (11, 19). Our MR analysis examined causal links between lipids with varying fatty acid chains and hemorrhagic stroke. Through stringent inclusion criteria and sensitivity analyses, we identified 11 lipids causally associated with ICH and 5 lipids with a causal relationship to SAH in the European population.

In our analysis, lipids of the same class displayed differing effects on the same disease, likely influenced by the specific fatty acid chains, particularly polyunsaturated fatty acids (PUFAs), except for sphingomyelin (SM). For instance, two PCs, PC 18:0;0_18:2;0 and PC 18:0;0_20:4;0, exhibited differing effects on ICH due to the presence of distinct PUFAs. Conversely, lipids from different classes with the same PUFA chain tended to exhibit similar effects. This is illustrated by CE 20:4;0 and PC 18:1;0_20:4;0, which both demonstrated risk-reducing effects on ICH. This could be due to the hydrolysis of PUFAs in the related lipids. However, an exception was noted with PE 16:0;0_20:4;0, which had an opposite effect on ICH.

In our study, lipids containing arachidonic acid (C20:4) and linoleic acid (C18:2), both omega-6 PUFAs, were found to be the most prevalent. Arachidonic acid (AA), a common omega-6 PUFA, is abundantly present in the phospholipids of cell membranes and is typically hydrolyzed into its free form by phospholipase A2 (32, 33). It plays various physiological roles, influencing the functions of endothelial cells and neurons (34). Lipids containing the AA chain were most abundant in hemorrhagic stroke subtypes and had protective effects, lowering the risk of ICH and SAH. This protective mechanism is possibly due to the reparative effects of hydrolyzed AA on the vascular endothelium. Previous research highlights AA's role in vascular biology via the cytochrome P450 (CYP) pathway (35), with CYP1B1 being the most prevalent enzyme subtype in brain microvessels (36, 37). Animal studies indicate that CYP1B1 deficiency leads to impaired AA metabolism, reducing cerebral microcirculation and compromising the blood–brain barrier (38, 39). Furthermore, AA supports vascular repair through 11,12-epoxyeicosatrienoic acid, a metabolite of CYP 2 J2, which significantly promotes neovascularization (40). However, PE 16:0;0_20:4;0 had an adverse effect on ICH, possibly due to its susceptibility to lipid peroxidation, leading to ferroptosis (41, 42). While a prospective study in China did not find an effect of AA on ICH (43), previous MR studies have consistently shown that higher AA levels significantly reduce the risk of ICH (44).

TABLE 2 Supplementary and sensitivity analysis of causality between plasma lipids and SAH in preliminary analysis.

Lipid		N	MR analysis			Heterogeneity		Pleiotropy		MR-PRESSO		Steiger test	
			Meth	OR (95% CI)	*p*	Q	*p*	Int	*p*	RSS	*p*	*p*	D
Cholesterol ester (CE)													
	CE 16:1;0	9	ME	0.79(0.55–1.11)	0.22	8.99	0.34	0.023	0.59	11.14	0.38	1.15E-123	T
			WME	0.83(0.71–0.98)	0.03								
			WMO	0.86(0.71–1.05)	0.18								
	CE 18:1;0	6	ME	0.43(0.19–1.01)	0.12	7.36	0.20	0.155	0.21	10.49	0.25	1.58E-51	T
			WME	0.80(0.62–1.02)	0.08								
			WMO	0.77(0.53–1.11)	0.22								
	CE 18:3;0	5	ME	0.64(0.40–1.01)	0.15	3.36	0.50	0.042	0.33	4.29	0.61	4.58E-60	T
			WME	0.82(0.66–1.01)	0.06								
			WMO	0.82(0.66–1.02)	0.15								
	CE 20:3;0	6	ME	0.57(0.34–0.98)	0.11	5.01	0.41	0.057	0.33	7.00	0.48	9.02E-83	T
			WME	0.81(0.69–0.96)	0.02								
			WMO	0.82(0.67–0.999)	0.11								
	CE 20:4;0	10	ME	1.03(0.92–1.15)	0.63	17.44	0.04	−0.06	0.02	58.43	0.28	0	T
			WME	0.93(0.87–1.01)	0.07								
			WMO	0.94(0.88–1.02)	0.15								
Phosphatidylcholine (PC)													
	PC 18:1;0_20:3;0	3	ME	1.07(0.39–2.99)	0.91	0.94	0.63	−0.056	0.67	NA	NA	1.24E-40	T
			WME	0.75(0.59–0.96)	0.02								
			WMO	0.74(0.54–1.02)	0.21								
	PC 18:1;0_20:4;0	4	ME	0.94(0.40–2.21)	0.89	1.07	0.78	−0.018	0.79	1.60	0.84	3.30E-46	T
			WME	0.83(0.66–1.04)	0.11								
			WMO	0.83(0.65–1.06)	0.24								
Phosphatidylethanolamine (PE)													
	PE 18:0;0_20:4;0	8	ME	0.83(0.65–1.06)	0.19	5.40	0.61	0.021	0.54	6.26	0.71	4.59E-245	T
			WME	0.91(0.82–1.02)	0.1								
			WMO	0.92(0.82–1.02)	0.16								

N, number of single nucleotide polymorphisms; Meth, methods; ME, MR-Egger; WME, Weighted median; WMO, Weighted mode; CI, confidence interval; OR, odds ratio; Int, MR-Egger intercept; MR-PRESSO, MR-Pleiotropy RESidual sum and outlier; RSS, RSSobs; D, Direction; T, True; NA, Not available.

An increasing number of epidemiological studies suggest that AA plays a critical role in neuroprotection following hemorrhagic stroke (45, 46). One study reported significantly elevated levels of AA and its metabolites in patients with ICH compared to healthy individuals, indicating a protective response (47). This mechanism involves metabolites from both the cytochrome P450 (CYP) enzyme pathway and the lipoxygenase pathway. Specifically, lipoxin A4, synthesized via the lipoxygenase pathway, inhibits inflammation and cell migration (32). In mouse models, activating the lipoxin A4 receptor has been shown to decrease neuroinflammation after ICH (48). In the cyclooxygenase pathway of prostaglandin synthesis, prostaglandin E2, a derivative of AA, may promote the proliferation of neural stem cells in the adult brain's subventricular zone following ICH, thus aiding in nervous system repair (45).

Linoleic acid (LA) is an omega-6 PUFA and an essential nutrient necessary for human growth and development, accounting for 1 to 2% of daily energy intake (49). However, excessive intake of LA can lead to various diseases (50). In the United Kingdom, vegetarians have a higher incidence of hemorrhagic stroke compared to meat eaters (51), mainly because LA is predominantly found in vegetable oils. A prospective study in China revealed that elevated blood LA levels increased the risk of ICH (43). Research has indicated that LA may reduce blood LDL-C and platelet aggregation (52), which could increase the risk of hemorrhagic stroke (53). Our studies also found that lipids with esterified LA chains are linked to an increased risk of ICH, likely due to the hydrolysis of these chains. Additionally, LA can increase the expression of adhesion molecules in endothelial cells, promote inflammatory cell migration, and inhibit microvascular dilation, leading to vascular diseases (54, 55). LA exhibits high-affinity binding to lipocalin-2, a proinflammatory adipokine that causes vascular inflammation and endothelial dysfunction in mice (56). It also amplifies TNF-α-induced oxidative stress and inflammatory mediators, which damage the vascular endothelium (57, 58). Although LA can be converted into gamma-linolenic acid and subsequently metabolized into AA, excessive intake results in an

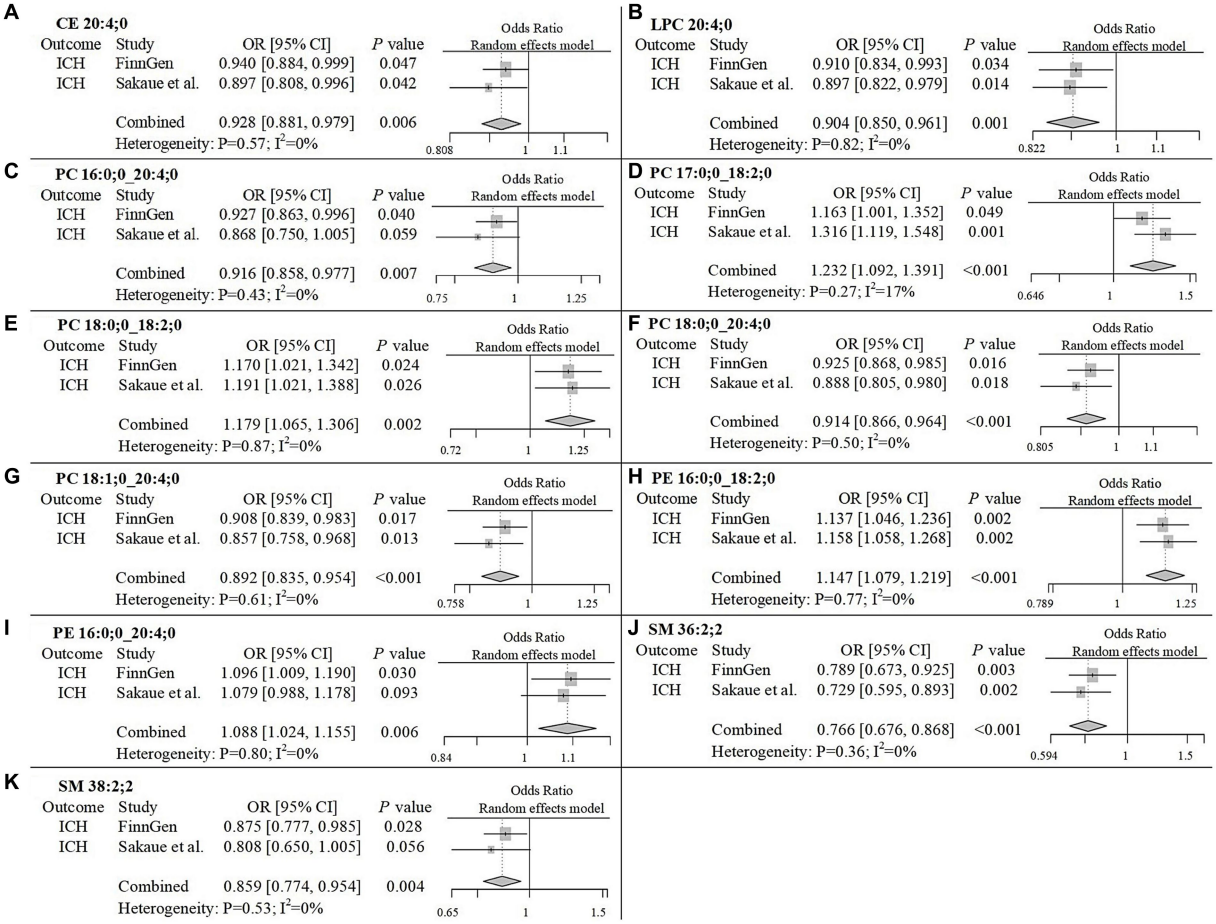

FIGURE 4
Meta-analysis of significantly associated candidate lipids in ICH. OR, odds ratio; 95%CI, 95% confidence interval.

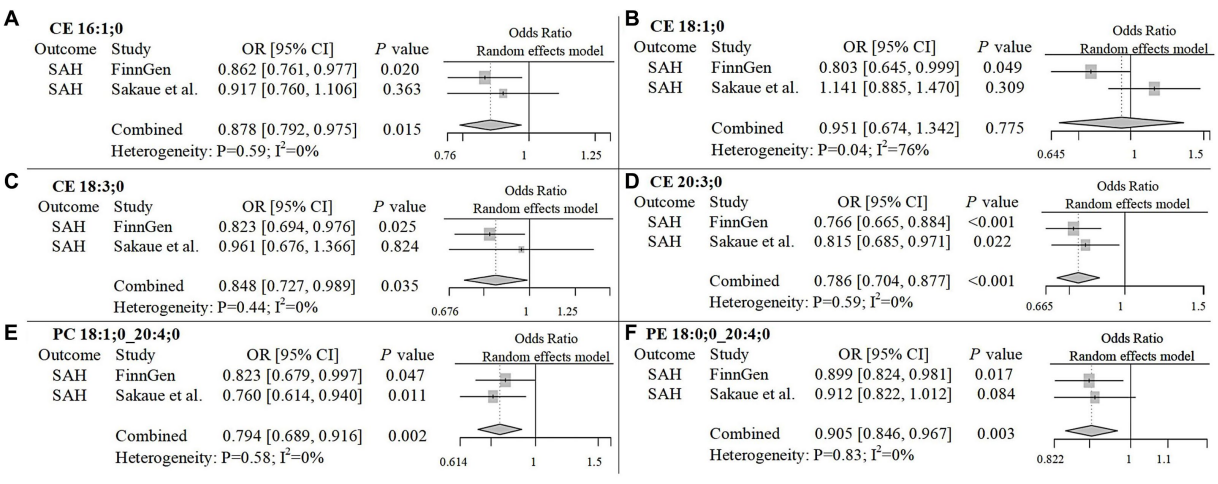

FIGURE 5
Meta-analysis of significantly associated candidate lipids in SAH. OR, odds ratio; 95%CI, 95% confidence interval.

accumulation of AA-derived pronociceptive lipid mediators (59, 60). However, a systematic review reported no correlation between LA intake and AA levels in human tissues (61). Our findings suggest that lipids containing esterified AA and LA have differing effects on ICH risk, indicating that the stroke risk associated with esterified LA may not be closely related to AA from a genetic standpoint.

Some research indicates that omega-3 PUFAs may lower the risk of coronary heart disease and ischemic stroke (62, 63). Nevertheless, our study discovered that lipids with esterified omega-3 PUFA side chains do not show a significant correlation with hemorrhagic stroke, except for CE 20:3;0 containing alpha-linolenic acid (ALA), which is relevant to SAH. It has been suggested that a higher dietary intake of ALA may help prevent stroke (64), though a cohort study found that while ALA supplementation was associated with improved overall mortality, it did not significantly affect the risk of coronary heart disease and stroke (65). Additionally, no significant association was observed between plasma phospholipid ALA levels and these conditions. In our study, ALA did not show a significant association with ICH risk. Likewise, other omega-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), demonstrated no significant causal effects. A meta-analysis of 29 prospective studies on omega-3 PUFAs and stroke incidence rates indicated that EPA and DHA were associated with reduced overall stroke and ischemic stroke risks, but not hemorrhagic stroke, which aligns with our findings (66). However, a case–control study in Korea suggested that low omega-3 PUFAs levels in red blood cells might increase the risk of acute ischemic and hemorrhagic strokes (67). In conclusion, more detailed research is necessary to examine the relationship between omega-3 PUFAs and the risks of ICH and SAH.

This study performed an in-depth analysis of genetic data and hemorrhagic stroke using the Mendelian randomization method. It included extensive genetic data containing SNPs information, combined with thorough MR analysis, effectively removing confounding factors to establish more accurate causal relationships. Multiple tests confirmed the robustness and reliability of the results. However, the study has certain limitations. First, the Mendelian randomization method relies on specific assumptions, such as genetic instrumental variables being unrelated to confounding factors, which may not always be valid. Second, most of the genetic data originate from European populations, which may not fully represent the genetic diversity of the broader human population. Finally, the study cannot provide precise individual risk predictions but can only offer general risk trends.

5 Conclusion

This study identified seven lipids that may be causally associated with an increased risk of ICH in European populations, and four lipids that may be causally associated with a reduced risk of ICH. Additionally, five lipids were found to be potentially causally associated with a reduced risk of SAH. However, no lipids were found to be associated with an increased risk of SAH. The effects of these plasma lipids on these diseases may be related to the lipids themselves, but are more likely associated with the unsaturated fatty acid side chains they carry. Plasma lipids with arachidonic acid side chains showed a risk-lowering effect on both intracerebral and subarachnoid hemorrhages, while those with linoleic acid side chains were associated only with an increased risk of intracerebral hemorrhage. These findings could contribute to the understanding of the mechanisms behind the onset and progression of hemorrhagic stroke.

Data availability statement

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

Ethics statement

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

Author contributions

XkZ: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. XyZ: Writing – original draft, Writing – review & editing, Visualization. QS: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

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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.2024.1432878/full#supplementary-material

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EDITED BY
Robert G. Kowalski,
University of Colorado Anschutz Medical Campus, United States

REVIEWED BY
Jayanta Gupta,
Florida Gulf Coast University, United States
Ai Feng He,
Binhai County People's Hospital, China

*CORRESPONDENCE
Yongkun Li
liyongkun721@139.com

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Systemic immune inflammation index and risk of stroke: a cross-sectional study of the National Health and Nutrition Examination Survey 2005–2018

Hua Xue[1], Yuqi Zeng[2], Xinyang Zou[1] and Yongkun Li[1]*

[1]Department of Neurology, Shengli Clinical Medical College of Fujian Medical University, Fuzhou, China, [2]Department of Neurology, Fujian Medical University Union Hospital, Fuzhou, China

Background: The incidence of stroke has increased globally, resulting in medical expenditures and social burdens over the past few decades. We aimed to explore the relationship between systemic immune inflammatory index (SII) and stroke using the National Health and Nutrition Examination Survey (NHANES) from 2005 to 2018.

Methods: Based on NHANES data, 902 stroke patients and 27,364 non-stroke patients were included in this study. SII was the independent variable and stroke was the dependent variable. Univariate and multivariate logistic regression analyses were used to explore the association between SII and stroke. Restricted cubic spline (RCS) method was used to test the nonlinear association between SII and stroke.

Results: Weighted logistic regression analysis showed a significant association between SII and stroke (OR: 1.985, 95% CI: 1.245–3.166, $p = 0.004$). The interaction test showed that the association between SII and stroke was not significant between strata ($p > 0.05$). A significant positive association between SII and stroke risk (OR >1, $p < 0.05$) was observed in the crude model, model I and model II. RCS analysis showed no nonlinear positive association between SII and stroke risk after adjusting for all confounders.

Conclusion: Our study determined that SII is associated with stroke risk. Given the inherent limitations of cross-sectional studies, further research is necessary to validate the causality of this association and to demystify the underlying mechanisms between inflammation and stroke.

KEYWORDS

NHANES, systemic immune inflammation index, stroke, logistic regression, cross-sectional study

1 Introduction

Stroke has emerged as a significant global public health concern, with a rising burden that impacts personal health, family, and societal economics (1). Despite advancements in diagnostic and therapeutic strategies for stroke over the past decades, data indicates a 2.1% increase in the global lifetime risk of stroke among adults aged 25 and above in 2016 compared

to 1990 (2). Stroke encompasses ischemic stroke, which constitutes approximately 87% of cases, and hemorrhagic stroke, characterized by higher mortality rates despite its lower prevalence (3). The pathogenesis of ischemic stroke involves factors such as atherosclerotic plaque formation, cardiac emboli, thrombosis, vasospasm, and hypoperfusion (4). On the other hand, hemorrhagic stroke is often linked to weakened blood vessel walls due to high blood pressure, ruptured aneurysms, or vascular malformations (5). The underlying mechanisms of stroke are intricate, influenced by various intrinsic and extrinsic factors. Recent research underscores the significance of systemic inflammation in stroke pathogenesis, which is intertwined with both infectious and non-infectious triggers (6). In ischemic stroke, the instability of atherosclerotic plaques and subsequent vascular blockages are closely associated with inflammatory regulation (7). Similarly, the inflammatory response plays a key role in neurological damage and prognosis after hemorrhagic stroke (8). In both types of stroke, there is a robust inflammatory reaction characterized by neutrophil and macrophage activation, along with the release of inflammatory mediators like tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) (9, 10). These processes contribute to initial damage and impact subsequent repair and regeneration (9, 10).

Systemic immune inflammation index (SII) is a novel comprehensive inflammatory index that considers three types of inflammatory immune cells (lymphocytes, neutrophils, and platelets), reflecting the balance between immunity and inflammatory status (11). Initially utilized for predicting tumor prognosis and identifying high-risk patients, SII has shown associations with disease severity and poor outcomes in various conditions, including diverse cancer, heart failure, and cardiovascular diseases (11–13). Recent studies have confirmed the relevance of SII in cerebrovascular diseases. For instance, Wang's et al. (14) research highlighted SII as an independent risk factor for stroke-associated pneumonia in patients with intracerebral hemorrhage, correlating with unfavorable results. Furthermore, a cross-sectional study suggested a connection between SII and cerebral small vessel disease, providing evidence for the prognostic relevance of SII (15). Notably, individuals with elevated SII levels were found to be at higher risk of modified white matter hyperintensity (WMH) burden and basal ganglia enlarged perivascular spaces (BG-EPVS) (15). The study by Kelesoglu et al. (16) confirmed that the increase in serum SII is closely related to the severity of carotid artery stenosis. Specifically, neutrophils can reinfiltrate the ischemic site within the first hours after stroke and induce brain tissue damage by activating the inflammatory response through the release of inflammatory mediators (17). Lymphocytes, especially T cells and B cells, participate in the repair process after stroke by regulating immune responses. A decrease in lymphocyte counts could suggest an immunosuppressed state, impacting the recovery from stroke (18). Platelets are not only key cells for coagulation, but also participate in inflammatory reactions, releasing inflammatory mediators, promoting cerebrovascular inflammation and aggravating brain tissue damage (19, 20). Consequently, the Systemic Immune-Inflammation Index (SII), as a biomarker incorporating counts of three cellular components, provides a more nuanced reflection of the immune inflammatory landscape, enabling a finer assessment of the equilibrium between inflammatory and immune states.

While some research has indicated that SII could be a useful inflammatory markers for diagnosing and predicting stroke outcomes, there is a lack of large-scale sampling studies. The National Health and Nutrition Examination Surveys (NHANES) database, with its complex multistage probability sampling design, provides a nationally representative and ethnically diverse cohort. Our study utilized data from 7 periods of the NHANES database spanning from 2005 to 2018. We employed weighted logistic regression to develop a model that accounted for confounding factors and investigated the relationship between SII and stroke risk.

2 Materials and methods

2.1 Study design and data source

We conducted an observational cross-sectional study using data from the National Health and Nutrition Examination Survey (NHANES) to investigate the casual relationship between systemic immune inflammation indexes (SII) and the risk of stroke. NHANES is a nationally representative survey project by the U.S. Centers for Disease Control and Prevention (CDC) aimed at assessing the health and nutritional status of individuals in the United States (21). The public can download all NHANES data for free at https://www.cdc.gov/nchs/nhanes/index.htm. Our study utilized data from seven survey cycles spanning from 2005 to 2018, totaling 70,190 participants. Exclusions criteria included: (i) aged <18 or ≥80 years ($n = 30,823$); (ii) missing complete blood routine count ($n = 3,435$); (iii) missing stroke diagnosis status ($n = 2,111$); (iv) missing covariates data, such as body mass index (BMI), smoke status, alcohol use status, family income ($n = 5,555$). After screening, 28,266 participants were selected for analysis. A detailed recruitment flowchart is provided in Figure 1.

2.2 Definition of SII

The exposure variable in our study was SII. The SII serves as a hematologic marker used to quantitatively assess both systemic inflammation and immune status within patients (22, 23). This index is calculated by the following formula: $SII = P \times N/L$. Herein, "P" symbolizes the platelet count, "N" denotes the neutrophil count, and "L" represents the lymphocyte count (22).

2.3 Stroke assessment

Stroke was defined as a previous diagnosis self-reported by a physician during a face-to-face interview. In NHANES, participants who answered the question on the medical conditions questionnaire, "Has a doctor or other health professional ever told you that you had a stroke?" where "yes" were considered to have had a stroke. In addition, although there is a lack of information on stroke type in the NHANES database, given the relatively high incidence of ischemic stroke in stroke patients, it is likely that most of the stroke participants included in this study had an ischemic stroke (24).

2.4 Covariates

Over the past few decades, extensive research has been conducted on the etiology and risk factors of stroke. We have endeavored to collect a comprehensive set of covariates that potentially confound the

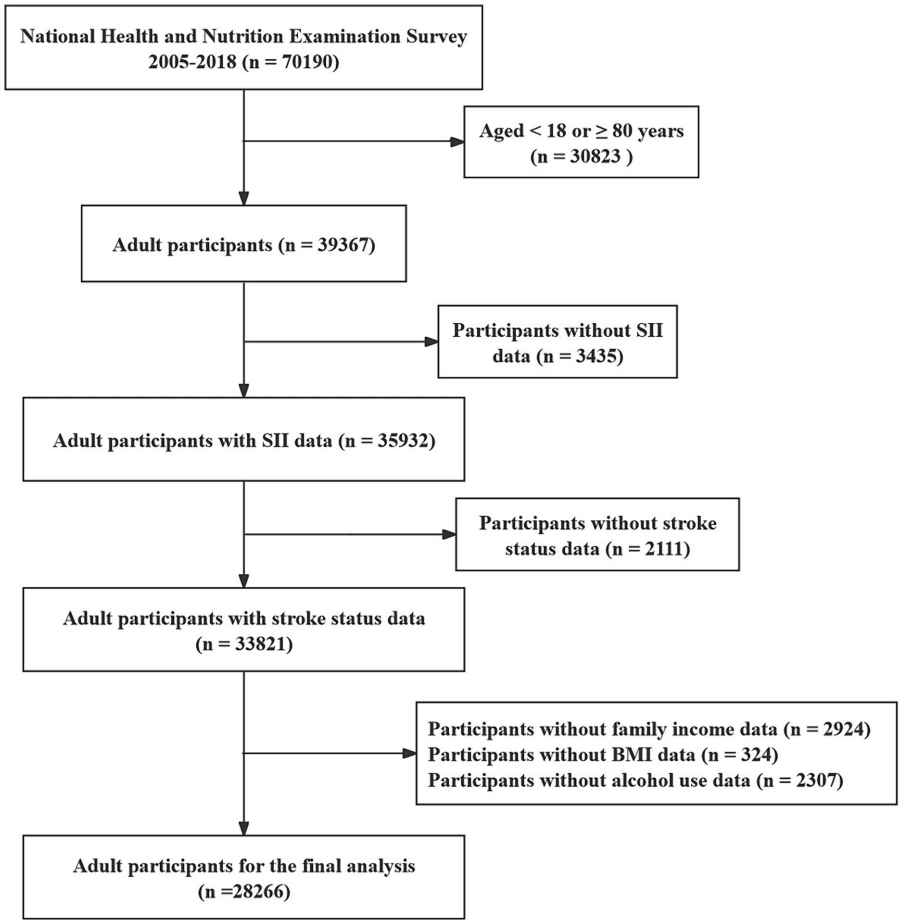

FIGURE 1
Flowchart of the participant selection from NHANES 2005–2018.

relationship with stroke, including age, gender, race/ethnicity, family income, smoke status, alcohol use, obesity, hypertension, diabetes, and coronary heart disease, all of which have been implicated in the occurrence of stroke. Race/ethnicity was categorized into five groups: non-Hispanic White, non-Hispanic Black, other Hispanic, Mexican American, and other races. Regarding smoke status, participants were defined as smokers if they answered "yes" to either the question "Have you smoked at least 100 cigarettes during your entire life?" or "Do you currently smoke?" in the questionnaire (25). With respect to alcohol consumption, participants were classified as drinkers if they responded "yes" to the question "Have you ever had at least 12 drinks of any type of alcoholic beverage in your lifetime?" in the survey (26). Hypertension was defined based on either self-reported prior diagnosis by a physician or measured blood pressure during the examination. Participants were considered hypertensive if they met at least one of the following criteria: (1) average systolic blood pressure (SBP) ≥130 mmHg; (2) average diastolic blood pressure (DBP) ≥90 mmHg; (3) self-reported history of hypertension diagnosis; or (4) current use of anti-hypertensive medication (27). Body mass index (BMI) is widely used to estimate overweight/obesity status. Clinically, BMI values greater than 25 and 30 kg/m^2 are generally regarded as the primary diagnostic thresholds for overweight and obesity, respectively (28). Participants were deemed to have diabetes if they met at least one of the following conditions: "told by a doctor that they have diabetes," "hemoglobin A1c (HbA1c) concentration >6.5%," or "fasting plasma glucose (FPG) level >126 mg/dL (7.0 mmol/L)" (29). Concentrations of fasting blood glucose (FBG), hemoglobin A1c (HbA1c), red blood cell (RBC) count, neutrophil count, monocyte count, lymphocyte count, and platelet count were all determined through standardized laboratory assays.

2.5 Statistical analysis

Data processing and analysis in this study were performed using R statistical software and MEC weights (WTMEC2YR). The NHANES surveys utilize various intricate sampling designs, thus we incorporated sample weights for different study periods in our analytical methods to ensure precise estimates of health-related statistics (30). Continuous variables are presented as weighted means and standard deviations (SD), whereas categorical variables are presented as frequencies and percentages. To identify variances in baseline characteristics between stroke and non-stroke participants, student's *t*-test was used for continuous variables and Chi-square test was used for categorical variables. A *p*-value <0.05 indicated statistically significant. We use the "survey" package to construct a weighted logistic regression model. Multivariable weighted logistic regression models were utilized to

investigate the relationship between SII and stroke risk. To assess the correlation and potential non-linear connection between SII and stroke, the continuous SII variable was categorized into quartiles, and trend p was calculated. Weighted logistic regression models were constructed using the survey package, both unadjusted and adjusted for confounders, with group analyses of confounders based on significant interaction terms. Initially, an unadjusted crude model was applied, followed by two multivariable logistic regression models that progressively controlled for covariates. Model 1 adjusted for age, race, smoking, and drinking status, while Model 2 adjusted for additional factors including gender, diabetes, hypertension, and coronary heart disease. The association strength was evaluated using odds ratios (OR) and 95% confidence intervals (CI). Furthermore, the restricted cubic splines (RCS) were used to explore the non-linear relationships. To explore the threshold effect of SII on the risk of stroke is and to find the inflection point, we used the smooth curve fitting and generalized additive models.

3 Results

3.1 Baseline characteristics

Details of the baseline characteristics of all participants grouped by stroke status are provided in Table 1. A total of 28,266 participants participated in the analysis, of which 50.92% were female and 49.08% were male. The average age of the sample is 47.57 (16.44). After classifying the participants according to stroke incidence, a total of 902 participants were identified as stroke patients, accounting for 3.2% of the total sample. The results showed that there were significant differences between the stroke group and the non-stroke group in terms of age, race, family income, BMI, smoking, hypertension, diabetes, coronary heart disease, platelets, neutrophils, lymphocyte, WBC count, monocyte, glycohemoglobin and SII ($p < 0.05$). Specifically, the average age of the stroke group was 62.19 (11.92), which was significantly higher than the average age of the non-stroke group, 47.09 (16.44) ($p < 0.001$). The SII of the stroke group was 588.76 (426.56), which was significantly higher than that of the non-stroke group, 536.25 (366.50) ($p < 0.001$). In addition, there was a significant difference in glycohemoglobin between the two groups. The glycohemoglobin in the stroke group was 6.19 (1.37), which was much higher than that in the non-stroke group 5.72 (1.08) ($p < 0.001$).

3.2 Univariate logistic regression analysis of stroke

After performing a weighted univariate logistic regression analysis (Table 2), our results indicate that older age (≥ 60 years), female, non-Hispanic White, non-Hispanic Black, other race, high BMI (>25), smoking, hypertension (yes), diabetes (yes) were at increased risk of stroke (OR >1, $p < 0.05$). However, participants who were other Hispanic, PIR (<1.3) show a reduced risk of stroke (OR <1, $p < 0.05$).

3.3 Relationship between stroke and SII

After performing a weighted multivariate logistic regression analysis (Table 3), our results indicate that a higher SII score is associated with an increased risk of developing stroke. This association was significant in our crude model (OR = 1.985; 95% CI = 1.245–3.166, $p = 0.004$) and model 1 (OR = 1.728; 95% CI = 1.118–2.672, $p = 0.014$). In the fully adjusted model, the positive association between SII and stroke remained stable (OR = 1.562; 95% CI = 1.020–2.394, $p = 0.040$), indicating that for every unit increase in log-formed SII, the risk of developing stroke increased by 16%. We further transformed the SII from a continuous variable into a categorical variable (quartiles) for sensitivity analysis (Table 3). Compared with the lowest quartile, the risk of developing stroke in the highest quartile increased by 48% (OR = 1.481; 95% CI = 1.121–1.958, $p = 0.006$) in the crude model, 39% (OR = 1.394; 95% CI = 1.057–1.838, $p = 0.018$) in the model 1 and 34% (OR = 1.348; 95% CI = 1.010–1.800, $p = 0.042$) in the model 2. In addition, there was a significant trend in SII and stroke risk with quartile (p for trend <0.05).

3.4 Subgroup analysis and interaction test

We also conducted stratified analyses to investigate whether the association between SII and stroke incidence remained consistent across different subgroups (Table 4). Our subgroup analyses revealed that the positive correlation was not significantly altered by stratification variables including gender (males and females), age (<60, ≥60), BMI (<25, 25–30, ≥30), PIR (<1.3, 1.3–3.5, >3.5), smoking status, alcohol use, diabetes, and hypertension. There was no statistically significant difference in the relationship between SII and stroke across these strata, as indicated by the interaction test p-values >0.05, suggesting that covariates have no significant effect on this association.

3.5 The nonlinear relationship between stroke and SII

In this study, restricted cubic splines (RCS) were employed to elucidate the nonlinear association between the SII and the risk of stroke (Figure 2). Covariates adjusted for in the analysis comprised gender, age, PIR, BMI, diabetes, hypertension, smoking status, and alcohol use. There was no statistically significant nonlinear relationship between SII and stroke risk ($p > 0.05$) either in the crude model (Figure 2A) or after adjustment for multiple confounders (Figure 2B). Of note, a threshold effect emerged, with a turning point observed at an SII value of 464.47. Below this critical threshold, the incidence risk of stroke remained relatively stable or even decreased; conversely, surpassing this threshold led to a marked escalation in the risk of stroke.

4 Discussion

This study ultimately included 28,266 participants from the NHANES 2005–2018 cohort for analysis, including 13,873 men and 14,393 women. Among them, 902 patients suffered from stroke. In baseline data, stroke patients had higher SII levels compared with normal subjects. The results of weighted univariate logistic analysis showed that there was a significant effect on the incidence of stroke among those age ≥60, BMI ≥30, diabetic, hypertensive, drinkers, and smokers. Furthermore, after adjusting for all covariates, we found that the relationship between SII levels and stroke was no nonlinear. When

TABLE 1 Characteristics of NHANES participants between 2005 and 2018.

Characteristic	Total	Non-stroke	Stroke	*p*-value
Overall	28,266	27,364	902	
Sex, *N* (%)				0.87
Female	14,393 (50.92)	13,936 (59.93)	457 (50.66)	
Male	13,873 (49.08)	13,428 (49.07)	445 (49.33)	
Age, (y), mean (SD)	47.57 (16.44)	47.09 (16.44)	62.19 (11.92)	<0.001
Race, *N* (%)				<0.001
Mexican American	4,512 (15.96)	4,414 (16.13)	98 (10.86)	
Other Hispanic	2,656 (9.39)	2,600 (9.50)	56 (6.21)	
Non-Hispanic White	12,038 (42.59)	11,640 (42.54)	398 (44.12)	
Non-Hispanic Black	6,000 (21.23)	5,714 (20.88)	286 (31.71)	
Other race	3,060 (10.83)	2,996 (10.95)	64 (7.10)	
BMI, (kg/m^2), *N* (%)				<0.001
<25	7,913 (28.03)	7,716 (28.19)	197 (21.84)	
25–30	9,194 (32.53)	8,931 (32.64)	263 (29.16)	
≥30	11,159 (39.48)	10,717 (39.16)	442 (49.00)	
PIR, *n* (%)				<0.001
<1.3	8,804 (31.15)	8,409 (30.73)	395 (43.79)	
1.3–3.5	10,511 (37.19)	10,152 (37.10)	359 (39.80)	
>3.5	8,951 (31.67)	8,803 (32.17)	148 (16.41)	
Smoke, *N* (%)				<0.001
No	15,453 (54.67)	15,130 (55.29)	323 (35.81)	
Yes	12,813 (45.33)	12,234 (44.71)	579 (64.19)	
Alcohol use, *N* (%)				0.09
No	7,048 (24.93)	6,802 (24.86)	246 (27.27)	
Yes	21,218 (75.06)	20,562 (75.14)	656 (72.72)	
Hypertension, *N* (%)				<0.001
No	18,603 (65.81)	18,389 (67.20)	214 (23.72)	
Yes	9,663 (34.18)	8,975 (32.80)	688 (76.28)	
Diabetes, *N* (%)				<0.001
No	24,743 (87.54)	24,160 (88.29)	583 (64.63)	
Yes	3,523 (12.46)	3,204 (11.71)	319 (35.37)	
CHD, *N* (%)				<0.001
No	27,319 (96.64)	26,566 (97.08)	753 (83.48)	
Yes	9,38 (3.36)	798 (2.92)	149 (16.52)	
Platelets, (10^9 cells/L), mean (SD)	249.36 (66.04)	249.56 (65.61)	243.19 (77.77)	0.015
Neutrophils, (10^9 cells/L), mean (SD)	4.29 (1.80)	4.28 (1.80)	4.52 (1.78)	<0.001
Lymphocyte, (10^9 cells/L), mean (SD)	2.19 (2.31)	2.19 (2.34)	2.11 (0.79)	0.007
WBC, (10^9 cells/L), mean (SD)	7.28 (3.28)	7.27 (3.30)	7.49 (2.28)	0.006
Monocyte, (10^9 cells/L), mean (SD)	0.55 (0.20)	0.55 (0.20)	0.59 (0.23)	<0.001
Glycohemoglobin, (%), mean (SD)	5.74 (1.09)	5.72 (1.08)	6.19 (1.37)	<0.001
SII, mean (SD)	537.93 (368.68)	536.25 (366.50)	588.76 (426.56)	<0.001

Continuous and categorical variables are expressed as mean (SD) and *n* (%), respectively. Mean (SD) and *n* (%) have been weighted. BMI, body mass index; PIR, income to poverty ratio; CHD, coronary atherosclerotic heart disease; WBC, white blood cell; SII, systemic immune inflammation index.

the SII was higher than 464.47, the risk of stroke increased significantly. Multiple imputation sensitivity analysis confirmed the association between SII and stroke.

Many epidemiologic studies have shown that the inflammatory response is associated with the stroke. Peripheral blood monocyte to lymphocyte ratio (MLR), neutrophil-to-lymphocyte ratio (NLR),

TABLE 2 Weighted univariate logistic analysis of stroke.

Characteristic	OR 95% CI	*p*-value
Age		
<60	ref	ref
≥60	5.306 (4.486, 6.267)	<0.001
Sex		
Male	ref	ref
Female	1.206 (1.011, 1.439)	0.037
Race		
Mexican American	ref	ref
Other Hispanic	0.904 (0.616, 1.326)	0.603
Non-Hispanic White	1.573 (1.232, 2.008)	<0.001
Non-Hispanic Black	2.553 (1.232, 2.008)	<0.001
Other race	1.799 (1.172, 2.762)	0.007
BMI, (kg/m^2)		
<25	ref	ref
25–30	1.130 (0.879, 1.425)	0.334
≥30	1.704 (1.332, 2.182)	<0.001
PIR		
<1.3	ref	ref
1.3–3.5	0.690 (0.557, 0.856)	<0.001
>3.5	0.301 (0.236, 0.386)	<0.001
Smoke		
No	ref	ref
Yes	2.105 (1.785, 2.482)	<0.001
Alcohol use		
No	ref	ref
Yes	1.206 (1.109, 1.149)	0.008
Hypertension		
No	ref	ref
Yes	6.383 (5.282, 7.714)	<0.001
Diabetes		
No	ref	ref
Yes	4.983 (4.088, 6.073)	<0.001

platelet-to-lymphocyte ratio (PLR), and SII, as emerging biomarkers of inflammation, have been associated with prognosis of stroke patients (18, 31–35). In a cross-sectional study involving the participation of 4,854 patients at high risk for acute nondisabling cerebrovascular events, patients with minor strokes were divided into 4 groups based on quartiles of neutrophil count or neutrophil ratio, 495 of whom had a recurrent stroke after 90 days of follow-up. The study demonstrated that high levels of neutrophil count and neutrophil ratio were associated with an increased risk of new stroke, composite events, and ischemic stroke in patients with minor ischemic stroke (31). Additionally, in a study involving 796 patients with acute ischemic stroke who underwent endovascular thrombectomy, higher NLR and PLR were significantly associated with adverse outcomes (33). Ordinarily, an upsurge in platelet and neutrophil counts correlates with heightened inflammatory processes, whereas a decline in lymphocyte levels can be indicative of immune suppression or exhaustion. As an inflammatory marker containing three cell component counts, a significant elevated SII value may signal a profound inflammatory reaction or a state of immune dysregulation (23). This versatile biomarker has found broad applications across various domains of clinical research. It plays a crucial role in estimating the prognosis of diseases, evaluating disease activity levels, and tracking therapeutic efficacy. Hu et al. (35) studied the relationship between in-hospital mortality and SII in 463 stroke patients. The results showed that in-hospital mortality was positively correlated with SII, but not linearly correlated. High SII was associated with poor prognosis in acute ischemic stroke (AIS) patients.

In ischemic stroke, damaged brain cells produce large amounts of inflammatory cytokines, chemokines, reactive oxygen species (ROS), and other neurotoxic substances, which mediate blood-brain barrier disruption and inflammatory cascade reactions, while directing immune inflammatory cells into brain tissue, further mediating secondary neuronal damage and aggravating neurological dysfunction (36). Neutrophils are the initial cells to infiltrate the ischemic brain tissue. Upon arrival at the ischemic site, they release pro-inflammatory mediators, proteases, ROS, and extracellular matrix metalloproteinase (MMP), leading to secondary damage in the ischemic brain cell (37). Monocytes are capable of secreting MMP-9, an enzyme that is involved in extracellular matrix (ECM) remodeling *in vivo* and is important for tissue repair, inflammatory response, angiogenesis, and disease progression (38). MMP-9 can infiltrate into infarcted foci and exacerbate brain damage (38). Increased monocyte count has been

TABLE 3 Weighted multivariate logistic analysis log-formed SII and stroke.

	Crude model		Model 1		Model 2	
	OR 95% CI	*p*-value		*p*-value		*p*-value
Log-formed SII	1.985 (1.245, 3.166)	0.004	1.728 (1.118, 2.672)	0.014	1.562 (1.020, 2.394)	0.040
Stratified by log-formed SII quartiles						
Q1	ref		ref			
Q2	1.116 (0.845, 1.472)	0.433	1.095 (0.829, 1.447)	0.515	1.189 (0.893, 1.583)	0.231
Q3	1.053 (0.763, 1.451)	0.750	1.021 (0.734, 1.419)	0.898	1.064 (0.767, 1.474)	0.706
Q4	1.481 (1.121, 1.958)	0.006	1.394 (1.057, 1.838)	0.018	1.348 (1.010, 1.800)	0.042
p for trend		0.011		0.033		0.080

TABLE 4 Subgroup analysis for the association between SII and stroke.

Characteristic	OR 95% CI	*p*-value	*p* for interaction
Age			0.197
<60	2.605 (1.296, 5.234)	<0.001	
≥60	1.523 (0.917, 2.530)	0.103	
Sex			0.699
Male	2.111 (1.141, 3.906)	0.017	
Female	1.775 (0.977, 3.223)	0.059	
BMI, (kg/m^2)			0.260
<25	2.434 (1.502, 5,580)	0.003	
25–30	1.543 (0.559, 4.260)	0.401	
≥30	1.463 (0.858, 2.495)	0.162	
PIR			0.056
<1.3	3.121 (1.885, 5.165)	<0.001	
1.3–3.5	2.348 (1.181, 4.669)	0.014	
>3.5	0.603 (0.226, 1.607)	0.312	
Smoke			0.968
No	1.791 (0.905, 3.542)	0.093	
Yes	1.840 (1.073, 3.155)	0.026	
Alcohol use			0.916
No	2.057 (0.961, 4.404)	0.063	
Yes	1.956 (1.175, 3.256)	0.009	
Hypertension			0.219
No	1.019 (0.476, 2.179)	0.960	
Yes	1.911 (1.178, 3.101)	0.008	
Diabetes			0.218
No	2.063 (1.168, 3.645)	0.012	
Yes	1.219 (0.684, 2.172)	0.501	

shown to be an independent predictor of poor stroke prognosis (39). Platelets interact directly with circulating leukocytes to form platelet-leukocyte aggregates through the alteration of P-selectin and CD40 expression on the cell surface (40). The release of fibrinogen, fibronectin, platelet factor-4, and other mediators from α-granules in platelets contributes to platelet adhesion, aggregation, and the coagulation process, potentially exacerbating thrombosis (41). Conversely, lymphocytes are believed to play a crucial role in inflammation-induced neuroprotection and serve as the primary immunomodulators for brain protection following ischemic stroke (42). The role of lymphocytes in AIS varies depending on the subtype. $CD4^+$ and $CD8^+$ T cells can exacerbate inflammatory reactions and contribute to neuronal death by producing inflammatory factors like interferon γ and interleukin-17 (IL-17) (43). On the other hand, regulatory T cells release IL-10 through various signaling pathways, such as signal transduction, phosphatidylinositol and transcription activator pathways, phosphatidylinositol-3-kinase pathway, and mitogen-activated protein kinase pathway, which have neuroprotective effects (44).

In the context of secondary brain injury following hemorrhagic stroke (45), immune inflammation can exacerbate cerebral edema, enlarge hematoma, raise intracranial pressure, and progressively deteriorate neurological function (45). This response involves the release of various inflammatory factors, including IL-6, IL-1β, and C-reactive protein (CRP) among others (46). These inflammatory factors can attract immune cells such as neutrophils, monocytes, and macrophages to accumulate in the affected area, intensifying local inflammation and tissue damage (47). Additionally, excessive oxidative stress and excitotoxicity can lead to nerve cell death and worsen brain injury (48). Luo et al. (49) conducted a study using the SII to forecast the prognosis of subarachnoid hemorrhage (SAH). The area under the curve (AUC) of SII in predicting poor prognosis was 0.692, indicating that SII could serve as a novel independent prognostic indicator for SAH patients in the initial stages of the condition.

In the clinical management of stroke patients, early detection and risk stratification can be conducted based on the SII level, allowing for personalized management and treatment according to the inflammatory response severity. Monitoring SII levels can also help evaluate intervention effectiveness and detect potential recurrence or exacerbation early during long-term follow-up, enabling timely adjustments in treatment options. The correlation between SII and stroke has significant implications for clinical guidance in screening, etiology research, treatment, prognosis evaluation, and also provides more targeted clinical treatment.

This study, based on the nationally representative NHANES database, analyzed 28,266 participants from the United States to determine that SII levels are positively associated with stroke risk. The use of a weighted logistic regression model in the analysis, adjusting for covariates, enhanced the accuracy and reliability of the conclusions. Additionally, the large sample size and subgroup analysis contributed to the reliability and representativeness of study. The study highlights the potential value of measuring systemic inflammatory biomarkers in identifying individuals at risk for stroke, offering new options for stroke diagnosis and treatment. However, this study has several limitations. First, the cross-sectional design employed made it challenging to establish a causal relationship between exposure factors and outcome variables. Future research should prioritize prospective studies to elucidate the causal link between SII and stroke to a deeper understanding of the association. Second, although we adjusted for covariates, residual confounding factors cannot be ruled out. Notable examples include hypercholesterolemia, physical activity status, and family history of stroke. Third, due to limitations in the database, we were unable to categorize the survey questions regarding alcohol consumption. Fourth, Q2 and Q3 are not related in all models. In the fully adjusted model, SII was associated with stroke in its highest quartile. It can be seen that SII can be used as a predictor of stroke only when SII is at the highest level. Since some typical inflammatory factors (such as TNF-α, IL-6, IL-10, etc.) are not recorded in NHANES, relevant indicators cannot be included to obtain more comprehensive results. Lastly, understanding the association between SII and various stroke subtypes (e.g., large-artery atherosclerotic stroke, cardiogenic stroke) is also constrained by limitations within the NHANES database.

5 Conclusion

This cross-sectional study based on the NHANES database demonstrated that SII is associated with stroke risk. Given the

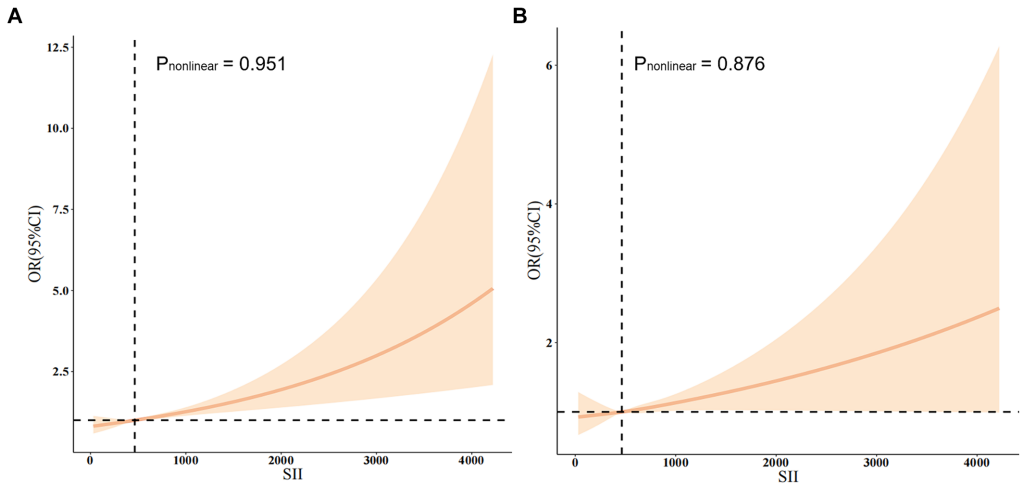

FIGURE 2
(A) The non-adjusted relationship between SII and stroke. **(B)** The full-adjusted relationship between SII and stroke.

inherent limitations of cross-sectional studies, further research is necessary to validate the causality of this association and to demystify the underlying mechanisms between inflammation and stroke.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/ index.htm.

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

HX: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. YZ: Writing – review & editing, Funding acquisition, Conceptualization. XZ: Writing – review & editing, Methodology, Investigation. YL: Writing – review & editing, Funding acquisition, Conceptualization.

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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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EDITED BY
Gerrit M. Grosse,
University Hospital of Basel, Switzerland

REVIEWED BY
Dong-Hua Yang,
New York College of Traditional Chinese Medicine, United States
Shenfeng Qiu,
University of Arizona, United States

*CORRESPONDENCE
Fenfei Gao
✉ ffgao@stu.edu.cn
Wenzhen He
✉ wenzhen_he@sina.com

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Genetic causality of lipidomic and immune cell profiles in ischemic stroke

Haohao Chen[1,2], Zequn Zheng[3,4], Xiaorui Cai[5], Shunxian Li[6], Manli Chen[6], Jiaming Wu[3], Wenzhen He[6]* and Fenfei Gao[2]*

[1]Department of Pharmacy, The First Affiliated Hospital of Shantou University Medical College, Shantou, China, [2]Department of Pharmacy, Shantou University Medical College, Shantou, China, [3]Department of Cardiology, First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, China, [4]Clinical Research Center, First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, China, [5]The Affiliated Cancer Hospital of Shantou University Medical College, Shantou, China, [6]Department of Neurology, The First Affiliated Hospital of Shantou University Medical College, Shantou, China

Background: Ischemic stroke (IS) is a global health issue linked to lipid metabolism and immune cell responses. This study uses Mendelian randomization (MR) to identify genetic risk factors for IS subtypes using comprehensive genetic data from lipidomic and immune cell profiles.

Methods: We assessed genetic susceptibility to IS across 179 lipids and 731 immune cell phenotypes using instrumental variables (IVs) from recent genome-wide association studies. A two-sample MR approach evaluated correlations, and a two-step MR mediation analysis explored the role of immune cell phenotypes in the lipid-IS pathway. Sensitivity analyses, including MR-Egger and Cochran *Q* tests, ensured robust results.

Results: Genetic IVs for 162 lipids and 614 immune cell phenotypes were identified. Significant genetic causality was found between 35 lipids and large artery stroke (LAS), with 12 as risk factors (sterol esters, phosphatidylcholines, phosphatidylethanolamines) and 23 as protective factors (phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols). For small vessel stroke (SVS), 8 as risk factors (sterol esters, phosphatidylcholines), and 2 as protective factors (phosphatidylinositol, sphingomyelin). For cardioembolic stroke (CS), 2 as risk factors, and 4 as protective factors. Mediation analysis revealed that CCR2 on granulocytes, CD11c on $CD62L^{+}$ myeloid dendritic cells, and FSC-A on granulocytes mediated the lipid-immune cell-LAS pathway, while CD4 on activated CD4 regulatory T cells and CD4 on activated & secreting CD4 regulatory T cells mediated the lipid-immune cell-SVS pathway.

Conclusion: This study identifies genetic links between specific lipids and IS subtypes, highlights immune cells' role in IS risk and mediation, suggests new therapeutic targets, and uncovers IS genetic drivers.

KEYWORDS

ischemic stroke, large artery stroke, small vessel stroke, cardioembolic stroke, lipidomic, immune cell phenotypes, Mendelian randomization, genetic causality

1 Introduction

Ischemic stroke (IS) is a leading cause of morbidity and mortality worldwide, presenting a significant public health challenge. The pathogenesis of IS is complex and multifactorial, involving genetic, environmental, and metabolic factors (1–3). Among these, lipid metabolism

and immune cell responses are crucial contributors to the onset and progression of IS (4–6).

Dyslipidemia, characterized by abnormal lipid levels in the blood, is a well-recognized modifiable risk factor for IS. Traditional lipid markers such as total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) are commonly used to assess stroke risk (7, 8). However, advances in mass spectrometry have facilitated the development of lipidomics, allowing for the simultaneous detection of multiple lipids. This technological progress has significantly enhanced our understanding of the role of lipid metabolism in disease processes, including IS (9–11).

Additionally, immune cells are important for the inflammatory response associated with IS. Immune cell phenotypes, including granulocytes, dendritic, and T cells, participate in the inflammatory cascade that exacerbates brain damage following ischemic events (4, 12, 13). The interplay between lipid metabolism and immune cell function is a burgeoning area of research with important implications for identifying novel therapeutic targets and improving stroke prognosis.

We utilized Mendelian randomization (MR) techniques to investigate the genetic basis of lipid metabolism and immune cell responses in the context of IS. By using genetic variants as instrumental variables (IVs), MR provides a robust method to infer causality and mitigate confounding inherent in observational studies (14, 15). We performed a two-sample MR analysis of 179 lipidomic traits and three IS subtypes—large artery stroke (LAS), small vessel stroke (SVS), and cardioembolic stroke (CS)—using genetic data from genome-wide association studies (GWAS) (16, 17). Additionally, we conducted a two-step MR (TSMR) mediation analysis to examine whether immune cell phenotypes mediate the causal relationship between lipid profiles and IS risk (18, 19).

This study aims to identify specific lipid species and immune cell phenotypes that contribute to IS risk, elucidate potential causal pathways, and highlight novel therapeutic targets for the prevention and management of IS. By integrating comprehensive genetic data with advanced MR methodologies, we seek to deepen our understanding of the complex interactions between lipid metabolism, immune responses and IS.

2 Materials and methods

2.1 Study overview

We performed a two-sample MR analysis utilizing genetic variants derived from the latest available GWAS of 179 plasma lipidomic and three subtypes of IS: LAS, SVS, and CS. We used inverse-variance weighted (IVW) and weighted median (WM) methods for the MR analyses, complemented by various sensitivity tests to ensure result robustness. Given the close relationship between immune cells and IS, we applied TSMR to determine if the identified effects were mediated through immune cell regulation. The first two-sample MR analysis examined 731 immune cell phenotypes as exposures and the three IS subtypes as outcomes. This was followed by a final two-sample MR analysis, where plasma lipids showing significant causality were used as exposures, and immune cell phenotypes with significant MR results in the GWAS were used as outcomes. The effectiveness of this MR approach depends on three key assumptions (1): genetic variants must strongly correlate with the exposure (2), variants influence the outcome only through the exposure, and (3) variants are free from confounding variables. The methodological workflow is depicted in Figure 1.

2.2 GWAS summary statistics

We accessed stroke-related data from the GIGASTROKE Consortium,[1] which includes three IS subtypes: LAS with 9,219 cases and 1,496,931 controls, SVS with 13,620 cases and 1,496,931 controls, and CS with 12,828 cases and 1,496,931 controls (17). Additionally, we utilized GWAS data for 731 immune cell phenotypes, cataloged from GCST90001391 to GCST90002121 (18), and for 179 lipid traits, spanning from GCST90277238 to GCST90277416 (see text footnote 1) (16). Each dataset adheres to the ethical standards of the original studies, ensuring that no additional ethical approval was required for this secondary analysis. These datasets are detailed in Supplementary Table S1.

2.3 Selection of instrumental variables

To ascertain causal connections between lipidomic and immune cell profiles (exposure) and IS subtype outcomes, we employed genetic proxies, specifically SNPs, associated with these phenotypes. We selected SNPs associated with lipidomic and immune cell phenotypes using thresholds ($p < 5 \times 10^{-8}$) and applied clumping criteria with an LD $r^2 > 0.001$ within a 10,000-kilobase window, based on the 1,000 Genomes European panel. To assess instrument strength and avoid weak instrument bias, we calculated the *F* statistic for each SNP, ensuring it was above 10, following the method outlined by Pierce and Burgess (20). Only SNPs exclusively related to the lipidomic and immune cell traits were included, ensuring no overlap with genes influencing ischemic stroke risk to adhere to the exclusion restriction criterion.

2.4 Statistical analysis

We initiated our investigation by conducting a two-sample MR analysis to investigate the causal relationship between lipidomic phenotypes and IS. The IVW and WM methods were employed for primary effect estimation. Analyses were conducted using a *p*-value threshold <0.05 to ascertain statistical significance, avoiding Bonferroni adjustments to preserve exploratory study objectives.

We utilized summary statistics of immune cell phenotypes, covering 731 immune cell levels in the blood, to explore potential immune cell levels as mediators between lipidomic profiles and ischemic stroke. We employed TSMR approach to delineate effects of lipidomic phenotypes and immune cell levels on IS subtypes. In addition to the estimation of the potential impact of lipidomic phenotypes on ischemic stroke derived from MR analyses (β_0), two additional estimates were calculated (1): the causal effect of 731 immune cell levels on ischemic stroke (β_1), and (2) the causal effect of exposure (lipid species significantly associated with IS

1 https://www.ebi.ac.uk/gwas/

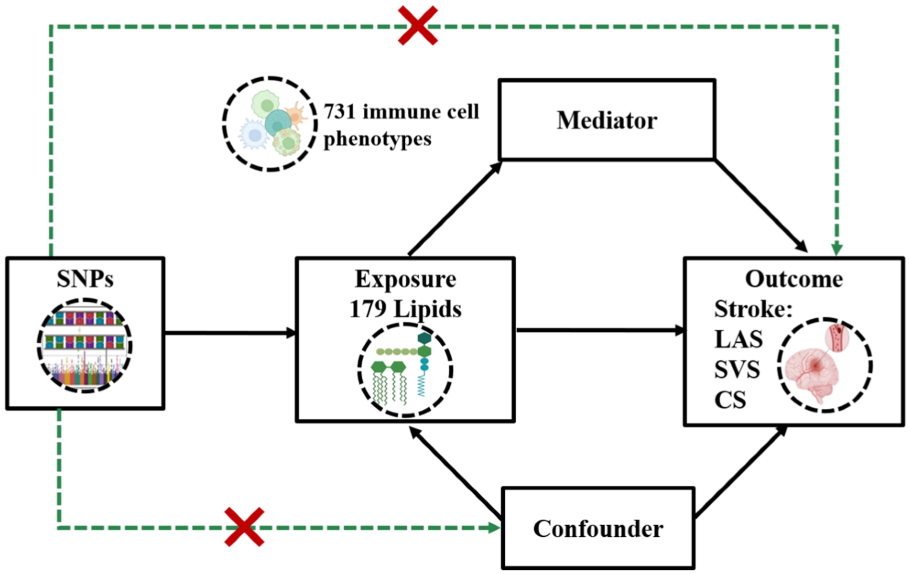

FIGURE 1
Assumptions and design of a two-step Mendelian randomization (TSMR) analyses. Firstly, a two-sample MR was performed to investigate the causal relationships between 179 lipid phenotypes and three distinct ischemic stroke subtypes. Secondly, 731 immune cell phenotypes were selected for subsequent mediation analyses. Finally, TSMR analysis was conducted to detect potential mediating immune cell phenotypes. TSMR, two-step Mendelian randomization; SNPs, single-nucleotide polymorphisms; LAS, large artery stroke; SVS, small vessel stroke; SC, cardioembolic stroke.

subtypes) on the mediator (immune cell level species significantly associated with IS subtypes) (β_2). Indirect effect, representing the causal effect of lipidomic profiles on IS subtypes via mediators, was estimated using the coefficient product method ($\beta_1 \times \beta_2$). Mediation ratio was calculated as the "indirect effect/total effect" ($[\beta_1 \times \beta_2]/\beta_0$) (19).

2.5 Sensitivity analyses

For sensitivity analyses, we employed three MR methods: IVW, WM, and MR-Egger, each based on different assumptions about pleiotropy to generate effect estimates. Evidence of horizontal pleiotropy was suggested if the MR-Egger intercept significantly differed from zero (p-value <0.05). Heterogeneity was evaluated using the Cochran Q test, where a p-value greater than 0.05 indicated an absence of heterogeneity (21, 22). To corroborate the conclusions on causality, we ensured that: (a) the MR-Egger intercept did not show significant directional pleiotropy, and (b) Cochran's Q test indicated no significant heterogeneity.

MR analyses were conducted in R (version 4.3.3; R Foundation for Statistical Computing, Vienna, Austria) with the "TwoSampleMR" packages.

3 Results

3.1 Causal associations between lipidomic profiles and IS subtypes

Our investigation probed the causal relationships between lipidomic profiles and IS subtypes, specifically focusing on three subtypes: LAS, SVS, and CS. We commenced by identifying IVs for 179 lipid species, ensuring each met the criteria for strong correlation and independence. IVs were successfully established for 162 lipid species, with F-statistic values ranging from 29.79 to 1946.15, effectively negating concerns of weak instrumental bias (Supplementary Table S2).

If the number of SNPs is greater than or equal to 3, we evaluated the data using both the IVW and WM methods; otherwise, only IVW was used. Our findings indicated that 35 lipid species are genetically causally associated with LAS; of these, 12 were identified as risk factors including 5 sterol esters, 6 phosphatidylcholines, and 1 phosphatidylethanolamine, and 23 as protective factors, comprising 16 phosphatidylcholines, 2 phosphatidylethanolamines, and 5 phosphatidylinositols (Figure 2A and Supplementary Table S3). Furthermore, 10 lipid species were associated with SVS, with 8 identified as risk factors (2 sterol esters and 6 phosphatidylcholines) and 2 as protective (1 phosphatidylinositol and 1 sphingomyelin) (Figure 2B and Supplementary Table S13). Additionally, 6 lipids demonstrated a causal association with CS, among which 2 sphingomyelins were risk factors, and 4 were protective including 1 sterol ester, 1 phosphatidylcholine, and 2 phosphatidylinositols (Figure 2C and Supplementary Table S22).

To assess and mitigate potential biases from directional horizontal pleiotropy in the MR results, Egger's intercept test was conducted for phenotypes supported by more than three IVs. The p-values of the MR-Egger intercept estimates consistently exceeded 0.05, indicating no significant pleiotropy bias. Moreover, the Cochran Q test, indicating a p-value greater than 0.05 across all analyses, affirmed the absence of heterogeneity, thus substantiating the robustness of our causal inferences (Table 1; Supplementary Tables S4, S5, S14, S15, S23, S24).

3.2 Causal associations between immune cell phenotypes and IS subtypes

Recognizing the significant involvement of immune cells in the pathogenesis of IS subtypes, our study sought to investigate the

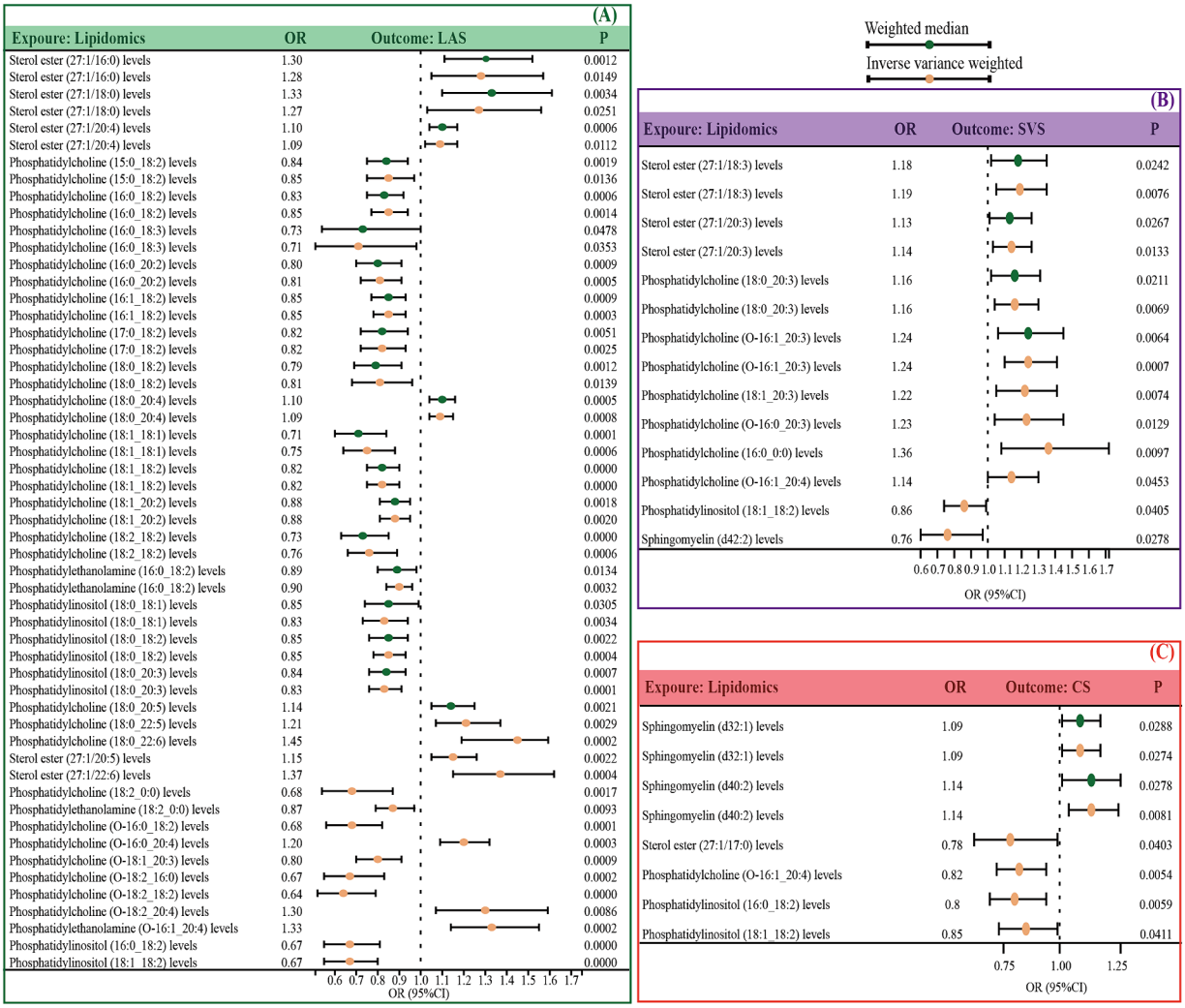

FIGURE 2
Significant MR estimates for specific lipids and IS subtypes (LAS, SVS, and CS) were assessed by IVW and WM. **(A)** The significant causal effect of lipids on LAS. **(B)** The significant causal effect of lipids on SVS. **(C)** The significant causal effect of lipids on CS. The dots colored in red and green indicate IVW and WM respectively. IS, ischemic stroke, IVW, inverse variance-weighted; MW, weighted median; MR, Mendelian randomization; OR, odds ratio; 95% CI, 95% confidence interval.

causal association between 731 immune cell phenotypes and IS subtypes. Employing criteria akin to those used for lipid species, we meticulously identified instrumental variables for these immune cell phenotypes. Out of 731 phenotypes, we successfully identified 614 species with eligible IVs, each characterized by *F*-statistic values surpassing 10, ranging from 29.85 to 5062.70 (Supplementary Table S6).

MR results unveiled genetic causal associations between 12 immune cell phenotypes and LAS, with 8 showing positive correlations and 4 showing negative correlations (Figure 3A and Supplementary Table S7). Similarly, 21 immune cell phenotypes were linked to SVS, with 4 exhibiting positive correlations and 17 displaying negative correlations (Figure 3B and Supplementary Table S16). Furthermore, 16 immune cell phenotypes demonstrated genetic causal associations with CS, with 4 manifesting positive correlations and 12 exhibiting negative correlations (Figure 3C and Supplementary Table S25).

Consistently, the *p*-values of MR-Egger intercept estimates exceeded 0.05, indicating the absence of significant pleiotropy bias. Additionally, the Cochran *Q* test yielded *p*-values greater than 0.05 across all analyses, reinforcing the lack of heterogeneity and thereby affirming the robustness of our causal inferences (see Table 1; Supplementary Tables S8, S9, S17, S18, S26, S27).

3.3 Immune cell-mediated pathways linking lipidomic profiles to IS subtypes

To explore the potential role of immune cell phenotypes as mediators in the causal pathway between lipidomic profiles and ischemic stroke subtypes, we employed TSMR approach. Specifically, we focused on lipids associated with LAS, SVS, and CS, and assessed their MR-estimated effects against immune cell phenotypes robustly associated with each subtype.

For LAS, our analysis revealed inverse genetic correlations between three lipid phenotypes (sterol ester (27:1/22:6) levels, phosphatidylcholine (O-16:0_20:4) levels, and phosphatidylcholine (18:2_0:0) levels) and CD11c on $CD62L^{+}$ myeloid dendritic cells, as

TABLE 1 Pleiotropy and heterogeneity assessment for significant results ($p < 0.05$).

Trait.exposure	Trait.outcome	Directional pleiotropy		Cochran's *Q* test	
		Egger_intercept	*p*-value	*Q*	*p*-value
Sterol ester (27:1/16:0) levels	LAS	−0.016	0.892	3.578	0.167
Sterol ester (27:1/18:0) levels		0.007	0.889	14.309	0.056
Sterol ester (27:1/20:4) levels		−0.039	0.118	6.105	0.191
Phosphatidylcholine (15:0_18:2) levels		0.014	0.634	9.290	0.098
Phosphatidylcholine (16:0_18:2) levels		0.026	0.253	8.276	0.219
Phosphatidylcholine (16:0_18:3) levels		−0.165	0.870	4.396	0.111
Phosphatidylcholine (16:0_20:2) levels		0.025	0.341	2.585	0.630
Phosphatidylcholine (16:1_18:2) levels		0.016	0.362	2.662	0.850
Phosphatidylcholine (17:0_18:2) levels		0.035	0.566	0.719	0.698
Phosphatidylcholine (18:0_18:2) levels		0.013	0.830	7.725	0.102
Phosphatidylcholine (18:0_20:4) levels		−0.009	0.703	2.936	0.402
Phosphatidylcholine (18:1_18:1) levels		0.031	0.571	4.183	0.242
Phosphatidylcholine (18:1_18:2) levels		−0.007	0.717	3.478	0.481
Phosphatidylcholine (18:1_20:2) levels		0.009	0.834	1.592	0.451
Phosphatidylcholine (18:2_18:2) levels		0.035	0.466	2.353	0.308
Phosphatidylethanolamine (16:0_18:2) levels		0.020	0.599	5.468	0.361
Phosphatidylinositol (18:0_18:1) levels		−0.103	0.317	5.696	0.223
Phosphatidylinositol (18:0_18:2) levels		0.028	0.555	2.435	0.487
Phosphatidylinositol (18:0_20:3) levels		−0.013	0.635	1.363	0.714
Sterol ester (27:1/18:3) levels	SVS	0.016	0.669	0.552	0.759
Sterol ester (27:1/20:3) levels		0.011	0.812	0.341	0.843
Phosphatidylcholine (18:0_20:3) levels		−0.014	0.770	0.235	0.889
Phosphatidylcholine (O-16:1_20:3) levels		0.004	0.945	0.099	0.952
Sphingomyelin (d32:1) levels	CS	0.001	0.954	0.993	0.803
Sphingomyelin (d40:2) levels		0.005	0.843	0.167	0.920
Phosphatidylcholine (18:2_0:0) levels	FSC-A on granulocyte	−0.116	0.663	0.404	0.817
Sphingomyelin (d40:2) levels	CD8 on CD39$^+$ CD8$^+$ T cell	0.064	0.215	7.588	0.180
CD14-CD16-absolute count	LAS	−0.094	0.449	1.675	0.433
CD3 on naive CD8$^+$ T cell		−0.006	0.816	0.273	0.965
CD3 on CD45RA$^+$ CD4$^+$ T cell		−0.005	0.855	0.414	0.937
CD40 on CD14$^+$ CD16-monocyte		−0.007	0.872	1.215	0.545
CD39 on granulocyte		−0.014	0.948	1.912	0.384
SSC-A on granulocyte		−0.018	0.659	2.121	0.548
CD62L-monocyte %monocyte	SVS	−0.036	0.837	2.192	0.334
BAFF-R on IgD$^+$ CD38-naive B cell		0.008	0.696	0.456	0.928
BAFF-R on IgD$^+$ CD38-unswitched memory B cell		−0.010	0.813	1.424	0.491
BAFF-R on IgD$^+$ CD38$^+$ B cell		0.001	0.927	0.574	0.966
BAFF-R on IgD$^+$ CD38dim B cell		0.008	0.675	0.405	0.939
BAFF-R on IgD-CD24-B cell		−0.011	0.530	2.315	0.510
BAFF-R on IgD-CD27-B cell		−0.011	0.533	2.258	0.521
BAFF-R on transitional B cell		0.009	0.674	0.417	0.937
BAFF-R on B cell		0.007	0.666	0.404	0.982
CD33 on granulocytic myeloid-derived suppressor cells		0.005	0.831	0.684	0.710
HLA DR on B cell		0.010	0.724	0.494	0.781

(Continued)

TABLE 1 (Continued)

Trait.exposure	Trait.outcome	Directional pleiotropy		Cochran's *Q* test	
		Egger_intercept	*p*-value	*Q*	*p*-value
$CD28^+$ $CD45RA^+$ $CD8^+$ T cell absolute count	CS	−0.086	0.416	1.954	0.376
$CD45RA^+$ CD28-$CD8^+$ T cell absolute count		0.005	0.636	39.417	0.541
BAFF-R on IgD^+ CD24-B cell		−0.002	0.903	1.976	0.853
BAFF-R on IgD^+ CD38-B cell		−0.010	0.624	1.834	0.766
BAFF-R on IgD^+ CD38-naive B cell		−0.004	0.851	1.968	0.742
BAFF-R on IgD^+ $CD38^+$ B cell		0.012	0.422	4.228	0.517
BAFF-R on IgD^+ CD38dim B cell		−0.002	0.911	2.064	0.840
BAFF-R on naive-mature B cell		−0.003	0.856	2.062	0.840
BAFF-R on transitional B cell		−0.007	0.741	1.727	0.786
CD25 on naive-mature B cell		−0.001	0.974	0.043	0.998

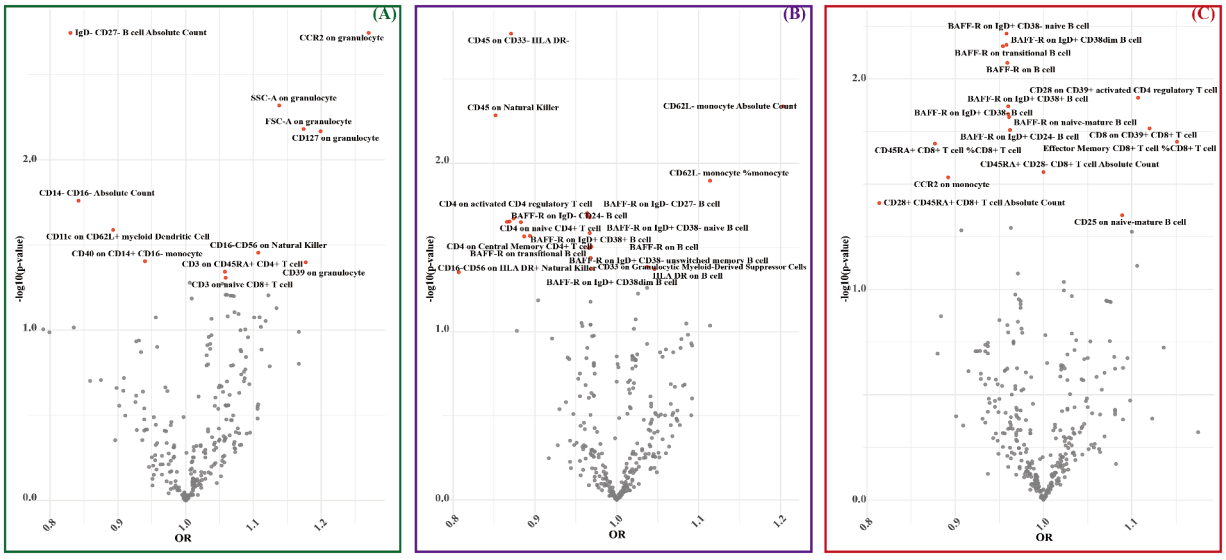

FIGURE 3
MR estimates for immune cell phenotypes and IS subtypes (LAS, SVS, and CS) were assessed by IVW. **(A)** The significant causal effect of immune cell phenotypes on LAS. **(B)** The significant causal effect of immune cell phenotypes on SVS. **(C)** The significant causal effect of immune cell phenotypes on CS. The dots colored in red indicate significant estimates by IVW ($p < 0.05$).

well as FSC-A on granulocytes (β_{IVW}: −0.406 to −0.121). Additionally, phosphatidylcholine (O-18:2_20:4) levels were positively correlated with CCR2 on granulocytes (β_{IVW}: 0.300) (Figure 4 and Supplementary Table S10). Regarding SVS, phosphatidylinositol (18:1_18:2) levels exhibited positive correlations with CD4 on activated CD4 regulatory T cells and CD4 on activated & secreting CD4 regulatory T cells, with β_{IVW} values of 0.408 and 0.383, respectively (Figure 5 and Supplementary Table S19). In the case of CS, sphingomyelin (d40:2) levels were inversely correlated with CD8 on $CD39^+$ $CD8^+$ T cells (β_{IVW}: −0.209). Furthermore, phosphatidylcholine (O-16:1_20:4) levels, phosphatidylinositol (16:0_18:2) levels, and phosphatidylinositol (18:1_18:2) levels showed positive correlations with $CD45RA^+$ CD28-$CD8^+$ T cell absolute count and CD28 on $CD39^+$ activated CD4 regulatory T cells (β_{IVW}: 0.343 to 37.991) (Figure 6 and Supplementary Table S28). No pleiotropy bias and heterogeneity were found by MR-Egger intercept estimates and Cochran *Q* test (Table 1; Supplementary Tables S11, S12, S20, S21, S29, S30).

A summary of STMR estimates revealed six robust causal pathways linking lipid levels, immune cell phenotypes, and IS subtypes. These pathways exhibited consistent directions of total, direct, and indirect effects. Three pathways involving phosphatidylcholine (O-18:2_20:4), phosphatidylcholine (O-16:0_20:4), and sterol ester (27:1/22:6) levels were positively associated with LAS, mediated by CCR2 on granulocytes and CD11c on $CD62L^+$ myeloid dendritic cells. Specifically, higher levels of phosphatidylcholine (O-18:2_20:4) and CCR2 on granulocytes correlated with increased LAS risk. Similarly, elevated levels of phosphatidylcholine (O-16:0_20:4) and sterol ester (27:1/22:6), along with lower levels of CD11c on $CD62L^+$ myeloid dendritic cells, were associated with increased LAS risk.

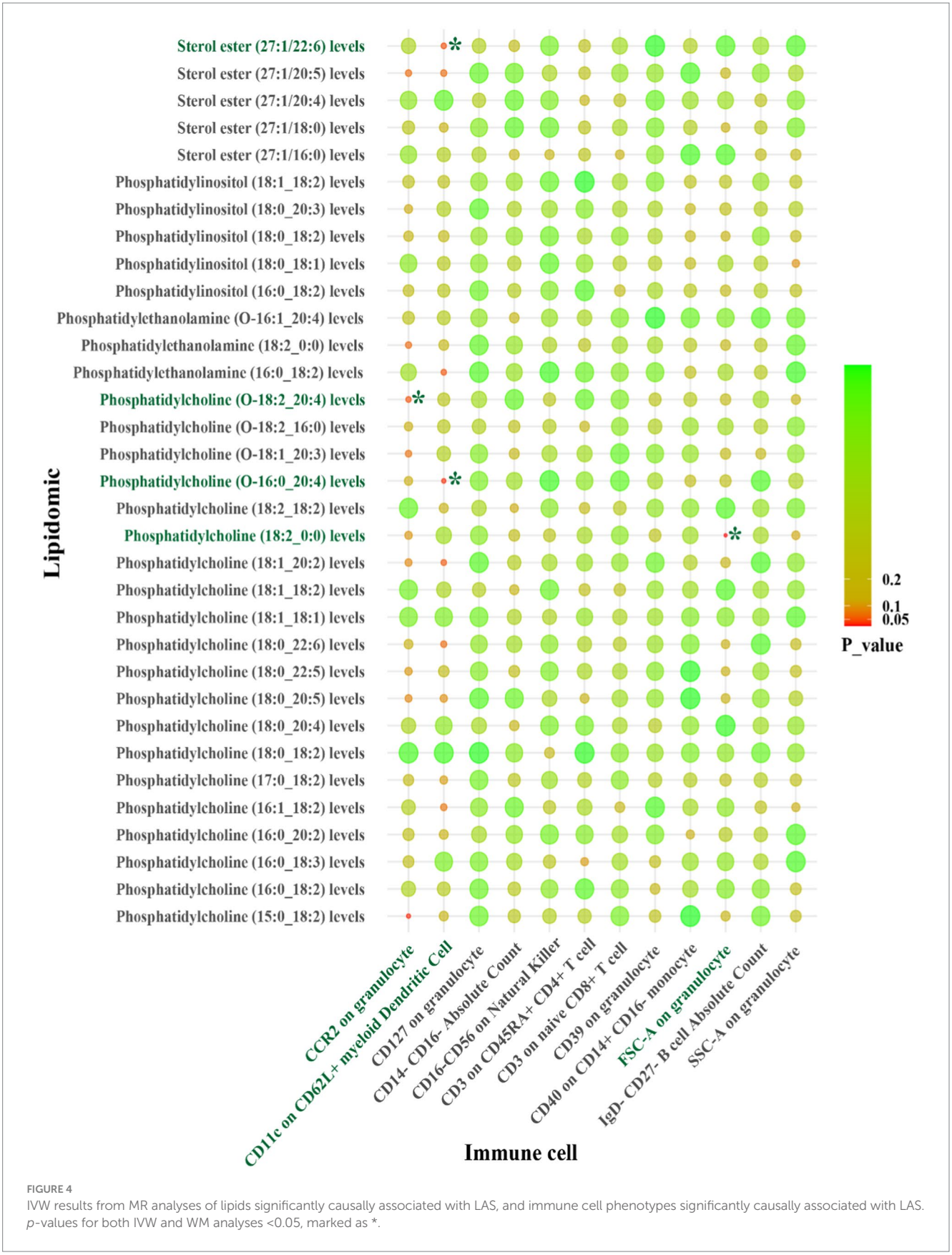

FIGURE 4
IVW results from MR analyses of lipids significantly causally associated with LAS, and immune cell phenotypes significantly causally associated with LAS. *p*-values for both IVW and WM analyses <0.05, marked as *.

Conversely, other pathways involving phosphatidylcholine (18:2_0:0) and phosphatidylinositol (18:1_18:2) levels were inversely related to LAS and SVS, mediated by FSC-A on granulocytes, CD4 on activated CD4 regulatory T cells and CD4 on activated & secreting CD4 regulatory T cells. Higher levels of phosphatidylcholine (18:2_0:0) and lower levels of FSC-A on granulocytes correlated with a decreased risk of LAS. Similarly,

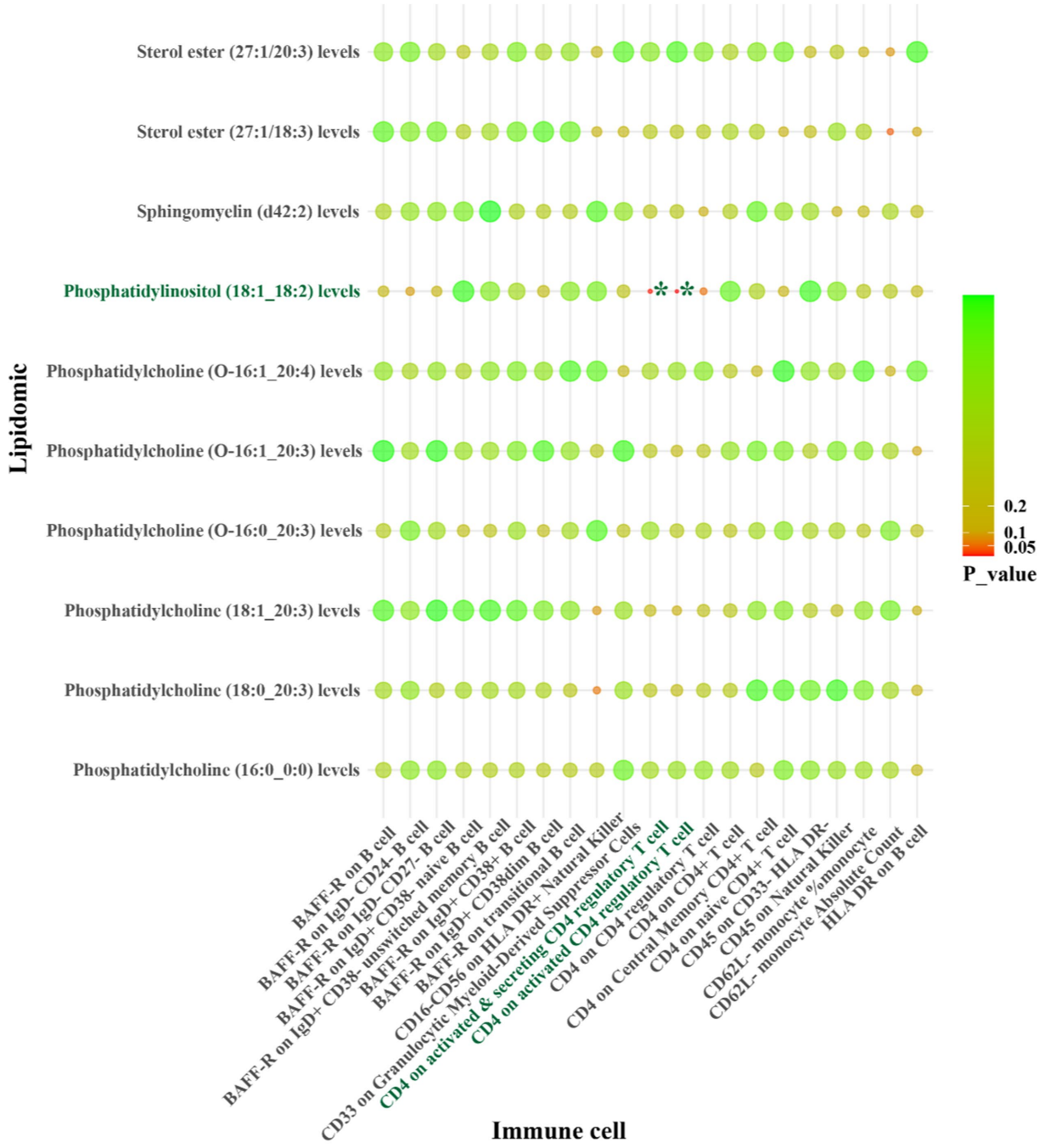

FIGURE 5
IVW results from MR analyses of lipids significantly causally associated with SVS, and immune cell phenotypes significantly causally associated with SVS. *p*-values for both IVW and WM analyses <0.05, marked as *.

higher levels of phosphatidylinositol (18:1_18:2) with lower levels of CD4 on activated CD4 regulatory T cells and CD4 on activated & secreting CD4 regulatory T cells were associated with a decreased risk of SVS. Detailed β values of the MR estimates are provided in Table 2.

In summary, CCR2 on granulocytes, CD11c on CD62L$^+$ myeloid dendritic cells, and FSC-A on granulocytes are identified as potential mediators in the lipid-LAS causal pathways. Levels of CD4 on activated CD4 regulatory T cells and CD4 on activated & secreting CD4 regulatory T cells are identified as potential mediators in the lipid-SVS causal pathways (Figure 7).

4 Discussion

We conducted STMR analysis to explore the causal relationship between lipidomic profiles, immune cell phenotypes, and the risk of three distinct types of IS. Our investigation revealed that phosphatidylcholine (O-18:2_20:4), phosphatidylcholine (O-16:0_20:4), and sterol ester (27:1/22:6) levels are associated with an increased risk of LAS. These associations are mediated through the CCR2 on granulocytes and CD11c on CD62L$^+$ myeloid dendritic cells. Moreover, we identified phosphatidylcholine (18:2_0:0) levels as a protective

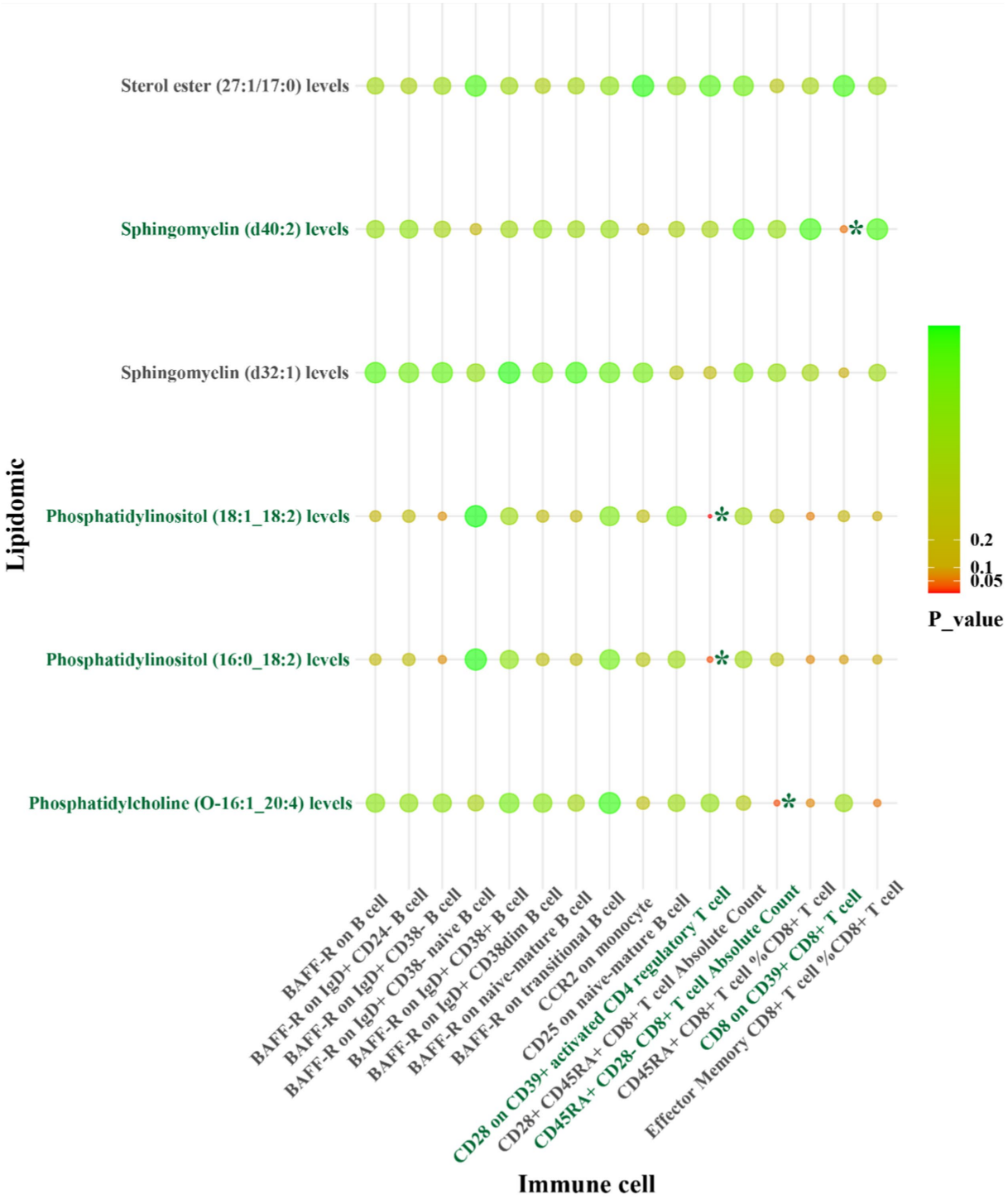

FIGURE 6
IVW results from MR analyses of lipids significantly causally associated with CS, and immune cell phenotypes significantly causally associated with CS. *p*-values for both IVW and WM analyses <0.05, marked as *.

factor against LAS, mediated by FSC-A on granulocytes. Additionally, phosphatidylinositol (18:1_18:2) levels emerged as a protective factor against SVS, mediated by CD4 on activated CD4 regulatory T cells and CD4 on activated & secreting CD4 regulatory T cells. Importantly, our analyses revealed no significant heterogeneity or evidence of horizontal pleiotropy in the data.

First, our findings establish a robust causal relationship between specific lipid species and LAS, SVS, and cardioembolic stroke (CS), with 35 out of 179 lipid species being genetically associated with LAS. Phosphatidylcholines and sterol esters were significant contributors to LAS risk. Similarly, 10 out of 179 lipid species were genetically associated with SVS, primarily phosphatidylinositol

TABLE 2 Two-step Mendelian randomization analyses of the causal effects between lipidomic, immune cell phenotypes, and ischemic stroke of LAS, SVS, and CS.

Exposure	Mediator	Outcome	Total effect (β_0)	Direct effect ($\beta_0 - \beta_1{*}\beta_2$)	Indirect effect ($\beta_1{*}\beta_2$)	Proportion mediated (%)
Phosphatidylcholine (18:2_0:0) levels	FSC-A on granulocyte	LAS	−0.381	−0.316	−0.065	17.05
Phosphatidylcholine (O-18:2_20:4) levels	CCR2 on granulocyte	LAS	0.265	0.193	0.072	27.06
Phosphatidylcholine (O-16:0_20:4) levels	CD11c on CD62L$^+$ myeloid Dendritic Cell	LAS	0.181	0.163	0.018	10.05
Sterol ester (27:1/22:6) levels	CD11c on CD62L$^+$ myeloid Dendritic Cell	LAS	0.312	0.283	0.029	9.34
Phosphatidylinositol (18:1_18:2) levels	CD4 on activated CD4 regulatory T cell	SVS	−0.156	−0.101	−0.055	35.05
Phosphatidylinositol (18:1_18:2) levels	CD4 on activated & secreting CD4 regulatory T cell	SVS	−0.156	−0.101	−0.055	35.35
Phosphatidylcholine (O-16:1_20:4) levels	CD45RA$^+$ CD28-CD8$^+$ T cell absolute count	CS	−0.193	−0.195	0.002	NA
Phosphatidylinositol (16:0_18:2) levels	CD28 on CD39$^+$ activated CD4 regulatory T cell	CS	−0.22	−0.255	0.035	NA
Phosphatidylinositol (18:1_18:2) levels	CD28 on CD39$^+$ activated CD4 regulatory T cell	CS	−0.161	−0.201	0.040	NA
Sphingomyelin (d40:2) levels	CD8 on CD39$^+$ CD8$^+$ T cell	CS	0.132	0.156	−0.024	NA

β, beta; β_0 is the causal effect of exposure on the outcome; β_1 is the causal effect of the mediator on the outcome; β_2 is the causal effect of mediator on outcome; indirect effect ($\beta_1{*}\beta_2$) is the effect of exposure on outcome via corresponding mediator; proportion mediated is calculated as the "indirect effect/total effect"; LAS, large artery stroke; SVS, small vessel stroke; SC, cardioembolic stroke; NA, data not available.

(18:1_18:2). This observation expands our understanding of lipid metabolism's role in IS beyond traditional markers like total cholesterol, LDL-C, and HDL-C, highlighting specific lipid molecules' crucial roles in IS development.

Phosphatidylcholines, phosphatidylinositol, and sterol esters containing different fatty acids have been less emphasized in IS risk assessment models. However, their significant association with LAS, SVS, and CS in our study points towards their potential role in IS pathophysiology. These lipids are critical components of cell membranes and lipoproteins, and alterations in their composition have been linked to changes in lipoprotein functionality and signaling (23–25). Phosphatidylcholine, phosphatidylinositol, and sterol esters are crucial components of membrane lipids, with many essential cellular processes depending heavily on their interactions. The membrane hypothesis suggests that dysfunction in membrane lipids may contribute to the development of diseases such as schizophrenia, Alzheimer's disease, autoimmune disorders, chronic fatigue syndrome, and cancer. The concept that cell membranes contain transient microdomains with distinct lipid compositions has led to the development of selective lipid-targeted therapies, known as membrane lipid therapy (26, 27). Lipid analogs such as perifosine, plasmalogens, and edelfosine have been developed for the treatment of solid tumors, hematological malignancies, and neurodegenerative diseases (28). Recent evidence suggests that unconventional lipids, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and sphingomyelin, are crucial in IS development (29–32). Our genetic informatics-driven identification of specific lipid profiles associated with IS risk may provide more accurate predictions due to their effects on inflammation, endothelial function, and plaque stability.

For the first time, sterol ester and phosphatidylinositol levels were established as significant causal risk factors for LAS and SVS, respectively. The risk of LAS increased by approximately 37% for each unit change in sterol ester (27:1/22:6), while the risk of SVS decreased by approximately 15% for each unit change in phosphatidylinositol (18:1_18:2). These unbiased results strengthen the genetic evidence beyond observational studies, emphasizing the intricate role of lipids in cerebrovascular disease beyond traditional pathways. While elevated LDL-C and HDL-C levels are well-established risk factors for IS, our study suggests that sterol ester (27:1/22:6) and phosphatidylinositol (18:1_18:2)'s specific role in LAS and SVS pathogenesis may involve complex interactions with immune cell pathways. Subclinical inflammation contributes to endothelial dysfunction and the buildup of immune-active cells in the vessel walls. These immune cells and lipids are crucial in forming and growing atherosclerotic lesions, leading to IS (11, 33, 34).

Our investigation into immune cell phenotypes revealed that CCR2 on granulocytes, CD11c on CD62L$^+$ myeloid dendritic cells, and FSC-A on granulocytes are genetically associated with LAS. Additionally, CD4 on activated CD4 regulatory T cells is genetically associated with SVS. Granulocytes, particularly neutrophils, are crucial in the pathophysiology of ischemic stroke, where they release neurotoxic agents such as reactive oxygen species (ROS) and matrix metalloproteinases (MMPs). These agents

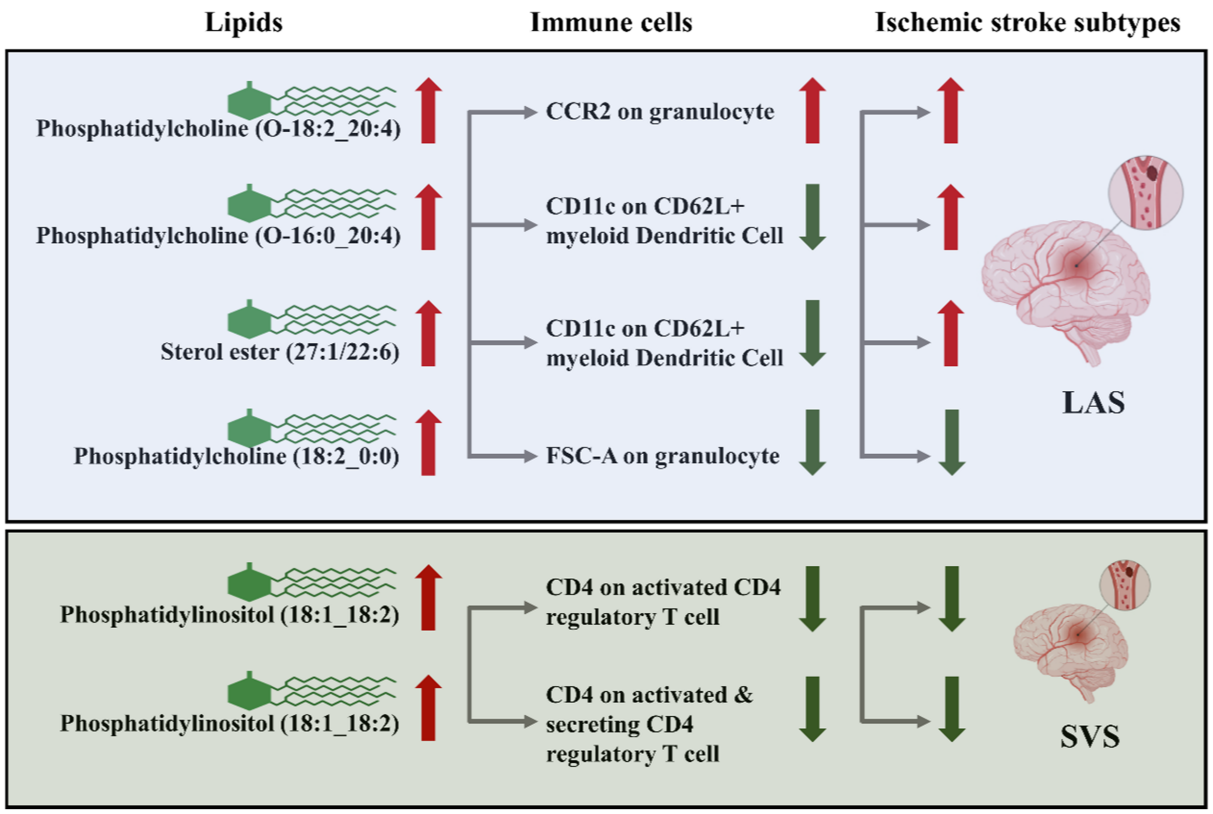

FIGURE 7
CCR2 on granulocytes, CD11c on CD62L^{+} myeloid dendritic cells, FSC-A on granulocytes, CD4 on activated CD4 regulatory T cells, and CD4 on activated & secreting CD4 regulatory T cells as immune cell mediators in the lipid-LAS/SVS causal pathways. The arrows represent the direction of lipids or immune cell levels and the risk effect of IS subtypes. For example, when phosphatidylcholine (O-18:2_20:4) and CCR2 on granulocyte levels are elevated, LAS risk is increased.

contribute to the disruption of the blood–brain barrier, thereby exacerbating tissue damage. Furthermore, neutrophils form neutrophil extracellular traps (NETs), which promote thrombosis and impede thrombolysis, complicating the ischemic injury (35). Dendritic cells are instrumental in antigen presentation within the ischemic brain. By presenting central nervous system (CNS) antigens to T cells, they amplify immune responses and drive inflammation. The interaction between dendritic cells and T cells, mediated by molecules such as MHC class II, is critical in the progression of post-stroke inflammation (36). These findings enrich the current understanding of immune cells' role in IS by identifying specific pathways that may contribute to IS pathogenesis.

Phosphatidylcholine mitigates the adverse effects of immune cell-mediated neuroinflammation on neuronal differentiation and plasticity. By modulating the inflammatory response, phosphatidylcholine enhances neuronal survival and proper differentiation, positioning itself as a potential therapeutic agent in cases of neuronal dysfunction arising from lipid-immune interactions. Additionally, bioactive lipids such as lysophosphatidylcholine (LPC) play a pivotal role in mediating the interaction between immune cells and apoptotic cells during efferocytosis. LPC acts as a "Find-Me" signal, guiding phagocytes to the site of inflammation, thereby facilitating the efficient clearance of apoptotic cells, which is crucial for resolving inflammation and promoting tissue repair. These findings reinforce the critical relationship between lipids, immune cells, and ischemic stroke, highlighting the potential therapeutic implications of targeting lipid-immune interactions in stroke treatment (9, 37–39). Based on the results of this study, it can be speculated that the prognosis of LAS may be improved by reducing the plasma levels of Phosphatidylcholine (O-18:2_20:4), Phosphatidylcholine (O-16:0_20:4), and Sterol ester (27:1/22:6). Conversely, increasing the levels of Phosphatidylcholine (18:2_0:0) may enhance the prognosis of LAS. Additionally, elevating the levels of Phosphatidylinositol (18:1_18:2) could potentially improve the prognosis of SVS. Additionally, the prognosis of ischemic stroke may be improved by modulating the activity of immune cells, such as granulocytes and myeloid dendritic cells.

The mediation analysis provided intriguing insights into how lipid levels could influence LAS and SVS risk through immune cell processes. We identified potential pathways whereby specific lipids might modulate LAS and SVS risk by altering immune cell counts, such as granulocytes, myeloid dendritic cells, and T cells. The absence of evidence for directional horizontal pleiotropy in our results supports the potential causal relationship between identified lipids and immune cell phenotypes with LAS and SVS. By using genetic instrumental variables (IVs) to infer causality, we circumvent the limitations of observational studies that can be confounded by lifestyle factors and

reverse causation. This methodological strength enhances the reliability of our findings and provides a stronger basis for developing intervention strategies based on genetic susceptibilities (19, 40).

While our study marks a significant step forward, further research is needed to elucidate the biological mechanisms through which these identified lipids and immune cells influence LAS and SVS risk. Experimental studies in cellular and animal models could provide deeper insights into the pathophysiological processes involved. Our findings identified immune cell phenotypes that may mediate the relationship between lipid levels and ischemic stroke subtypes. However, the mediation analysis was constrained by the available data, limiting our ability to capture all potential mediators or interactions. Future research should aim to include a broader spectrum of immune cell phenotypes. Additionally, clinical trials designed to modulate these specific lipid and immune cell pathways could validate the therapeutic potential of our findings. Including populations from diverse ethnic backgrounds could enhance the generalizability of our findings and uncover population-specific genetic risk factors for IS.

In conclusion, our study reveals an intricate landscape of genetic factors influencing LAS and SVS risk, involving specific lipid species and immune cells. These findings not only advance our understanding of IS pathogenesis but also point toward novel therapeutic targets that could transform IS management. As we move towards a more personalized medicine approach, integrating genetic risk factors with clinical strategies will be crucial in combating the global burden of ischemic stroke.

5 Conclusion

This study identifies significant genetic associations between specific lipids—namely phosphatidylcholine, sterol ester, and phosphatidylinositol—and the risk of LAS and SVS. Additionally, we identified key immune cell phenotypes that contribute to LAS and SVS risk and act as mediators in the lipid-LAS/SVS causal pathway. These findings enhance our understanding of the genetic factors influencing LAS and SVS pathophysiology and suggest potential targets for therapeutic intervention. This work advances the current knowledge by providing new insights into the complex interactions between lipid metabolism, immune response, and ischemic stroke risk, highlighting novel avenues for treatment strategies.

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

HC: Conceptualization, Writing – original draft, Visualization, Methodology, Investigation, Data curation. ZZ: Writing - original draft, Conceptualization, Investigation, Data curation, Methodology, Visualization. XC: Writing – original draft, Validation, Investigation. SL: Conceptualization, Methodology, Writing – original draft. MC: Data curation, Methodology, Writing – original draft. JW: Writing – original draft, Methodology, Data curation. WH: Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – review & editing. FG: Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

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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.2024.1437153/full#supplementary-material

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EDITED BY
Ramona Schuppner,
Hannover Medical School, Germany

REVIEWED BY
Nandavar Shobha,
Manipal Hospitals, India
William C. Y. Leung,
Queen Mary Hospital, China
Heling Chu,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Yan Li
liyanmanu@163.com
Chunsheng Yang
cyang01@tmu.edu.cn

†These authors have contributed equally to this work

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Triglyceride-glucose index prediction of stroke incidence risk in low-income Chinese population: a 10-year prospective cohort study

Xiao Li[1†], Juan Hao[1†], Qingqing Han[2], Di Wang[2], Yuting Lu[3], Jun Tu[1,4,5,6], Lifeng Wang[6], Jinghua Wang[1,4,5,6], Xianjia Ning[1,4,5,6], Chunsheng Yang[1*] and Yan Li[6,7*]

[1]Department of Neurology, Tianjin Medical University General Hospital, Tianjin, China, [2]College of Anesthesiology, Shanxi Medical University, Taiyuan, China, [3]School of Basic Medical Sciences, Tianjin Medical University, Tianjin, China, [4]Laboratory of Epidemiology, Tianjin Neurological Institute, Tianjin, China, [5]Tianjin Neurological Institute, Key Laboratory of Post-Neuroinjury Neuro-repair and Regeneration in Central Nervous System, Ministry of Education and Tianjin City, Tianjin, China, [6]Institute of Clinical Epidemiology & Evidence-Based Medicine, Tianjin Jizhou People's Hospital, Tianjin, China, [7]Department of Anesthesiology, Tianjin Jizhou People's Hospital, Tianjin, China

Aim: The Triglyceride-Glucose (TyG) index, an indicator of insulin resistance, has been proposed as a predictor of cardiovascular diseases. However, its role in predicting stroke risk, particularly in low-income populations, is not well understood. This study aimed to investigate the predictive value of the TyG index for stroke incidence in a low-income Chinese population, with a focus on gender and age-specific differences.

Methods: This 10-year prospective cohort study included 3,534 participants aged ≥45 years from rural areas in northern China. Baseline data on demographic characteristics, lifestyle factors, and clinical measurements were collected. Participants were followed for stroke incidence, categorized into ischemic and hemorrhagic stroke. Multivariate logistic regression models were used to assess the association between the TyG index and stroke incidence, adjusting for potential confounders.

Results: During the follow-up period, 368 participants (10.4%) experienced a stroke, with 327 ischemic and 31 hemorrhagic strokes. TyG index was significantly associated with total and ischemic stroke incidence but not hemorrhagic stroke. After adjusting for confounding factors, for every one standard deviation increase in TyG index, the risk of stroke increased by 32% for overall stroke (RR: 1.32; 95% CI: 1.08-1.61; P=0.006) and 39% for ischemic stroke (RR: 1.39; 95% CI: 1.12-1.73; P=0.003). The risk of stroke in the highest TyG tertile levels (tertile 3) increased by 49% (RR: 1.49; 95% CI 1.11-1.99; P=0.007) for overall stroke, compared to those in the lowest tertile levels (tertile 1). For ischemic stroke, the risk of stroke increased by 53% (RR: 1.53; 95% CI 1.12-2.11; P=0.008) in the highest TyG tertile levels (tertile 3) compared to those in the lowest tertile levels (tertile 1).

Conclusion: This 10-year prospective cohort study has established the TyG index as an independent predictor of both total and ischemic stroke incidence in a low-income Chinese population. The findings indicate that the TyG index is particularly effective in predicting stroke risk among women and older adults (≥60 years), but not for hemorrhagic stroke. These insights are crucial for improving clinical practice and stroke prevention strategies.

KEYWORDS

TyG index, stroke, risk factors, epidemiology, metabolism

Introduction

Stroke is the second leading cause of disability and death worldwide, particularly affecting individuals over 50 years old, with ischemic stroke being the most common subtype, accounting for about 80% of cases (1). In China alone, there were 3.94 million new stroke cases in 2019, marking an 86.0% increase in incidence since 1990, reaching 276.7 cases per 100,000 people (2). This trend is expected to continue, with the American Heart Association projecting that 3.4 million U.S. adults will have a stroke by 2030, a 20.5% increase from 2012 (3). This growing burden underscores the critical need for early identification of stroke risk factors and targeted prevention strategies.

Recent advancements in stroke research have identified various risk factors and biomarkers. One such biomarker, the TyG index, was first proposed in 2008 as a simple, convenient, and low-cost alternative to assess insulin resistance (IR) (4). Elevated TyG index levels have been independently associated with an increased risk of cardiovascular diseases (CVD) and mortality (5–8). Additionally, emerging evidence suggests a link between higher TyG index levels and an increased risk of cerebrovascular diseases, including ischemic stroke (9). However, most studies have focused on urban populations, leaving a gap in understanding its impact on low-income rural populations.

Despite these advancements, there remains considerable debate regarding the TyG index's predictive value for different stroke subtypes. Some studies suggest a strong association between elevated TyG index and ischemic stroke but not hemorrhagic stroke (10). Moreover, the lack of research focusing on low-income rural populations, who may have different risk profiles and healthcare access, highlights a significant gap in the current literature.

While large-scale studies such as the PURE study by Lopez-Jaramillo et al. have extensively examined cardiovascular outcomes across diverse countries and income levels (5), specific attention to stroke risk in rural, low-income populations remains limited. This population faces unique challenges, such as limited healthcare access, low health literacy, and a higher prevalence of untreated cardiovascular risk factors, all of which can significantly contribute to stroke incidence. Therefore, the generalizability of findings from broader studies to these underrepresented groups may be limited.

Therefore, this study aims to investigate the predictive effect of the TyG index on stroke incidence risk in a low-income Chinese population. By focusing on this underrepresented group, we hope to provide insights that can inform targeted prevention strategies and ultimately reduce the stroke burden in similar communities.

Methods

Study design and population

This study was a 10-year prospective cohort study, with the baseline survey conducted from April to June 2014. All participants were recruited as part of the Tianjin Brain Study, a population-based surveillance program for cardiovascular and cerebrovascular diseases (11–13). The study population was drawn from 18 administrative villages, where approximately 95% of participants were low-income farmers, with a per capita disposable income of less than $1,600 in 2014 (14). Eligible participants were residents aged 45 years and older who had no prior history of cardiovascular or cerebrovascular diseases. The population was characterized by advanced age, low income, and limited education, as well as low levels of health awareness. All participants have been continuously followed since enrollment.

The study protocol was approved by the Tianjin Medical University General Hospital Ethics Committee (IRB2018-100-01), and all participants provided written informed consent.

Definition and monitoring of stroke and CVD

From June 30, 2014, to December 31, 2023, all participants were followed to identify stroke events, CVD events, and all-cause mortality. A first stroke event was defined as the rapid onset of symptoms and signs of focal brain injury, lasting more than 24 hours, and confirmed by imaging evidence. Stroke events were

identified through three sources: local licensed village doctors reporting according to a predetermined procedure, medical records for hospital inpatients, and the all-cause death registry.

CVD events included newly diagnosed coronary atherosclerotic heart disease, myocardial infarction, and cardiovascular death. Coronary atherosclerotic heart disease was defined as the presence of more than 50% narrowing in one or more coronary arteries, as demonstrated by coronary angiography.

Information on stroke and CVD events came from three sources as described previously (11, 13). Briefly, local licensed village doctors (LLVD) reporting according to a predetermined procedure, medical records for hospital inpatients, and the all causes of death registry.

Risk factor assessment

Data on gender, age, and years of education were collected from existing records. Participants were categorized into three age groups (45-59 years, 60-74 years, and ≥75 years) and four education groups (illiterate, 1-6 years, 7-9 years, and ≥10 years). Smoking status was defined as smoking more than one cigarette per day for at least one year and categorized into never smoked, quit smoking, and current smoking. Alcohol consumption was defined as drinking more than 500 grams per week for at least one year and categorized into never drank, quit drinking, and current drinking. Additionally, data on participants' history of atrial fibrillation and medication use, including lipid-lowering agents, antiplatelets, and anticoagulants, were collected through self-reported information.

Clinical measurements and definitions

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded as the average of two measurements. Hypertension was defined as SBP above 140 mmHg, DBP above 90 mmHg, or the use of antihypertensive drugs. Diabetes was defined as fasting blood glucose (FPG) of 7.0 mmol/L or higher, or the use of insulin or oral hypoglycemic agents. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2) and categorized into normal weight (18.5 Kg/m^2 ≤ BMI < 24.0 Kg/m^2), overweight (24.0 Kg/m^2≤ BMI < 28.0 Kg/m^2), and obesity (BMI ≥ 28.0 Kg/m^2).

Biochemical measurements

Participants were instructed to fast from midnight (after 24:00) the day before blood collection. Fasting blood samples were collected at 8:00 a.m. the following morning, ensuring a fasting period of at least 8 hours. Measurements included complete blood count, FPG, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). The TyG index was calculated as Ln[TG (mg/dL) × FPG (mg/dL)/2] (4). All samples were processed in the central laboratory of Tianjin Jizhou People's Hospital within 2 hours of collection.

Statistical analyses

The normality of continuous variables was tested using the single-sample Kolmogorov-Smirnov test. For normally distributed continuous variables, the Student's t-test was applied to compare group differences, with results expressed as mean (standard deviation). For non-normally distributed continuous variables, the rank-sum test was used, and results were expressed as medians (25th and 75th percentiles). Categorical variables were expressed as numbers (percentages), and the chi-square test was used to compare the distribution difference between groups.

To evaluate the predictive value of the TyG index for stroke onset, receiver operating characteristic (ROC) curves were generated, and the area under the curve (AUC) was calculated. Multivariate logistic regression analysis was employed to examine the association between stroke and its subtypes, with stroke onset as the dependent variable, the TyG index as the main independent variable, and other covariates selected based on statistical significance in the univariate analysis. The TyG index was analyzed both as a continuous variable and by tertile grouping to assess its relationship with stroke risk. Results were reported as adjusted relative risks (RR) with 95% confidence intervals (95% CI). Additionally, a restricted cubic spline (RCS) analysis was performed to explore potential non-linear relationships between the TyG index and stroke risk. All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA) and R, version 4.2.3 (R Development Core Team, Vienna, Austria). A two-tailed p-value of <0.05 was considered statistically significant.

Results

A total of 3992 participants were recruited during study periods, and 3648 participants met the inclusion criteria after excluded 344 participants with the previous CVD histories. Moreover, the basic data of TG or FPG in 62 participants was missing, and 52 participants were less than 45 years were excluded. Finally, 3534 participants were analyzed in this study (Figure 1).

Baseline characteristics

The study included 3,534 participants, of whom 1,419 (40.2%) were men and 2,115 (59.8%) were women. The median age of the participants was 59.05 years (25th percentile: 51.75, 75th percentile: 65.67), with a mean follow-up period of 9.04 years. Participants were divided into three groups based on tertiles of the TyG index: tertile 1 (TyG index < 8.51), tertile 2 (8.51 ≤ TyG index ≤ 9.04), and tertile 3 (TyG index > 9.04). A total of 19 participants (0.54%) self-reported taking oral lipid-lowering statins. None of the participants

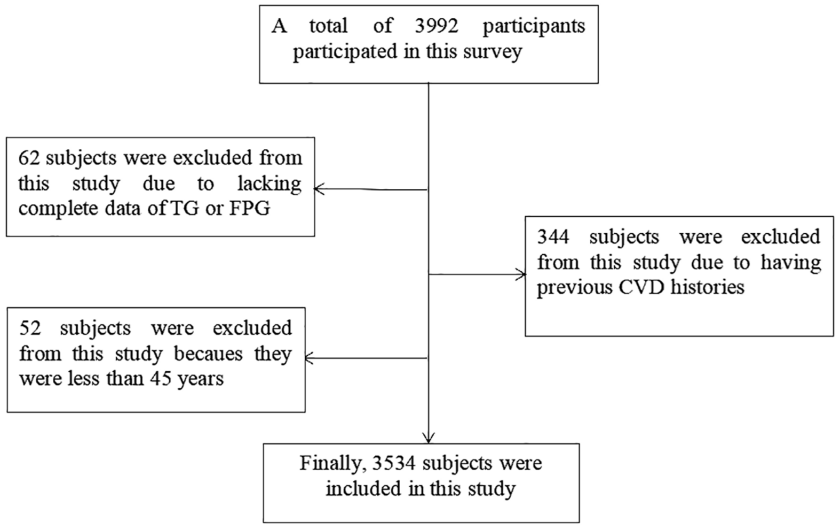

FIGURE 1
Flow chat of participants selection. Figure showed that total of 3992 participants were recruited during study periods, and 3648 participants met the inclusion criteria after excluded 344 participants with the previous CVD histories. Moreover, the basic data of TG or FPG in 62 participants was missing, and 52 participants were less than 45 years were excluded. Finally, 3534 participants were analyzed in this study.

used antiplatelet drugs or anticoagulants, and no participant had a history of atrial fibrillation. Compared to those in the lowest tertile, participants in the higher tertiles were more likely to be female, obese, hypertensive, or diabetic, and less likely to have a history of smoking or alcohol consumption. Higher TyG index levels were associated with elevated SBP, DBP, LDL-C, TC, TG, and FPG levels, and lower HDL-C levels (Table 1).

Incidence of stroke and CVD

During the 10-year follow-up period, a total of 368 participants (10.4%) experienced a new stroke, 32 participants (0.9%) were diagnosed with a new CVD and 406 participants (11.5%) died from all causes. Of these, 327 were ischemic strokes, 31 were hemorrhagic strokes, and 10 were unclassified strokes.

The ROC curve for TyG index in predicting stroke incidence is presented in Figure 2. The AUC for the TyG index was 0.554 (95% CI: 0.522–0.586; P < 0.001), with a cut-off point of 9.04. These results suggest that the TyG index serves as an important predictor of stroke, with stroke risk increasing significantly when the TyG index exceeds 9.04.

Association of TyG index with stroke incidence in the univariate study

In univariate analysis, several factors were significantly associated with stroke onset, including male gender, older age, smoking, hypertension, diabetes, higher SBP, DBP, FBG, TC, LDL-C, and TyG index (all P<0.05; Table 2). However, no significant association was observed between the TyG index and CVD incidence in the univariate analysis (P > 0.05; Supplementary Table S1).

Association of TyG index with stroke incidence in the multivariate study

TyG index was significantly associated with total and ischemic stroke incidence but not hemorrhagic stroke. After adjusting for confounding factors, for every one standard deviation increase in TyG index, the risk of stroke increased by 32% for overall stroke (RR: 1.32; 95% CI: 1.08-1.61; P=0.006) and 39% for ischemic stroke (RR: 1.39; 95% CI: 1.12-1.73; P=0.003).

After adjusting for sex, age group, smoking status, LDL-C, and history of hypertension, participants in the highest TyG tertile (tertile 3) had a 49% higher risk of overall stroke (RR: 1.49; 95% CI: 1.11–1.99; P = 0.007) compared to those in the lowest tertile (tertile 1). Similarly, after adjusting for sex, age group, BMI, smoking status, LDL-C, alcohol consumption, and history of hypertension, participants in the highest TyG tertile (tertile 3) had a 53% higher risk of ischemic stroke (RR: 1.53; 95% CI: 1.12–2.11; P = 0.008) compared to those in the lowest tertile. No significant association was found between the TyG index and hemorrhagic stroke incidence (Figure 3).

Association of TyG index with stroke incidence in the subgroup analysis

Further analysis was stratified by sex and age, with the univariate results presented in Supplementary Tables S2, S3.

The multivariate analysis revealed that among women, after adjusting for age, BMI groups, LDL-C, and history of hypertension,

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TABLE 1 Baseline characteristics of the study population according to tertile groups of TyG index .

Characteristic	Total	TyG index			P
		Tertile 1	Tertile 2	Tertile 3	
Case, n (%)	3534 (100)	1177 (33.3)	1178 (33.3)	1179 (33.4)	
Gender, n (%)					<0.001
Men	1419 (40.2)	573 (40.4)	459 (32.3)	387 (27.3)	
Women	2115 (59.8)	604 (28.6)	719 (34.0)	792 (37.4)	
Age*, years	59.05 (51.75, 65.67)	59.24 (51.34, 66.07)	59.05 (52.12, 66.02)	58.92 (52.00, 65.04)	0.556
Age groups, n (%)					0.433
45-59 years	1925 (54.5)	620 (32.2)	643 (33.4)	662 (34.4)	
60-74 years	1326 (37.5)	456 (34.4)	437 (33.0)	433 (32.7)	
≥75 years	283 (8.0)	101 (35.7)	98 (34.6)	84 (29.7)	
Education groups, n (%)					0.229
0 year	678 (19.3)	208 (30.7)	224 (33.0)	246 (36.3)	
1-6 years	1512 (43.1)	526 (34.8)	517 (34.2)	469 (31.0)	
7-9 years	1070 (30.5)	354 (33.1)	344 (32.1)	372 (34.8)	
≥10 years	249 (7.1)	82 (32.9)	82 (32.9)	85 (34.1)	
BMI groups, n (%)					<0.001
Normal	1225 (34.7)	576 (47.0)	432 (35.3)	217 (17.7)	
Overweight	1486 (42.1)	442 (29.7)	498 (33.5)	546 (36.7)	
Obesity	817 (23.2)	156 (19.1)	246 (30.1)	415 (50.8)	
Smoking status, n (%)					<0.001
Current smoking	737 (20.9)	293 (39.8)	232 (31.5)	212 (28.8)	
Quit smoking	152 (4.3)	61 (40.1)	49 (32.2)	42 (27.6)	
Never smoked	2645 (74.8)	823 (31.1)	897 (33.9)	925 (35.0)	
Alcohol consumption, n (%)					0.007
Current drinking	510 (14.4)	196 (38.4)	174 (34.1)	140 (27.5)	
Quit drinking	40 (1.1)	18 (45.0)	10 (25.0)	12 (30.0)	
Never drank	2984 (84.4)	963 (32.3)	994 (33.3)	1027 (34.4)	
Hypertension, n (%)					<0.001
Yes	2442 (69.1)	705 (28.9)	822 (33.7)	915 (37.5)	
No	1091 (30.9)	472 (43.3)	355 (32.5)	264 (24.2)	
Diabetes, n (%)					<0.001
Yes	671 (19.0)	74 (11.0)	176 (26.2)	421 (62.7)	
No	2863 (81.0)	1103 (38.5)	1002 (35.0)	758 (26.5)	
Statins use, n (%)					0.367
Yes	19 (0.5)	6 (31.6)	4 (21.1)	9 (47.4)	
No	3515 (99.5)	1171 (33.3)	1174 (33.4)	1170 (33.3)	
SBP*,mmHg	144.00 (130.33, 160.00)	140.67 (127.67, 157.83)	144.00 (131.00, 159.50)	146.50 (133.00, 162.00)	<0.001
DBP*,mmHg	86.00 (79.00, 93.67)	84.00 (77.00, 91.00)	86.50 (79.00, 94.00)	88.00 (81.00, 95.50)	<0.001
Hb*, g/L	138.00 (129.00, 147.00)	138.00 (129.75, 148.00)	137.00 (129.00, 146.50)	138.00 (129.00, 147.00)	0.841

(Continued)

TABLE 1 Continued

Characteristic	Total	TyG index			P
		Tertile 1	Tertile 2	Tertile 3	
Plt*, 10^9/L	231.00 (197.00, 271.00)	229.00 (198.00, 270.00)	231.00 (195.50, 272.00)	232.00 (197.00, 272.00)	0.925
FPG*, mmol/L	5.60 (5.10, 6.10)	5.30 (4.90, 5.65)	5.60 (5.17, 6.10)	5.93 (5.40, 7.10)	<0.001
TC*, mmol/L	4.79 (4.15, 5.50)	4.44 (3.86, 5.06)	4.75 (4.18, 5.44)	5.20 (4.56, 5.99)	<0.001
TG*, mmol/L	1.40 (1.01, 2.11)	0.89 (0.73, 1.04)	1.42 (1.25, 1.64)	2.55 (2.09, 3.36)	<0.001
HDL-C*, mmol/L	1.39 (1.15, 1.70)	1.54 (1.30, 1.84)	1.39 (1.16, 1.73)	1.25 (1.05, 1.49)	<0.001
LDL-C*, mmol/L	2.59 (2.04, 3.22)	2.44 (1.96, 3.00)	2.63 (2.10, 3.27)	2.72 (2.06, 3.39)	<0.001

*Continuous variables were expressed as medians (percentile25, percentile75). TyG index, triglyceride-glucose index; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hb, hemoglobin; Plt, platelet; FPG, fasting plasma glucose; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

every one standard deviation increase in the TyG index was associated with a 76% higher risk of stroke (RR: 1.76; 95% CI: 1.31–2.38; P < 0.001). Compared to participants in tertile 1, the risk of stroke was significantly increased by 90% (RR: 1.90; 95% CI: 1.21–3.00; P = 0.006) in tertile 3.

For participants aged ≥60 years, after adjusting for sex, LDL-C, and history of hypertension, every one standard deviation increase in the TyG index was associated with a 33% higher risk of stroke (RR: 1.33; 95% CI: 1.03–1.74; P = 0.028). In this age group, the risk of stroke was 59% higher in tertile 3 compared to tertile 1 (RR: 1.59; 95% CI: 1.11–2.29; P = 0.012).

No significant associations were found among men and younger participants after adjusting for confounding factors (Figure 4).

Non-linear relationship between TyG index and stroke incidence

We conducted a RCS analysis to assess the potential non-linear relationship between the TyG index and stroke incidence (Figure 5). The P-value for non-linearity was 0.02, indicating a statistically significant non-linear relationship between TyG index levels and stroke risk in participants aged 60 years and older. A distinct U-shaped pattern was observed in this age group. Stroke risk initially decreased as the TyG index increased, up to a threshold of 8.47. However, beyond this threshold, stroke risk began to rise, becoming more pronounced at higher TyG index levels. This suggests that 8.47 may represent a critical inflection point in the relationship between TyG index and stroke risk in older adults.

In contrast, no significant non-linear relationships were observed between the TyG index and overall stroke, ischemic stroke, stroke in females, or stroke in individuals aged below 60 years.

Discussion

This is the first studies to explore the predictive value of the TyG index for stroke and CVD in a low-income rural Chinese population. This 10-year prospective cohort study investigated the predictive value of the TyG index for stroke incidence in a low-income Chinese population, focusing on gender and age-specific differences. Both the value of TyG and its tertile grouping were confirmed to be independent predictors of stroke and ischemic stroke. Notedly, the association was not found in hemorrhagic stroke. Moreover, the TyG index was a stronger predictor of stroke in women and older adults (≥60 years). However, the relationship between TyG index and CVD risk was not found in this study.

The relationship between the TyG index and the incidence of different types of stroke has been previously reported in various studies. These studies have generally found that the TyG index is a significant predictor of ischemic stroke, but not hemorrhagic stroke.

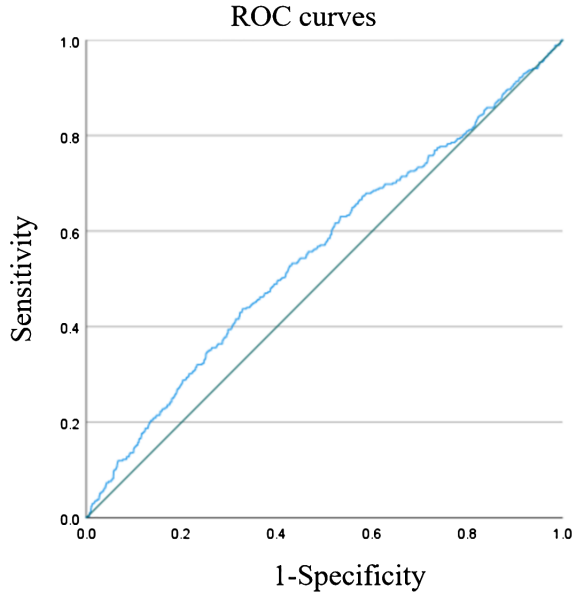

FIGURE 2
ROC curve for TyG index to predict stroke. Figure showed that the AUC of TyG index in predicting stroke was 0.554 (95% CI: 0.522-0.586; P<0.001). The cut-off point was 9.04. It was suggested that TyG index is an important predictor of stroke, and when TyG index > 9.04, the risk of stroke would increase.

TABLE 2 The associated factors of stroke onset in the study population by types in univariate analysis.

Characteristic	Stroke			Ischemic stroke			Hemorrhagic stroke		
	No	Yes	P	No	Yes	P	No	Yes	P
Case, n (%)	3166 (89.6)	368 (10.4)	—	3207 (90.7)	327 (9.3)	—	3503 (99.1)	31 (0.9)	—
Gender, n (%)			<0.001			<0.001			0.191
Men	1231 (86.8)	188 (13.2)		1252 (88.2)	167 (11.8)		1403 (98.9)	16 (1.1)	
Women	1935 (91.5)	180 (8.5)		1955 (92.4)	160 (7.6)		2100 (99.3)	15 (0.7)	
Age*, years	58.52 (51.42,64.99)	63.20(58.03,69.79)	<0.001	58.65 (51.47, 65.16)	62.82 (57.88, 68.83)	<0.001	59.01 (51.71, 65.63)	64.75 (57.53, 69.97)	0.008
Age groups, n (%)			<0.001			<0.001			0.058
45-59 years	1800 (93.5)	125 (6.5)		1811 (94.1)	114 (5.9)		1914 (99.4)	11 (0.6)	
60-74 years	1130 (85.2)	196 (14.8)		1153 (87.0)	173 (13.0)		1308 (98.6)	18 (1.4)	
≥75 years	236 (83.4)	47 (16.6)		243 (85.9)	40 (14.1)		281 (99.3)	2 (0.7)	
BMI groups, n (%)			0.057			0.030			0.928
Normal	1114 (90.9)	111 (9.1)		1128 (92.1)	97 (7.9)		1215 (99.2)	10 (0.8)	
Overweight	1330 (89.5)	156 (10.5)		1349 (90.8)	137 (9.2)		1473 (99.1)	13 (0.9)	
Obesity	716 (87.6)	101 (12.4)		724 (88.6)	93 (11.4)		809 (99.0)	8 (1.0)	
Smoking status, n (%)			<0.001			<0.001			0.289
Current smoking	633 (85.9)	104 (14.1)		645 (87.5)	92 (12.5)		727 (98.6)	10 (1.4)	
Quit smoking	131 (86.2)	21 (13.8)		132 (86.8)	20 (13.2)		151 (99.3)	1 (0.7)	
Never smoked	2402 (90.8)	243 (9.2)		2430 (91.9)	215 (8.1)		2625 (99.2)	20 (0.8)	
Alcohol consumption, n (%)			0.102			0.016			0.808
Current drinking	445 (87.3)	65 (12.7)		450 (88.2)	60 (11.8)		506 (99.2)	4 (0.8)	
Quit drinking	38 (95.0)	2 (5.0)		40 (100)	0 (0)				
Never drank	2683 (89.9)	301 (10.1)		2717 (91.1)	267 (8.9)		2997 (99.1)	27(0.9)	
Hypertension, n (%)			<0.001			<0.001			<0.001
Yes	2111 (86.4)	331 (13.6)		2149 (88.0)	294 (12.0)		2412 (98.8)	30 (1.2)	
No	1054 (96.6)	37 (3.4)		1058 (97.0)	33 (3.0)		1090 (99.9)	1 (0.1)	
Diabetes, n (%)			<0.001			<0.001			0.058
Yes	550 (82.0)	121 (18.0)		561 (83.6)	110 (16.4)		661 (98.5)	10 (1.5)	

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TABLE 2 Continued

Characteristic	Stroke			Ischemic stroke			Hemorrhagic stroke		
	No	Yes	P	No	Yes	P	No	Yes	P
No	2616 (91.4)	247 (8.6)		2646 (92.4)	217 (7.6)		2842 (99.3)	21 (0.7)	
SBP*,mmHg	142.50 (129.00, 158.50)	155.00 (142.00, 173.00)	<0.001	142.50 (129.50, 158.50)	154.33 (141.50, 173.00)	<0.001	143.50 (130.00, 160.00)	155.50 (145.50, 173.00)	<0.001
DBP*,mmHg	85.50(78.50, 93.00)	91.33(83.13,99.00)	<0.001	85.50 (78.67, 93.00)	91.00 (83.50, 99.00)	<0.001	86.00 (79.00, 93.67)	91.67 (81.50, 97.00)	0.014
Hb*, g/L	137.00 (129.00, 147.00)	138.00 (130.00, 147.75)	0.356	137.00 (129.00, 147.00)	138.00 (130.00, 148.00)	0.270	137.50 (129.00, 147.00)	139.50 (128.75, 147.00)	0.791
Plt*, 10^9/L	231.00 (197.00, 273.00)	223.00 (200.00, 258.50)	0.255	231.00 (197.00, 272.00)	223.00 (198.00, 260.00)	0.285	231.00 (197.00, 271.75)	223.00 (200.25, 259.75)	0.557
FPG*, mmol/L	5.56 (5.10, 6.10)	5.80 (5.30, 6.70)	<0.001	5.56 (5.10, 6.10)	5.80 (5.36, 6.77)	<0.001	5.60 (5.10, 6.10)	5.60 (5.23, 6.20)	0.650
TC*, mmol/L	4.77 (4.13, 5.48)	4.95 (4.27, 5.77)	<0.001	4.77 (4.14, 5.49)	4.94 (4.27, 5.76)	0.002	4.79 (4.15, 5.50)	5.30 (4.24, 6.07)	0.096
TG*, mmol/L	1.39 (1.01, 2.09)	1.52 (1.01, 2.21)	0.151	1.39 (1.01, 2.09)	1.52 (1.04, 2.21)	0.054	1.40 (1.01, 2.11)	1.39 (0.81, 1.92)	0.402
HDL-C*, mmol/L	1.39 (1.15, 1.70)	1.38 (1.14, 1.67)	0.529	1.39 (1.15, 1.70)	1.36 (1.14, 1.67)	0.430	1.39 (1.15, 1.70)	1.42 (1.13, 1.70)	0.624
LDL-C*, mmol/L	2.57 (2.02, 3.20)	2.71 (2.21, 3.37)	0.001	2.58 (2.03, 3.20)	2.70 (2.20, 3.38)	0.007	2.59 (2.04, 3.21)	2.75 (2.20, 3.38)	0.312
TyG index*	8.75 (8.38, 9.20)	8.88 (8.44, 9.37)	0.001	8.75 (8.38, 9.20)	8.91 (8.45, 9.40)	<0.001	8.77 (8.39, 9.22)	8.73 (8.18, 9.30)	0.560
TyG index tertile groups, n (%)			<0.001			<0.001			0.401
Tertile 1	1072 (91.1)	105 (8.9)		1088 (92.4)	89 (7.6)		1164 (98.9)	13 (1.1)	
Tertile 2	1073 (91.1)	105 (8.9)		1084 (92.0)	94 (8.0)		1171 (99.4)	7 (0.6)	
Tertile 3	1021 (86.6)	158 (13.4)		1035 (87.8)	144 (12.2)		1168 (99.1)	11 (0.9)	

*Continuous variables were expressed as medians (percentile25, percentile75). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hb, hemoglobin; Plt, platelet; FPG, fasting plasma glucose; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TyG index, triglyceride-glucose.

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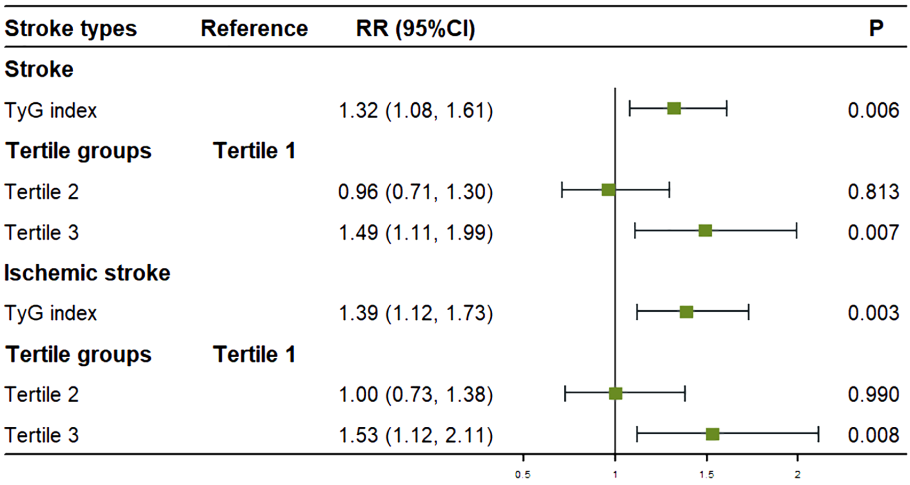

FIGURE 3
Association between the TyG index and stroke onset in multivariate analysis. Figure showed that after adjusting for covariates, for every one standard deviation increase in TyG index, the risk of stroke increased by 32% for overall stroke and 39% for ischemic stroke. Compared to tertile 1, the risk of stroke was increased by 49% for overall stroke and 53% for ischemic stroke in tertile 3.

Several notable studies have highlighted the role of the TyG index in predicting stroke risk. For instance, a study by Wang et al. found that higher TyG index levels were associated with an increased risk of both total and ischemic stroke in a general Chinese population, but no significant association was observed with hemorrhagic stroke (15). Similarly, Hoshino et al. demonstrated that the TyG index is a valuable prognostic marker for major adverse cardiovascular events, including ischemic stroke, in Japanese patients with a history of ischemic stroke or transient ischemic attack (TIA) (16). Moreover, a meta-analysis confirmed that elevated TyG index levels were linked to a higher risk of cerebrovascular diseases, particularly ischemic stroke, across various populations (9). In another cohort study observed that the TyG index was a significant predictor of incident ischemic stroke, especially in older adults, but did not find a similar association with hemorrhagic stroke (17). Finally, the Kailuan study reported that long-term increases in the TyG index were associated with an elevated risk of ischemic stroke in a hypertensive population, while no significant link was found with hemorrhagic stroke (10). Consistent to these previous studies, our findings demonstrated that the TyG index is an independent predictor of total and ischemic stroke incidence, but not hemorrhagic stroke, in a low-income Chinese population. The potential mechanisms underlying these associations can be attributed to the role of IR in vascular health. Elevated TyG index levels indicate higher IR, which leads to oxidative stress, chronic inflammation, endothelial

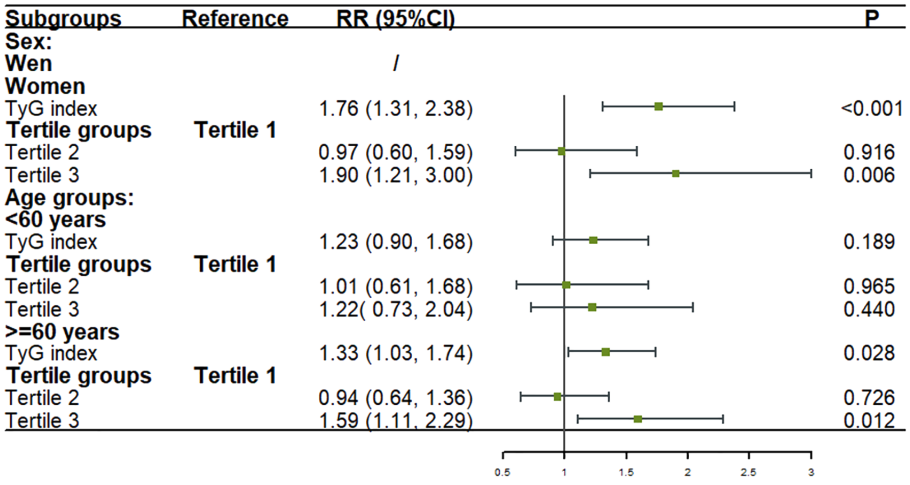

FIGURE 4
Association between the TyG index and stroke onset in sex and age subgroups in logistic models. Figure showed that after adjusting for covariates, for every one standard deviation increase in TyG index, the risk of stroke increased by 76% among women and 33% among participants aged ≥60 years. Compared to tertile 1, the risk of stroke was significantly increased by 90% among women and 59% among participants aged ≥60 years in tertile 3. No significant associations were found among men and younger participants after adjusting for confounding factors.

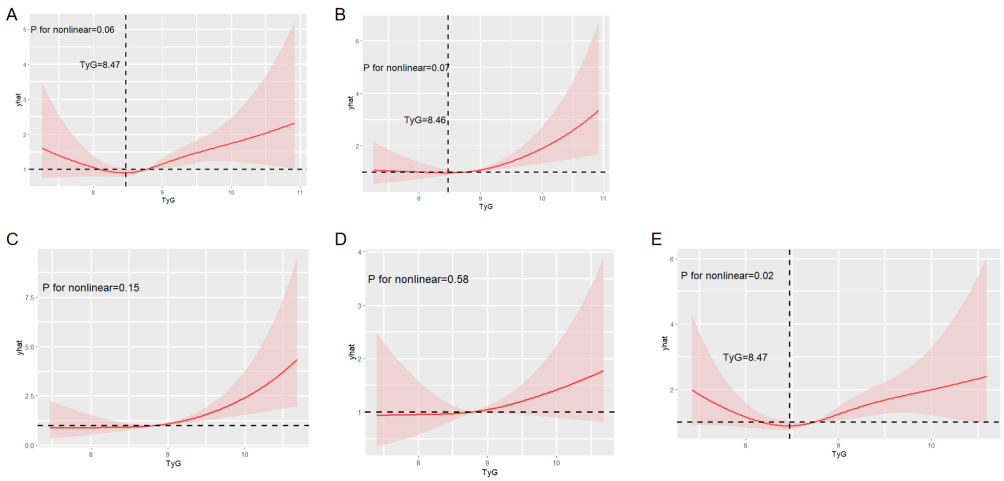

FIGURE 5
Restricted cubic spline analysis to evaluate the potential non-linear relationship between the TyG index and stroke incidence. Figure showed the potential non-linear relationship between overall stroke incidence **(A)**, ischemic stroke incidence **(B)**, stroke incidence in female subgroup **(C)**, stroke incidence in <60 years subgroup **(D)**, stroke incidence in ≥60 years subgroup **(E)** and the TyG index.

dysfunction, and accelerated atherosclerosis—all contributing factors to ischemic stroke (18–22). IR also increases thrombotic potential through enhanced platelet activity and impaired fibrinolysis, further exacerbating the risk of ischemic stroke (23–25). The lack of association with hemorrhagic stroke may be due to different pathophysiological processes involved, as hemorrhagic stroke is more related to factors such as hypertension and vascular integrity rather than metabolic disturbances like IR.

The relationship between the TyG index and the incidence of different types of stroke, particularly its stronger predictive value in women, has been reported in previous studies. Several studies have highlighted gender-specific differences in the association between the TyG index and stroke risk. The Rural Chinese Cohort Study found that the TyG index was significantly associated with an increased risk of ischemic stroke in women, but not in men, after adjusting for traditional risk factors (17). Similarly, a study reported that higher TyG index levels were more strongly associated with the risk of ischemic stroke in women compared to men in a large Chinese cohort (15). Furthermore, Zhao et al. observed that the TyG index was a significant predictor of stroke, particularly ischemic stroke, in women, with no significant association found in men (17). Additionally, a meta-analysis supported these findings, indicating that the TyG index was a more potent predictor of cerebrovascular events in women than in men (9). However, the Kailuan study showed a positive association between the TyG index and ischemic stroke in hypertensive men, but not in women, suggesting possible differences in study populations and comorbid conditions (10). Consistent to these previous studies, our findings also demonstrate that the TyG index is a stronger predictor of stroke, particularly ischemic stroke, in women. Several potential mechanisms may explain why the TyG index is a stronger predictor of stroke in women. Women generally have higher body fat percentages and different fat distribution patterns compared to men, which may influence IR and its metabolic consequences (18). Hormonal differences, particularly the protective effects of estrogen on vascular function and lipid metabolism, may also play a role. Post-menopausal women, who experience a decline in estrogen levels, may be more susceptible to IR and its associated vascular risks, thereby amplifying the predictive value of the TyG index for stroke (19, 20). Additionally, women may have different lifestyle factors, such as dietary habits and physical activity levels, that interact with metabolic risk factors differently than men (21, 22).

Several studies have highlighted age-specific differences in the association between the TyG index and stroke risk. TyG index was significantly associated with an increased risk of ischemic stroke in older adults, particularly those aged 60 years and above, in a large Chinese cohort (17), higher TyG index levels were more strongly associated with the risk of ischemic stroke in older adults compared to younger individuals (15), elevated TyG index levels were linked to a higher risk of cerebrovascular diseases, particularly in older populations (9). The Kailuan study showed that long-term increases in the TyG index were associated with an elevated risk of ischemic stroke in older adults with hypertension (10). Similar association was found in this study. Age-related increases in IR are well-documented, and older adults often exhibit higher levels of IR due to changes in body composition, decreased physical activity, and altered metabolic function (18, 19). These factors contribute to an increased risk of atherosclerosis and other vascular complications, which are major risk factors for ischemic stroke. Additionally, the cumulative exposure to cardiovascular risk factors over time may exacerbate the impact of IR on vascular health in older adults, making the TyG index a more potent predictor of stroke in this age group (20, 21). Furthermore, age-related endothelial dysfunction and increased arterial stiffness, both of which are associated with IR, may further enhance the risk of ischemic stroke in older individuals (22). These potential mechanisms may explain why the TyG index is a stronger predictor of stroke in older adults.

The association between the TyG index and the incidence of CVD has been well-documented in several studies, which generally

indicate that the TyG index is a significant predictor of CVD. For instance, a cohort study by Laura Sánchez-Íñigo et al. found that the TyG index could predict the development of cardiovascular events (6). Similarly, the Kailuan study, which considered longitudinal changes in the TyG index over time, showed that cumulative TyG index was associated with an increased risk of CVD (8). Additionally, the PURE study, which examined populations in urban and rural areas across five continents, found that the TyG index was significantly associated with myocardial infarction and cardiovascular mortality (5). However, in our study, no significant association was observed between the TyG index and CVD incidence, including coronary atherosclerotic heart disease, myocardial infarction, and cardiovascular death. A potential explanation for this discrepancy is the absence of uniform coronary angiography in our cohort, which may have led to missed diagnoses of new coronary heart disease, myocardial infarction, and cardiovascular death, thereby affecting the accuracy of our findings.

This study has several limitations that need to be considered when interpreting the results. First, the study population was comprised of middle-aged and elderly individuals from low-income rural areas in northern China, which may limit the generalizability of our findings to other regions and socioeconomic groups. While this population is representative of other low-income rural populations in China, the specific environmental, lifestyle, and genetic factors in different regions may influence the association between the TyG index and stroke risk. The findings may not be directly applicable to urban populations or those with different socioeconomic backgrounds. Future studies should include diverse populations from various regions and socioeconomic statuses to enhance the generalizability of the results. Second, we assessed the predictive effect of the baseline TyG index on stroke incidence. Although this approach provides valuable insights into the initial risk assessment, it does not account for changes in the TyG index over time. IR and associated metabolic parameters can fluctuate, potentially altering stroke risk. The lack of multiple measurements over time may lead to an underestimation or overestimation of the true association between the TyG index and stroke risk. Therefore, future research should incorporate longitudinal measurements of the TyG index to capture its dynamic changes and better reflect its long-term impact on stroke incidence. Third, this study focused exclusively on first-time stroke events and did not investigate the relationship between the TyG index and recurrent strokes. Secondary stroke prevention is a critical aspect of managing patients who have already experienced a stroke, and understanding the role of the TyG index in predicting recurrent stroke risk is essential. By not including recurrent stroke events, we may have overlooked important information that could further elucidate the role of the TyG index in stroke prevention. Future studies should follow stroke survivors to explore the association between the TyG index and recurrent stroke risk, thereby providing a more comprehensive understanding of its utility in secondary prevention. Fourth, the absence of longitudinal data on the TyG index and the management of cardiovascular (CVS) risk factors throughout the 10-year follow-up period is a significant limitation. Only baseline measurements of the TyG index, TG, and FBG were collected, and no data were obtained on how these risk factors were managed over time, such as whether participants received treatment or achieved adequate control. This lack of longitudinal data hinders our ability to fully assess the dynamic relationship between the TyG index and stroke risk. For example, it remains unclear whether participants with high baseline TyG index levels improved their risk profiles through medication or lifestyle changes, potentially influencing long-term stroke outcomes. Consequently, this may lead to either an underestimation or overestimation of the true association between the TyG index and stroke risk, as the baseline measurement may not accurately capture the participant's CVS risk over the 10-year period. Future studies should incorporate repeated measurements of the TyG index and other CVS risk factors to better capture how changes in these parameters over time affect stroke risk. Additionally, tracking the management of CVS risk factors, including medication use and lifestyle interventions, would provide valuable insights into the long-term impact of these factors on stroke outcomes. Furthermore, we did not collect data on whether participants with elevated triglyceride or fasting glucose levels received treatment or achieved optimal control during the follow-up period. This limitation restricts our ability to evaluate the effectiveness of managing CVS risk factors in individuals with high TyG index values, and whether such management mitigated stroke risk. Without information on the management of CVS risk factors, it is difficult to determine whether a high TyG index predicts poor control of these factors (e.g., persistent hyperglycemia or dyslipidemia) and consequently an increased risk of stroke. Participants with high baseline TyG index who received appropriate treatment may have reduced their risk, while those who remained untreated could have experienced worse outcomes. This lack of data introduces uncertainty into our interpretation of the predictive value of the TyG index. Future research should include detailed data on the treatment and control of CVS risk factors, including medication adherence and lifestyle modifications. Such data would provide a more comprehensive understanding of how managing these factors influences the relationship between the TyG index and stroke risk over time. Finally, potential confounding factors such as diet, physical activity, and medication use were not thoroughly controlled for in this study. These factors can significantly influence IR and stroke risk, and their omission may lead to residual confounding. Although we adjusted for several major confounders in our analysis, more detailed data on lifestyle factors and comprehensive adjustments are needed in future research to minimize potential biases and provide more accurate estimates of the TyG index's predictive value.

Conclusion

In this 10-year prospective cohort study, we demonstrated that the TyG index is an independent predictor of both total and ischemic stroke incidence in a low-income Chinese population. Our findings revealed that the TyG index was particularly effective in predicting stroke risk among women and older adults (≥60 years), but not hemorrhagic stroke. These findings highlight the

potential of the TyG index as a valuable tool for stroke risk assessment. The TyG index, as a simple, cost-effective, and non-invasive measure of IR, can be readily integrated into routine clinical practice. Its use can facilitate early identification of individuals at high risk for ischemic stroke, allowing for timely intervention and personalized prevention strategies. Given its particular predictive value in women and older adults, the TyG index can aid clinicians in targeting these high-risk groups more effectively. Especially, the TyG index's ability to predict stroke risk in a low-income rural population highlights its potential utility in resource-limited settings. By adopting the TyG index, healthcare providers in these areas can improve stroke prevention efforts and reduce the burden of stroke in vulnerable populations.

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 humans were approved by The Tianjin Medical University General Hospital Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XL: Writing – original draft, Investigation. JH: Writing – original draft, Investigation. QH: Writing – original draft, Investigation. DW: Writing – original draft, Investigation. YTL: Writing – original draft, Investigation. JT: Data curation, Writing – original draft. LW: Investigation, Writing – original draft. JW: Methodology, Formal analysis, Writing – original draft. XN: Writing – original draft, Methodology, Formal analysis. CY: Writing – review & editing, Conceptualization. YL: Writing – review & editing, Conceptualization.

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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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EDITED BY
Joachim Eduard Weber,
Charité University Medicine Berlin, Germany

REVIEWED BY
Miao Chen,
Hainan Medical University, China
Davide Norata,
Campus Bio-Medico University, Italy

*CORRESPONDENCE
Chuanchen Hu
✉ hcc20030323@126.com

†These authors share first authorship

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The HALP (hemoglobin, albumin, lymphocyte, and platelet) score is associated with hemorrhagic transformation after intravenous thrombolysis in patients with acute ischemic stroke

Jiahao Chen[1†], Rui Hu[2†], Lejia Shang[3], Xiaoqin Li[1], Yisi Lin[4], Yu Yao[1] and Chuanchen Hu[1*]

[1]Department of Neurology, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, China, [2]Department of Neurology, Yongkang First People's Hospital, Jinhua, China, [3]Ruao Town Health Service Center, Shaoxing, China, [4]Department of Neurology, The Third Affiliated Hospital of Wenzhou Medical University (Ruian People's Hospital), Wenzhou, China

Background: Hemorrhagic transformation (HT) after intravenous thrombolysis (IVT) with rt-PA can precipitate rapid neurological deterioration, poor prognosis, and even death. The HALP score (hemoglobin, albumin, lymphocyte, and platelet) is a novel indicator developed to reflect both systemic inflammation and the nutritional status of patients. The goal of this study was to reveal the relationship between the HALP score and the risk of HT after IVT in people with acute ischemic stroke (AIS).

Methods: A total of 753 patients with AIS were included in this study. Patients were divided into quartiles according to baseline HALP score. The HALP score was calculated as follows: hemoglobin (g/L) × albumin (g/L) × lymphocytes (/L)/platelets (/L). Binary logistic regression was used to reveal the connection between HALP score and HT.

Results: The baseline HALP score were significantly lower in the HT than non-HT patients ($p < 0.001$). The HALP score were divided into four quartiles: Q1 (<27.4), Q2 (27.4–37.6), Q3 (37.7–49.6), Q4 (>49.6), respectively. Moreover, the severity of HT increased with decreasing HALP level ($p < 0.001$). In multivariable logistic regression, taking the Q4 as the reference, the association between Q1 and HT remained, after adjusting for confounding variables [odds ratio (OR) = 3.197, 95% confidence interval (CI) = 1.634–6.635, $p = 0.003$].

Conclusion: The HALP value can predict the HT risk after IVT in patients with AIS. A lower HALP level was associated with an increased severity of HT post-IVT.

KEYWORDS

acute ischemic stroke, hemorrhagic transformation, inflammation, nutrition status, predictor

1 Introduction

Acute ischemic stroke (AIS) is a main cause of death and disability worldwide. Currently, in the very early stage of AIS, intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (rt-PA) is the main method of treatment, which can effectively improve functional outcomes of patients with AIS (1). However, despite improving neurological prognosis, IVT with rt-PA also increases the risk of hemorrhagic transformation (HT) (2), a common post-IVT complication with a prevalence of 27–37% (3). HT can precipitate rapid neurological deterioration, poor prognosis, and even death (4). Consequently, the accurate and convenient assessment of HT risk post-IVT in patients with AIS is paramount.

After the onset of AIS, inflammatory status plays a pivotal role in neurologic function injury and protection. Recently, neutrophil to lymphocyte ratio (NLR), an important inflammatory index, of which level has been seemed like a substantial risk factor for HT after IVT (5). In addition, platelet to lymphocyte ratio (PLR) is another inflammatory measure, is associated with HT and In-hospital mortality in AIS patients with large-artery occlusion (6). Albumin level was employed as a primary indicator for the patient's nutritional status. In a cohort analysis, researchers discovered a link between low serum albumin levels and an increased risk of HT post-IVT (7). On this basis, we aggregated these common indications and explored the association between them and HT after IVT in this study.

A novel marker, HALP, comprising hemoglobin, albumin, lymphocyte, and platelet levels, has been developed to reflect both systemic inflammation and the nutritional status of patients (8–11). Inflammation is a known pathophysiological mechanism of HT, especially following reperfusion post-IVT (12, 13). Additionally, malnutrition is recognized as a risk factor for disability and long-term mortality in patients with AIS (14). Yet, no studies have examined the association between the HALP score and HT risk post-IVT in patients with AIS.

Therefore, this study aimed to investigate the relationship between HALP score and HT in patients with AIS following IVT treatment.

2 Methods

2.1 Patients

This retrospective study was conducted at the Affiliated Jinhua Hospital, School of Medicine of Zhejiang University. The study included all consecutive patients over 18 years who underwent IVT with rt-PA (0.9 mg/kg, maximum 90 mg) between January 2020 and January 2024. The study was approved by the Institutional Review Board and Ethics Committee of the Affiliated Jinhua Hospital, School of Medicine of Zhejiang University, with informed consent waived due to the retrospective nature of the study and the anonymity of all data.

AIS diagnosis was confirmed via CT or magnetic resonance imaging (MRI) upon admission. The IVT with rt-PA was administered within 4.5 h of symptom onset for all suitable participants, strictly adhering to the ESO guidelines (15). The exclusion criteria were as follows: (1) data unavailable; (2) absence of follow-up CT or MRI within 24 h; (3) severe diseases, including tumors and trauma; (4) suffered from infection within 2 weeks before admission.

2.2 Data collection

Demographic characteristics (age and gender) and medical history of atrial fibrillation (AF), diabetes mellitus (DM), hypertension, coronary heart disease (CHD), hyperlipidemia, cigarette smoking, and alcohol consumption were extracted from medical records. Based on the National Institutes of Health Stroke Scale (NIHSS), stroke severity was evaluated upon admission (16). Short-term functional outcome was assessed by modified Rankin Scale (mRS) and Barthel index (BI) score at discharge. Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria were used to classify the AIS subtypes (17). Fasting peripheral venous blood samples were collected within 24 h after admission to obtain laboratory data, including serum albumin, hemoglobin, lymphocyte, and PLT levels. The HALP score was calculated as follows: hemoglobin (g/L) × albumin (g/L) × lymphocytes (/L)/platelets (/L).

2.3 Definition and classification of HT subtypes

Patients with possible AIS underwent cranial CT examination prior to IVT, and subsequent cranial CT or MRI was performed within 24 h post-IVT to screen for HT. In case of clinical symptom deterioration, an immediate imaging examination was conducted. HT was radiologically classified into four subtypes based on follow-up CT/MRIs by two experienced neuroradiologists blinded to the clinical data, following the criteria of the European Cooperative Acute Stroke Study (ECASS) (18): hemorrhagic infarction (HI)-1 (small petechiae along the periphery of the infarct), HI-2 (more confluent petechiae around the infarcted area without a space-occupying effect), parenchymal hematoma (PH)-1 (hematoma <30% of the infarcted area with a mild space-occupying effect), and PH-2 (hematoma >30% of the infarcted area with a significant space-occupying effect).

2.4 Statistical analyses

Data distribution normality was tested using the Kolmogorov–Smirnov test. Baseline characteristics were expressed as mean ± standard deviation, those with non-normal distributions as median with interquartile range, and categorical variables as relative frequency and percentage. Continuous variables were compared using the student's *t*-test or the Mann–Whitney *U* test; categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Based on the quartiles of the HALP scores, all patients in the study were divided into four groups. One-way analysis of variance (ANOVA) or Kruskal–Wallis test was used to perform statistical comparisons of HALP score stratification for continuous variables. For significantly different variables, we employed Tukey's honestly significant difference (HSD) test as the post-hoc test. To evaluate whether the HALP stratification was an independent predictor of HT, a multivariate-adjusted binary logistic regression was performed after adjusting for conventional confounding factors and significant variables ($p < 0.1$) identified in univariate logistic regression analysis. The predictive capacity of the HALP score in discriminating possible HT was assessed by receiver operating characteristic curves (ROC). Spearman's rank correlation

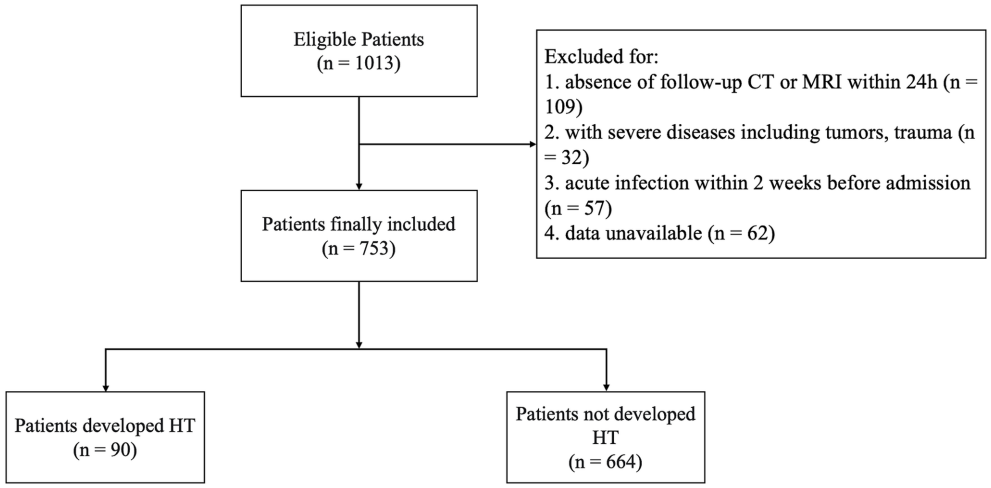

FIGURE 1
Flowchart of patient recruitment.

test was used to analyze the correlations between the HALP score and ECASS subtypes. Two-sided $p < 0.05$ was considered significant. All statistical analyses were performed using R for MacOS, version 4.1.2.

3 Results

3.1 Baseline characteristics

This study included 753 patients with AIS (Figure 1), with a median age of 70 (59–77) years; 66.7% (503) were male. Among these, 11.8% (89) developed HT, while 88.1% (664) did not.

3.2 Baseline characteristics of patients with and without HT

Table 1 presents the baseline characteristics and laboratory findings of the patients with AIS. The HT group had a significantly lower HALP score (29.8 versus 38.7, $p < 0.001$) compared to the non-HT group. Significant differences were also observed in age, baseline NIHSS score, mRS score at discharge, BI score at discharge, TOAST classification, AF, white blood cell (WBC) count, CRP, HDL-C, and LDL-C ($p < 0.05$). The HT group also exhibited lower hemoglobin, albumin, and lymphocyte ($p < 0.05$).

3.3 Baseline characteristics of patients according to HALP score quartiles

Patients were categorized into four groups based on HALP score quartiles: Q1 (<27.4), Q2 (27.4–37.6), Q3 (37.7–49.6), Q4 (>49.6). Table 2 displays demographics, vascular risk factors, clinical information, laboratory findings, TOAST classifications, and laboratory signs according to HALP quartiles. Significant differences were found in age, gender, baseline NIHSS score, mRS score at discharge, BI score at discharge, TOAST classification, history of smoking, HDL, LDL, CRP, hemoglobin, albumin, lymphocytes, and platelets ($p < 0.05$).

3.4 The relationship between HALP score and HT after IVT

An analysis of HALP score quartiles according to HT subtype revealed that the most severe type of HT (PH-2) was significantly more prevalent in the lowest quartile (Q1) than in other types of HT or without HT (Figure 2A). The highest HALP score quartile (Q4) contained the highest proportion of patients without HT. HT severity increased with decreasing HALP score ($p < 0.001$) (Figure 2B). A negative association was found between elevated HALP score and HT severity (Spearman correlation coefficient −0.189, $p < 0.001$) (Figure 2C). The predictive capacity of the HALP score in discriminating possible HT was assessed by ROC with an area under the curve of 0.667 (0.609–0.724) (Figure 2D).

Univariate binary logistic analysis (Table 3) showed that HALP score quartiles (Q1 and Q2) were significantly associated with the risk of HT in patients with AIS (Q1: OR = 5.206, 95% CI = 2.845–10.234, $p < 0.001$; Q2: OR = 3.032, 95% CI = 1.600–6.101, $p = 0.006$). Other factors significantly associated with HT were age, AF, baseline NIHSS, WBC counts, CRP, and LDL. After adjusting for these variables, the association between Q1 and HT remained (OR = 3.197, 95% CI = 1.634–6.635, $p = 0.003$) (Figure 3).

4 Discussion

Our study assessed the correlation between the HALP score and the risk of HT in 753 patients with AIS post-IVT. The findings indicated a significant increase in HT risk in patients with AIS after IVT with a low HALP score at admission, even after adjusting for potential and known confounders. This suggests that a low HALP score could be a potential risk factor for HT post-IVT.

TABLE 1 Comparison of baseline characteristics between patients with or without HT.

Variables	Non-HT (*n* = 664)	HT (*n* = 89)	*p*-value
Demographics			
Age, years (IQR)	68.0 (58.0–68.0)	76.5 (69.5–83.8)	**<0.001**
Gender (male, %)	441 (66.4%)	62 (68.8%)	0.728
Vascular risk factor			
Hypertension, *n* (%)	536 (80.7%)	74 (82.2%)	0.844
DM, *n* (%)	151 (22.7%)	21 (23.3%)	1.000
AF, *n* (%)	109 (16.4%)	39 (43.3%)	**<0.001**
CHD, *n* (%)	44 (66.3%)	6 (66.7%)	1.000
Hyperlipidemia, *n* (%)	122 (18.4%)	9 (10.0%)	0.689
History of stroke, *n* (%)	99 (14.9%)	10 (11.2%)	0.445
History of smoking, *n* (%)	267 (40.2%)	28 (31.1%)	0.122
History of drinking, *n* (%)	301 (45.3%)	31 (34.4%)	0.066
Clinical information			
SBP at admission, mmHg (IQR)	152.0 (136.8–167.0)	158.0 (139.0–170.8)	0.178
DBP at admission, mmHg (IQR)	86.0 (76.0–95.0)	88.0 (73.3–97.0)	0.901
NIHSS at admission (IQR)	3.0 (1.0–5.0)	6 (3.0–11.8)	**<0.001**
mRS at discharge (IQR)	2.0 (1.0–3.0)	3.0 (2.0–5.0)	**<0.001**
BI at discharge (IOR)	80 (60–95)	50 (20–70)	**<0.001**
DNT, min (IQR)	38.0 (29.0–50.0)	39.0 (33.5–50.5)	0.257
TOAST classification			**<0.001**
Large artery atherosclerosis, *n* (%)	218 (32.8%)	27 (30.3%)	
Cardioembolism, *n* (%)	116 (17.5%)	43 (48.3%)	
Small vessel occlusion, *n* (%)	260 (39.2%)	10 (11.2%)	
Other determined, *n* (%)	6 (0.9%)	0 (0.0%)	
Undetermined, *n* (%)	64 (9.6%)	9 (10.1%)	
Laboratory signs			
Hemoglobin, g/L (IQR)	136.0 (125.0–148.0)	129.0 (117.0–141.0)	**0.002**
Albumin, g/L (IQR)	38.5 (36.5–40.5)	37.6 (34.8–40.4)	**0.032**
Lymphocyte, 10^9/L (IQR)	1.4 (1.1–1.8)	1.1 (0.8–1.4)	**<0.001**
Platelet, 10^9/L (IQR)	195.0 (155.0–234.0)	189.0 (141.0–233.0)	0.174
HALP (IQR)	38.7 (28.3–50.8)	29.8 (21.8–39.4)	**<0.001**
WBC, 10^9/L (IQR)	6.7 (5.5–8.3)	7.7 (6.3–10.0)	**<0.001**
Neutrophile, 10^9/L (IQR)	3.3 (2.3–4.7)	5.3 (3.8–7.7)	**<0.001**
CPR, mg/L (IQR)	1.2 (0.5–4.2)	6.7 (1.2–14.9)	**<0.001**
Glucose, mmol/L (IQR)	5.1 (4.6–6.1)	5.5 (4.8–6.6)	0.065
LDL, mmol/L (IQR)	2.9 (2.3–3.5)	2.6 (2.1–3.2)	**0.004**
HDL, mmol/L (IQR)	1.2 (1.0–1.4)	1.3 (1.0–1.4)	**0.027**

Data are expressed as mean ± SD, median (interquartile, range), or *n* (%) as appropriate. Comparisons among groups were performed using student's *t*-test, or Mann–Whitney *U* test, chi-square test or Fisher's exact test, as appropriate. Bold indicates $p < 0.05$. HT, hemorrhagic transformation; DM, diabetes mellitus; AF, atrial fibrillation; CHD, coronary heart disease; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; DNT, door to needle time; WBC, white blood count; CPR, C reactive protein; LDL, low density lipoprotein.

HT was identified in 11.8% of all patients with AIS post-IVT, aligning with the 10–43% range reported in previous studies (19). Factors such as age, baseline NIHSS score, WBC count, and AF were independently associated with HT, corroborating previous findings (20, 21).

Initially, the HALP score was a combined scoring system predicting patient prognosis across various tumors (8–11). Recent studies have increasingly focused on the relationship between the HALP score and stroke. A Chinese study suggested that a decreased HALP score correlated with an increased mortality risk within 90 days

TABLE 2 Comparison of baseline characteristics between patients according to HALP quartiles.

Variables	HALP quartiles					
	All Patients (*n* = 753)	Quartile 1 (*n* = 188)	Quartile 2 (*n* = 189)	Quartile 3 (*n* = 188)	Quartile 4 (*n* = 188)	*p*-value
Demographics						
Age, years (IQR)	70 (59–77)	74 (65–82)	69 (61–76)	70 (57–77)	66 (54–73)	**<0.001**
Gender (male, %)	503 (66.7%)	114 (60.6%)	122 (64.6%)	125 (64.5%)	142 (75.5%)	**0.018**
Vascular risk factor						
Hypertension, n (%)	609 (80.8%)	155 (82.4%)	160 (84.7%)	145 (77.1%)	149 (79.3%)	0.254
DM, *n* (%)	171 (22.7%)	38 (20.2%)	45 (23.8%)	44 (23.4%)	44 (23.4%)	0.825
AF, *n* (%)	148 (19.6%)	43 (22.9%)	41 (21.7%)	38 (20.2%)	26 (13.8%)	0.121
CHD, *n* (%)	50 (6.6%)	11 (5.9%)	13 (6.9%)	11 (5.9%)	15 (8.0%)	0.816
Hyperlipidemia, *n* (%)	130 (17.2%)	22 (11.7%)	35 (18.5%)	33 (17.6%)	40 (21.3%)	0.093
History of stroke, *n* (%)	109 (14.4%)	34 (18.1%)	28 (14.8%)	27 (14.4%)	20 (10.6%)	0.237
History of smoking, *n* (%)	295 (39.1%)	64 (34.0%)	70 (37.0%)	82 (43.6%)	79 (42.0%)	0.200
History of drinking, *n* (%)	332 (44.0%)	73 (38.8%)	72 (38.1%)	88 (46.8%)	99 (52.7%)	**0.012**
Clinical information						
SBP at admission, mmHg (IQR)	152.0 (138.0–167.0)	156.0 (138.8–168.0)	152.0 (139.0–166.0)	151.0 (135.0–164.0)	148.0 (137.0–168.0)	0.558
DBP at admission, mmHg (IQR)	86.0 (76.0–95.0)	83.0 (72.0–94.3)	87.0 (76.0–94.0)	87.0 (76.0–95.3)	87.0 (78.8–96.0)	0.103
NIHSS at admission (IQR)	3.0 (1.0–6.0)	4.0 (1.0–8.0)	3.0 (1.0–6.0)	3.0 (1.8–6.0)	2.0 (1.0–4.5)	**0.001**
mRS at discharge (IQR)	2.0 (1.0–3.0)	2.0 (1.0–4.0)	2.0 (1.0–3.0)	2.0 (1.0–3.0)	1.0 (1.0–2.0)	**<0.001**
BI at discharge (IQR)	75.0 (50.0–95.0)	60.0 (30.0–86.3)	80.0 (50.0–95.0)	77.5 (55.0–95.0)	85.0 (60.0–95.0)	**<0.001**
DNT, min (IQR)	38.0 (29.5–50.0)	39.5 (30.0–50.3)	38.0 (29.0–51.5)	37.0 (29.0–48.5)	37.0 (29.0–50.0)	0.791
TOAST classification						**0.001**
Large artery atherosclerosis, *n* (%)	245 (32.5%)	73 (38.8%)	54 (28.6%)	69 (36.7%)	49 (26.1%)	
Cardioembolism, *n* (%)	159 (21.1%)	47 (25.0%)	45 (23.8%)	40 (21.3%)	27 (14.4%)	
Small vessel occlusion, *n* (%)	270 (35.8%)	49 (26.1%)	76 (40.2%)	61 (32.4%)	84 (44.7%)	
Other determined, *n* (%)	6 (0.7%)	1 (0.5%)	0 (0.0%)	1 (0.5%)	4 (2.1%)	
Undetermined, *n* (%)	73 (9.6%)	18 (9.6%)	14 (7.4%)	17 (9.0%)	24 (12.8%)	
Laboratory signs						
Hemoglobin, g/L (IQR)	136.0 (124.0–147.0)	123.5 (112.8–135.0)	134.0 (123.0–145.0)	138.5 (129.0–149.0)	146.0 (135.8–155.3)	**<0.001**
Albumin, g/L (IQR)	38.4 (36.4–40.5)	37.1 (35.0–39.1)	38.2 (36.0–39.9)	39.3 (37.0–40.8)	39.1 (37.3–41.5)	**<0.001**
Lymphocyte, 10^9/L (IQR)	1.37 (1.1–1.7)	0.9 (0.7–1.1)	1.3 (1.1–1.5)	1.5 (1.3–1.8)	1.9 (1.6–2.3)	**<0.001**
Platelet, 10^9/L (IQR)	193.0 (154.0–234.0)	204.5 (165.5–242.5)	203.0 (167.0–238.0)	193.0 (154.8–228.5)	175.5 (135.8–211.8)	**<0.001**
HALP (IQR)	37.6 (27.5–37.6)	21.1 (16.8–23.9)	32.8 (30.0–35.3)	42.5 (40.0–45.5)	60.3 (54.9–71.6)	**<0.001**
WBC, 10^9/L (IQR)	6.9 (5.6–8.5)	6.6 (5.3–8.8)	6.5 (5.4–8.1)	6.8 (5.8–8.3)	7.4 (5.9–8.9)	**0.006**
Neutrophile, 10^9/L (IQR)	4.8 (3.6–6.3)	5.1 (3.8–7.3)	4.6 (3.5–6.1)	4.7 (3.6–5.9)	4.8 (3.5–6.1)	0.088
CRP, mg/L (IQR)	1.4 (0.5–5.0)	3.0 (0.5–12.1)	1.7 (0.5–5.7)	1.0 (0.5–3.8)	0.9 (0.5–3.0)	**<0.001**
Glucose, mmol/L (IQR)	5.2 (4.6–6.2)	5.2 (4.6–6.1)	5.2 (4.6–6.2)	5.1 (4.6–6.6)	5.1 (4.6–6.0)	0.075
LDL, mmol/L (IQR)	1.2 (1.0–1.4)	2.8 (2.2–3.2)	2.8 (2.2–3.3)	2.8 (2.3–3.5)	3.0 (2.5–3.5)	**0.008**
HDL, mmol/L (IQR)	2.8 (2.3–3.4)	1.2 (1.0–1.4)	1.2 (1.0–1.4)	1.2 (1.0–1.3)	1.1 (1.0–1.4)	**0.034**

Data are expressed as mean ± SD, median (interquartile, range), or *n* (%) as appropriate. Comparisons among groups were performed using One-way analysis of variance (ANOVA) or Kruskal–Wallis test, chi-square test or Fisher's exact test, as appropriate. Bold indicates $p < 0.05$. HT, hemorrhagic transformation; DM, diabetes mellitus; AF, atrial fibrillation; CHD, coronary heart disease; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; DNT, door to needle time.

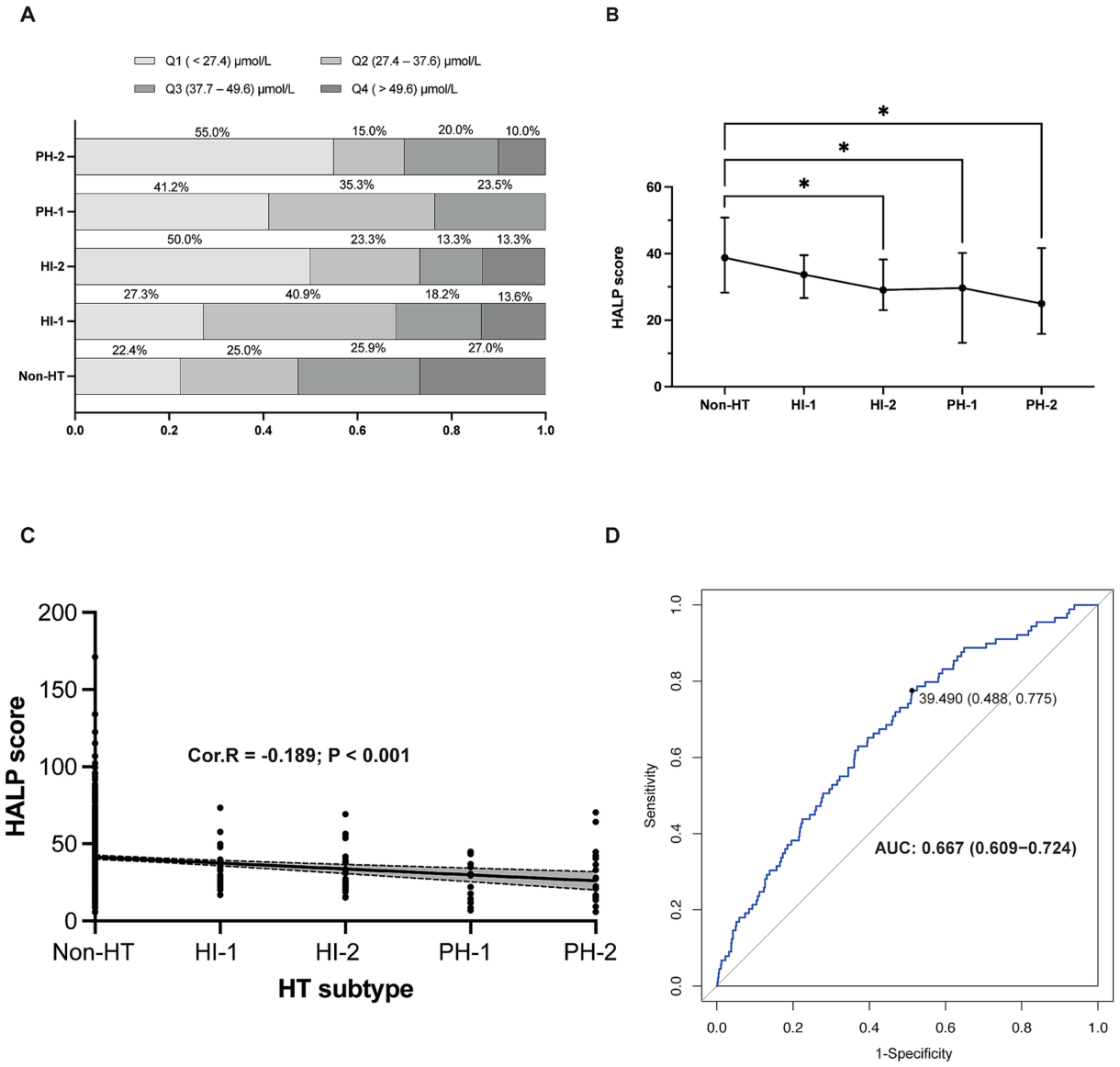

FIGURE 2
The relationship between HALP score and HT. **(A)** Proportion of patients in each HALP score with different HT subtypes. **(B)** HALP score in different subgroups of HT. **(C)** The negative relationship between HALP score and HT subtypes. **(D)** Receiver operating characteristic (ROC) curve showing the predictive ability of HALP score for HT. HALP, the hemoglobin, albumin, lymphocyte, and platelet score; HI, hemorrhagic infarct; HT, hemorrhagic transformation; PH, parenchymal hematoma; Cor. R, Spearman's rank man's correlation rank correlation test was test was used to analyze the correlations between HALP score and HT subtypes.

and 1 year in a cohort of patients with AIS (22). A subsequent study in patients with cerebral venous sinus thrombosis (CVST) found that a lower HALP score was associated with a worse prognosis (23), reinforcing our findings.

Increased blood-brain barrier (BBB) permeability, particularly post-IVT, underpins post-stroke HT, accompanied by leukocyte infiltration and heightened oxidative stress (13). Brain damage can be exacerbated by a series of systemic inflammatory chemicals and cells triggered by AIS. Lymphocytes play a crucial role in resolving inflammation and tissue regeneration. Lower lymphocyte levels have consistently been associated with increased infarction volume, accelerated neurological deterioration, and unfavorable functional outcomes in patients with AIS (24). One possible mechanism is that reduced lymphocyte counts may sever as an indicator of systemic response to acute stress (25). Another suggested mechanism is that reduced lymphocyte counts means increased pre-stroke cortisol levels and sympathetic tone (26), which can secrete more pro-inflammatory cytokines that can aggravate BBB injury and finally lead to HT (27). Besides, CRP, a significant inflammation indicator (28), is typically elevated in patients with AIS. In our study, the CRP level in the HT group was higher than in the non-HT group, aligning with previous studies (29, 30). Interestingly, our study observed lower platelet levels in the HT group compared to the non-HT group, although this difference was not statistically significant ($p = 0.174$), consistent with

TABLE 3 Univariate logistic regression analysis to identify relationships between variables and HT.

Variables	OR (95% CI)	*p*-value
Demographics		
Age, years	1.054 (1.037–1.073)	**<0.001**
Gender (male)	0.861 (0.570–1.279)	0.542
Vascular risk factor		
Hypertension	1.090 (0.685–1.803)	0.770
DM	0.985 (0.622–1.517)	0.955
AF	3.972 (2.680–5.970)	**<0.001**
CHD	1.019 (0.452–2.029)	0.967
Hyperlipidemia	0.439 (0.221–0.791)	**0.032**
History of ischemic stroke	0.722 (0.389–1.251)	0.357
History of smoking	0.683 (0.455–1.008)	0.114
History of drinking	0.645 (0.434–0.945)	**0.062**
Clinical information		
SBP at admission, mmHg	1.006 (0.998–1.014)	0.235
DBP at admission, mmHg	0.999 (0.987–1.012)	0.947
NIHSS at admission	1.129 (1.095–1.125)	**<0.001**
DNT, min	1.000 (0.992–1.008)	0.921
TOAST classification	0.830 (0.702–0.974)	**0.060**
Laboratory signs		
Hemoglobin, g/L	0.982 (0.972–0.993)	**0.005**
Albumin, g/L	0.947 (0.898–0.997)	0.084
Lymphocyte, 10^9/L	0.285 (0.185–0.431)	**<0.001**
Platelet, 10^9/L	0.997 (0.993–1.000)	0.135
WBC, 10^9/L	1.207 (1.125–1.295)	**<0.001**
Neutrophile, 10^9/L	1.277 (1.187–1.376)	**<0.001**
Glucose, mmol/L	0.994 (0.964–1.003)	0.551
CRP, mg/L	1.023 (1.014–1.032)	**<0.001**
LDL, mmol/L	0.640 (0.507–0.802)	**0.001**
HDL, mmol/L	1.397 (0.912–2.064)	0.168
HALP score		
HALP Q1	5.206 (2.845–10.234)	**<0.001**
HALP Q2	3.032 (1.600–6.101)	**0.006**
HALP Q3	1.850 (0.925–3.857)	0.153
HALP Q4	Ref	

HT, hemorrhagic transformation; DM, diabetes mellitus; AF, atrial fibrillation; CHD, coronary heart disease; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; DNT, door to needle time. The bold values were taken as statistically significant confounders as we mentioned in the "Statistical analyses" section ($p < 0.1$).

previous studies (31, 32). This might be due to the higher prevalence of AF among patients with AIS in the HT group compared to the non-HT group. Platelets play a dual role in stroke progression, contributing to hemostasis and BBB preservation while also displaying proinflammatory effects that can exacerbate stroke outcomes (33). Recent studies have shown that inflammatory cells, including lymphocytes and platelets, play a role in cerebral ischemic injury, potentially worsening ischemic brain damage and neurological impairments (34, 35). Previous studies have established a link between decreased lymphocyte counts and increased incidence of cardiovascular disease (36). In animal models, stroke-induced immunosuppression has been shown to lead to lymphocytopenia (37). Similarly, human studies have observed a decline in lymphocyte activity within peripheral blood following a stroke (38). Regarding platelets, previous studies have shown that platelet depletion resulting from cerebral ischemia-reperfusion exacerbates brain tissue damage (39). In the acute phase of AIS, platelets guide lymphocytes to vascular injury sites, and T cells, a lymphocyte subgroup, secrete cytokines to modulate platelet activation. This process may trigger thrombo-inflammatory reactions, deteriorating tissue integrity, disrupting the BBB, and elevating oxidative stress levels (40). Hence, the acute inflammatory response is likely intricately linked with HT post-IVT.

Hemoglobin, an erythrocyte-specific protein, transports oxygen to various organs. Abnormal hemoglobin levels are associated with atherosclerosis and may pose a risk factor for AIS (41). Notably, low hemoglobin levels significantly correlate with poor outcomes and mortality post-AIS (42). Besides, a machine learning-based prediction model for HT post-IVT identified hemoglobin as a crucial indicator (43). The specific mechanism by which a low hemoglobin level is more likely to lead to HT is currently still unclear. There are several possible reasons. First, low hemoglobin level can influence energy metabolism in the infarct area, further lead to an increase in the area of infarction (42). Second, anemia could produce inflammation and oxidative stress, which could impair BBB in individuals with AIS (44). Third, coagulation disorders and extended bleeding tendencies in patients with anemia were common in patients with anemia, which were also risk factors of HT (45, 46).

Serum albumin levels serve as a useful nutritional status indicator. Studies have revealed that between 6.1 and 62% of patients with AIS are at malnutrition risk, which correlates with poor functional outcomes (47). Che et al. (7) proposed albumin as a predictor of HT post-IVT in patients with AIS and associated it with short-term good clinical outcomes, as evaluated by the BI score at 7 days. The possible mechanism may be that albumin possesses a neuroprotective effect as it can counteract oxidation, blood stasis, thrombosis, and leukocyte adhesion according to previous studies (48, 49).

HALP is a novel index reflecting the combined inflammatory and nutritional status of patients. To our knowledge, no study has reported the significance of HALP in patients with AIS undergoing IVT. The HALP score, derived from a composite calculation involving hemoglobin concentration (g/L), albumin levels (g/L), lymphocyte count (10^9/L), and platelet count (10^9/L), is a cost-effective and straightforward parameter for evaluating inflammation-nutrition status. This insight is crucial as immediate assessment of the inflammation-nutritional state allows neurologists to identify patients with AIS vulnerable to HT post-IVT.

This study has several limitations. First, our study was a single-centered, retrospective cohort analysis, which could not prove cause and effect. Second, the small sample size of the HT group precluded the performance of a regression analysis between the radiological HT subtypes and HALP levels. Third, HALP was measured only once upon admission, despite its potential variability pre- and post-IVT and during hospitalization. In summary, further well-designed, large-scale, prospective, multicenter cohort studies are required to validate this association.

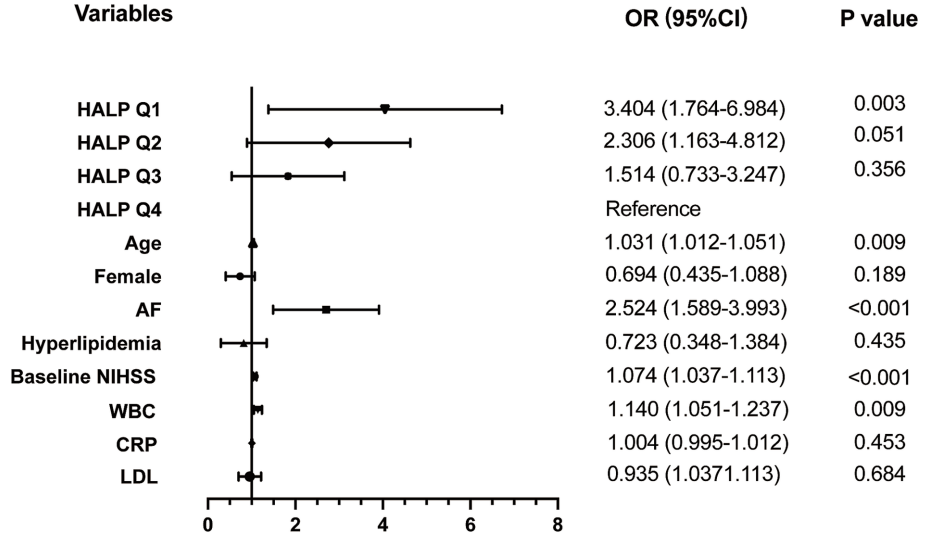

FIGURE 3
Adjusted odds ratios (OR) for the risk of HT by HALP score. HALP, the hemoglobin, albumin, lymphocyte, and platelet score; AF, atrial fibrillation; NIHSS, National Institutes of Health Stroke Scale; WBC, white blood count; CRP, C reactive protein; LDL, low density lipoprotein.

5 Conclusion

In conclusion, our study revealed that the HALP value can predict the HT risk after IVT in patients with AIS. A lower HALP level was closely associated with an increased severity of HT post-IVT.

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 humans were approved by Institutional Review Board and Ethics Committee of the Affiliated Jinhua Hospital, School of Medicine of Zhejiang University. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because the retrospective nature of the study and the anonymity of all data.

Author contributions

JC: Conceptualization, Investigation, Software, Writing – original draft. RH: Data curation, Software, Writing – review & editing. LS: Data curation, Investigation, Writing – review & editing. XL: Data curation, Writing – review & editing. YL: Data curation, Methodology, Writing – review & editing. CH: Conceptualization, Funding acquisition, Writing – review & editing. YY: Writing – review & editing.

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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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EDITED BY
Gerrit M. Grosse,
University Hospital of Basel, Switzerland

REVIEWED BY
Maria Magdalena Gabriel,
Hannover Medical School, Germany
Alexander Heinrich Nave,
Charité University Medicine Berlin, Germany

*CORRESPONDENCE
Saana Pihlasviita
✉ saana.pihlasviita@helsinki.fi

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Plasma symmetric dimethylarginine as a metabolite biomarker of severe acute ischemic stroke

Saana Pihlasviita[1]*, Olli S. Mattila[1], Tiina Nukarinen[1], Markku Kuisma[2], Heini Harve-Rytsälä[2], Juhani Ritvonen[1], Gerli Sibolt[1], Sami Curtze[1], Daniel Strbian[1], Mikko Pystynen[2], Turgut Tatlisumak[3,4] and Perttu J. Lindsberg[1]

[1]Neurology and Clinical Neurosciences, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [2]Emergency Medicine and Services, Department of Emergency Care, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [3]Department of Clinical Neuroscience/Neurology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden, [4]Department of Neurology, Sahlgrenska University Hospital, Gothenburg, Sweden

Introduction: After severe ischemic stroke (IS), circulating levels of symmetric dimethylarginine (SDMA) increase. We investigated the early dynamics of SDMA in stroke to potentially aid with prehospital identification of severe IS from hemorrhagic stroke (HS).

Methods: We performed targeted mass spectrometry (MS) measurements of SDMA in two sequential acute plasma samples (early and secondary) of 50 IS patients with LVO and 49 HS patients. Secondary samples of 227 IS and 84 HS patients with moderate to severe symptoms (NIHSS $\geq$ 7) subsequently underwent ELISA validation.

Results: The median (IQR) last-known-well (LKW) to sampling times were 43 min (35–67) for early samples in the MS analysis, and 83 min (65–113) for secondary samples in MS and ELISA analyses. No inter-group differences existed in early samples, but IS patients had significantly higher mean (IQR) SDMA levels in secondary samples in both analyses: 5.8 (5.3–6.9) vs. 5.1 (4.2–5.8) A.U. for HS, $p < 0.001$, with MS; and 0.82 (0.72–1.01) vs. 0.71 (0.58–0.85) nmol/mL for HS, $p < 0.001$, with ELISA. For IS patients, higher SDMA levels were associated with cardioembolic stroke: 0.84 (0.73–1.09) vs. 0.79 (0.71–0.91) nmol/mL for other etiologies, $p = 0.042$, and poor outcome: modified Rankin Scale (mRS) 4–6; 0.90 (0.73–1.06) vs. 0.80 (0.72–0.97) nmol/mL for mRS 0–3 ($p = 0.045$).

Conclusion: In a large clinical cohort of stroke patients with moderate to severe symptoms, our data suggest that SDMA can assist in differentiation of IS and HS patients already 1 h and a half after symptom onset. SDMA may prove to have future value in a diagnostic stroke biomarker panel.

KEYWORDS

stroke, diagnosis, acute management, biomarkers, SDMA

1 Introduction

The prehospital management of both ischemic and hemorrhagic stroke (IS and HS) is highly time-dependent and benefit from early differential diagnosis to provide optimal prehospital care and direct transfer to an adequately equipped hospital for immediate therapy. In most hospital districts, because deployment of high-cost mobile stroke units has been limited to metropolitan areas, emergency medical services (EMS) lack affordable diagnostic methods for differentiating IS from HS. The recently published INTERACT 4 trial (1) reported divergent effect of prehospital blood-pressure reduction in IS and HS patients, indicating that ideal prehospital management differs greatly between stroke subtypes. The routine use of mechanical thrombectomy in IS patients with large vessel occlusion (LVO) has further emphasized the importance of precision in early triage. Prehospital recognition of LVO would enable timely preparation of an angiography suite prior to hospital arrival, but clinical prehospital LVO scales have shown limited performance in ruling out HS (2). Thus, novel diagnostic methods are needed to optimize prehospital care, to improve triage, and to select patients for future prehospital therapeutic studies.

One promising biomarker candidate for ischemic stroke is the proteolytic metabolite symmetric dimethylarginine (SDMA). This is produced in all nucleated cells during proteolysis when methylated arginine residues of proteins are released into the cytosol (3), with preclinical studies finding the highest tissue concentrations in the brain (4). Upon intracellular accumulation, SDMA is readily liberated into the extracellular space and systemic circulation and can be measured in plasma as a circulating metabolite. SDMA, eliminated mainly via renal excretion, can also serve as a marker for renal function (5).

Preliminary studies have proposed that, in the acute phase of IS, circulating levels of SDMA rise within 6 h of symptom onset (6), and may associate with cardioembolic (CE) stroke etiology (7, 8). In contrast, SDMA levels have not been found to significantly increase in the acute phase of HS (8, 9). Further, elevated levels of SDMA following IS have predicted poor outcome as well as all-cause mortality (10–12). Elevated circulating concentrations of SDMA have also been associated across differing patient populations with cardiovascular disease and all-cause mortality (13). Because circulating levels of SDMA elevate in acute IS, SDMA may have potential as a metabolite biomarker, either on its own or within a biomarker panel, in early differential diagnosis and prognostication of acute stroke patients. Because most diagnostic stroke biomarkers are released by HS (14, 15), those elevated in IS are rarer and of great value. We thus set out to define the very early dynamics of plasma SDMA in acute stroke and its usefulness as a biomarker for early differentiation of IS from HS patients, with a focus on severe stroke cases, the patient group requiring most urgent optimal treatment and transport decisions.

2 Methods

2.1 Study design

The Helsinki Ultra-Acute Stroke Biomarker Study is an observational project aiming to improve diagnostics of acute stroke through discovery of novel blood biomarkers (16, 17). In the hospital district of Helsinki and Uusimaa, all patients considered to be candidates for stroke recanalization therapies, known as stroke code (SC) patients, are transported to the emergency department of Helsinki University Hospital (HUH), the district's only 24/7 neurological service. Emergency medical services (EMS) of the region operate under central management, and all units are staffed by professional emergency medicine technicians or paramedics or both. Prehospital identification of stroke symptoms is based on the Face Arm Speech Test (FAST), and a phone consultation with an on-call neurologist or EMS physician is always available. Contrary to the practice of most other stroke biomarker studies using in-hospital sampling, we trained our EMS personnel to collect prehospital blood samples on site from all SC patients before transportation (17).

Of the 2,392 SC patients admitted to HUH during the study enrolment period between May 20, 2013, and November 19, 2015, 1,015 were included in the final Helsinki Ultra-Acute Stroke Biomarker Study cohort (Figure 1) (16). Following completion of all follow-up investigations, chart review allowed data collection into the study database (16). The National Institutes of Health Stroke Scale (NIHSS) was recorded upon admission, and previously described cut-off limits of moderate and severe strokes (NIHSS > 8 and NIHSS > 15, respectively) served for univariate and area under the receiver-operating characteristics curve (AUC) analyses (18). The definition of poor outcome was 3-month modified Rankin Scale (mRS) score 4–6 (19). The final diagnosis group (IS vs. HS) was based on all available patient records (16). The Field Assessment Stroke Triage for Emergency Destination (FAST-ED) scores to detect LVO (20) were counted retrospectively based on recorded admission NIHSS evaluations, in our district, gaze palsy and neglect not being routinely evaluated by EMS.

We analyzed the diagnostic performance of plasma SDMA in two phases with two complimentary measuring techniques (Figure 1). First, to compare prototypical cases, we selected a representative screening sample of 100 cases with either severe ischemic or hemorrhagic stroke and performed targeted measurements of SDMA in a mass spectrometry (MS) analysis in two sequential acute samples: early samples collected in the prehospital setting and secondary samples collected on hospital admission. To select patients with the most severe strokes in the MS analysis, we only included patients with a positive LVO screening score (FAST-ED ≥ 4) (21) and excluded IS patients without a verified LVO on admission CT-angiography (ICA or M1 occlusion). Of the remaining IS and HS patients, we selected 50 cases with the highest NIHSS scores in each diagnosis group.

In the second phase of the study, we aimed to further validate the differential diagnostic performance of SDMA in an ELISA analysis of a larger series of 227 IS and 84 HS patients, including 98 patients from the MS analysis. We used a threshold of NIHSS ≥ 7, a previously described optimal cut-off value of total NIHSS score to predict LVO (22).

2.2 Sample collection and storage

We have described the EMS training and prehospital sample collection (16, 17). Briefly, EMS personnel collected prehospital

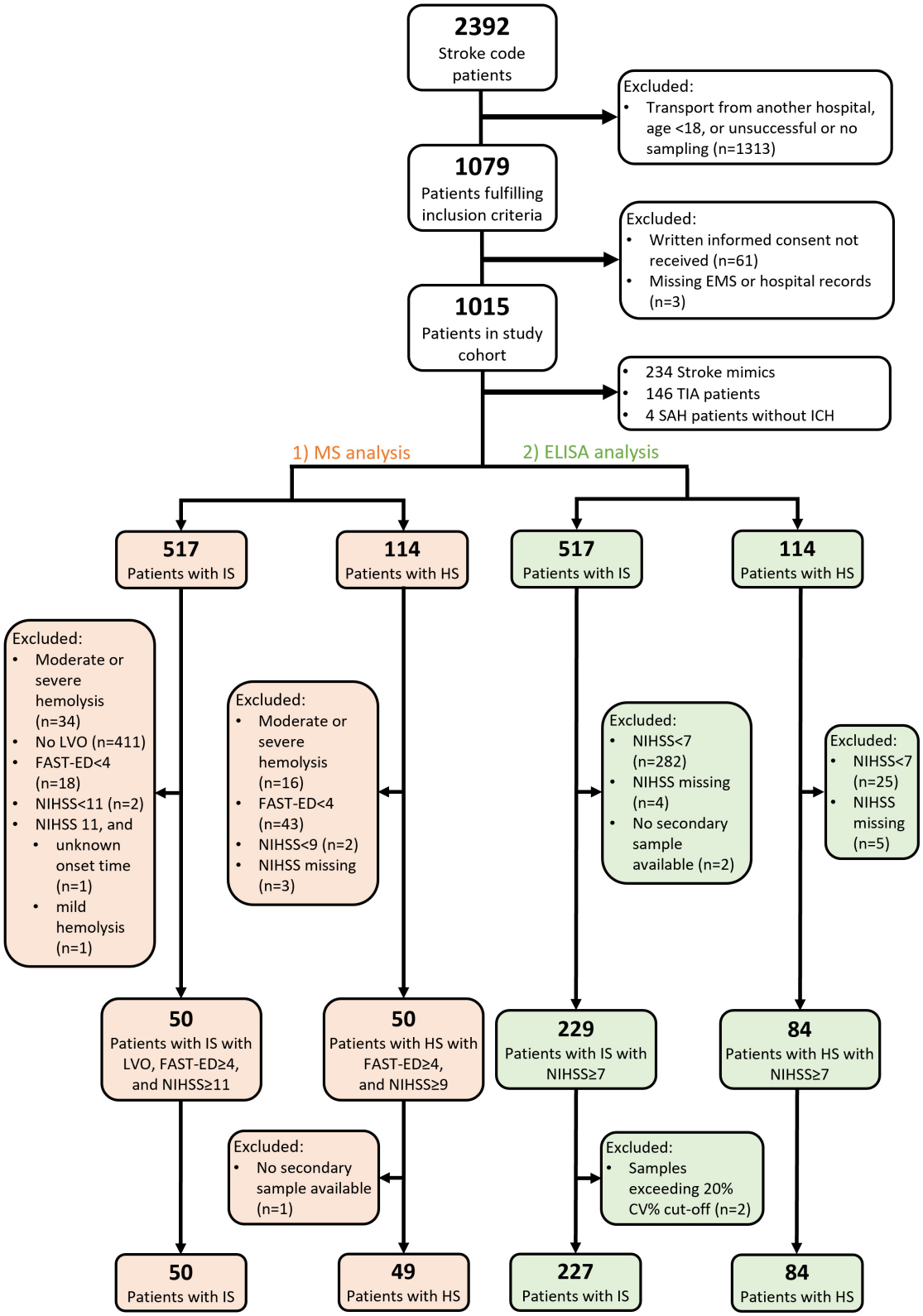

FIGURE 1
Flowchart of patient selection for MS and ELISA analyses. EMS, emergency medical services; TIA, transient ischemic attack; SAH, subarachnoid hemorrhage; ICH, intracranial hemorrhage; IS, ischemic stroke; HS, hemorrhagic stroke; LVO, large vessel occlusion; FAST-ED, Field Assessment Stroke Triage for Emergency Destination; NIHSS, National Institutes of Health Stroke Scale; CV, coefficient of variation.

serum and plasma samples immediately after the on-scene identification of a SC patient by using a cannula adapter and vacuum tubes. In addition to primary prehospital samples, secondary acute samples were collected immediately on hospital arrival. Once in the hospital laboratory, samples were centrifuged at 2,000 g for 10 min at 20°C and divided into cryotubes for storage at −80°C.

2.3 Targeted mass spectrometry analysis of plasma metabolites

In the first phase of the study, the Metabolomics Unit of the Institute for Molecular Medicine Finland (FIMM) performed targeted measurement of plasma metabolites, including SDMA by means of multiple reaction monitoring (MRM) on a ACQUITY UPLC-MS/MS system (Waters Corporation, Milford, MA). Overall, 100 metabolites were measured in a targeted manner, of which SDMA was analyzed for this sub-study. The metabolic profiling protocol has been published (23).

The targeted MS analyses occurred in two separate runs, first for early samples collected in the prehospital setting, then for subsequent secondary samples collected on admission from the same patients. To ensure the comparability of the measurements performed in separate assay runs, 10 samples from the first run were re-analyzed in the second run. We calculated the median percent change of SDMA for the ten control samples between the two runs. Final measurement values of the study samples from the second run were then corrected using the median percent change derived from these control samples [corrected concentration = 2nd run value × (1 + median percent change in 134 controls/100)].

One HS patient without an available secondary sample was excluded from all analyses. Although mass spectrometry measurements were performed with a standard curve for quantitation, due to the screening nature of the assay, we could not verify the absolute level of quantitation, and thus reported results as arbitrary units (A.U.).

2.4 ELISA

For the second phase of the study, we used a commercially available human SDMA ELISA kit from DLD Diagnostika GmbH which is based on acetylation of sample SDMA. Measurements took place in the Neurology Research Unit at the University of Helsinki according to kit inserts provided by the manufacturer. One sample with SDMA measurement above the highest point on the standard curve was re-analyzed at a 5-fold dilution. Inter-assay and intra-assay coefficients of variation (CV%) were 14.9% and 6.1%, respectively. Each sample was measured in duplicate. We determined a 20% CV% cut-off for variation between each measured duplicate. Samples with duplicates exceeding this limit were reanalyzed, and samples from two patients still exceeding this CV% limit after replication were excluded.

2.5 Statistical analysis

Continuous variables are presented as medians and interquartile ranges, and categorical variables as absolute counts and percentages. Univariate analyses utilized the Mann-Whitney U, $\chi 2$, or Fisher exact test where appropriate. The non-parametric Spearman rank test served for measuring correlation. Significance we set at $p \leq 0.05$. We used univariate analyses to analyze intergroup differences of SDMA levels, and linear regression models to correct for possible confounding factors. Due to SDMAs strong elevation in renal impairment, (5) univariate and correlation analyses were repeated for patients with normal creatinine levels. We performed area under the receiver-operating characteristics curve (AUC) analyses to evaluate how well plasma SDMA levels differentiate IS cases from HS. AUCs of 0.9–1.0 have been considered excellent, 0.8–0.9 good, 0.7–0.8 fair, and <0.7 poor. (24) AUC analysis and plotting served to determine optimal cut-off values, and cross-tabulations to calculate diagnostic measures. We performed all analyses with SPSS (v.25, IBM).

3 Results

This study utilized two complimentary measuring techniques: MS and ELISA analyses. We included 99 patients (50 with IS, 49 with HS) in the initial targeted MS analysis and 311 patients (227 with IS, 84 with HS) in the larger ELISA analysis (Figure 1). Ninety eight samples analyzed in both MS and ELISA analyses showed a significant correlation ($\rho = 0.605$, $p < 0.001$). For baseline characteristics (Table 1), no statistically significant differences emerged between IS and HS patients in the MS cohort, but in the ELISA cohort, IS patients had higher rates of underlying coronary artery disease (CAD; $p = 0.016$), of myocardial infarction (MI; $p = 0.032$), and of elevated creatinine ($p = 0.019$). HS patients presented with more severe symptoms, with a median admission NIHSS (IQR) of 15 (11–19) compared to 12 (9–17) in the IS group ($p = 0.002$).

3.1 MS analysis

The median (IQR) delay from last-known-well (LKW) to sampling was 43 min (35–67) for early samples, and 83 min (65–113) for secondary samples. We found no statistically significant difference in plasma SDMA between IS and HS groups in early samples (unadjusted $p = 0.826$). However, in secondary samples, IS patients had significantly higher plasma SDMA levels: 5.8 (5.3–6.9) vs. 5.1 (4.2–5.8) A.U. in HS (unadjusted $p < 0.001$). Notably, we found a significant correlation between plasma SDMA levels and stroke severity (NIHSS) on admission in IS patients (ρ 0.546, $p < 0.001$), but not in HS patients (ρ −0.184, $p = 0.204$). Correspondingly, the inter-group difference was more distinct in patients with severe stroke (NIHSS > 15), being 6.0 (5.6–7.6) for IS vs. 4.9 (4.1–5.3) A.U. for HS (unadjusted $p < 0.001$).

When comparing SDMA levels at two time points, no significant differences emerged in the rate of change between IS and HS groups: 0.13 (0.08–0.20) for IS vs. 0.12 (0.07–0.15) nmol/mL/min for HS group ($p = 0.123$, $n = 96$). However, rate of

TABLE 1 Univariate comparisons of baseline characteristics between IS and HS patients in MS and ELISA analyses.

Variable	MS analysis			ELISA analysis		
	IS ($n = 50$)	HS ($n = 49$)	p-Value	IS ($n = 227$)	HS ($n = 84$)	p-Value
Male, n (%)	29 (58.0)	35 (71.4)	0.162	120 (52.9)	54 (64.3)	0.072
Age, years, median (IQR)	65 (58–71)	67 (59–74)	0.493	71 (63–81)	69 (60–77)	0.075
Time from LKW to secondary sample, min, median (IQR)	77 (65–102)	93 (65–119)[a]	0.182	87 (65–133)[b]	91 (65–116)[c]	0.925
NIHSS on admission, median (IQR)	17 (14–20)	16 (13–20)	0.611	12 (9–17)	15 (11-19)	**0.002**
Creatinine level above reference range, n (%)	9 (18.0)	5 (10.2)	0.388	52 (22.9)[d]	9 (10.7)[e]	**0.019**
Hypertension, n (%)	25 (50.0)	33 (67.3)	0.103	144 (63.4)	59 (70.2)	0.286
Diabetes, n (%)	5 (10.0)	7 (14.3)	0.554	34 (15.0)	10 (11.9)	0.584
Hyperlipidemia, n (%)	16 (32.0)	16 (32.7)	1.000	100 (44.1)	28 (33.3)	0.093
Previous IS, n (%)	5 (10.0)	6 (12.2)	0.760	36 (15.9)	7 (8.3)	0.098
Atrial fibrillation, n (%)	12 (24.0)	7 (14.3)	0.308	59 (26.0)	15 (17.9)	0.177
Congestive heart failure, n (%)	4 (8.0)	3 (6.1)	1.000	22 (9.7)	5 (6.0)	0.369
Coronary artery disease, n (%)	7 (14.0)	4 (8.2)	0.525	46 (20.3)	7 (8.3)	**0.016**
Previous myocardial infarction, n (%)	4 (8.0)	2 (4.1)	0.678	23 (10.1)	2 (2.4)	**0.032**
Current smoking, n (%)	19 (38.0)	12 (24.5)	0.194	64 (28.2)	21 (25.0)	0.668

IS, ischemic stroke; HS, hemorrhagic stroke; MS, mass spectrometry; IQR, interquartile range; LKW, last known well; NIHSS, National Institutes of Health Stroke Scale. [a]$n = 46$; [b]$n = 225$; [c]$n = 81$; [d]$n = 226$; [e]$n = 83$. Bold indicates $p < 0.05$.

change was significantly correlated with admission NIHSS score in IS patients ($\rho = 0.337$, $p = 0.017$, $n = 50$), but not in HS patients ($\rho = -0.015$, $p = 0.919$, $n = 46$). Furthermore, there was no significant correlation with hemorrhage volume in HS patients ($\rho = -0.225$, $p = 0.132$, $n = 98$).

3.2 ELISA

The delay from LKW to secondary sampling was 89 (65–125) min ($n = 306$). Again, plasma SDMA levels were significantly higher in IS patients: 0.82 (0.72–1.01) vs. 0.71 (0.58–0.85) nmol/mL for HS, unadjusted ($p < 0.001$). Box plots of plasma SDMA levels in the secondary samples of IS and HS patients in both MS and ELISA analyses are shown in Figure 2.

3.3 Regression analysis

To correct for possible confounding factors, we assessed results using linear regression in basic (age and sex) and full models (age, sex, creatinine level, NIHSS, CAD, and MI), as in Table 2. Diagnosis group (IS vs. HS) was a significant predictor of SDMA levels in the admission phase in both MS and ELISA models but not in the early phase in MS models. Of note, in the MS models, small sample size limited model stability; in three models we observed a non-normality of residuals, and in one model we observed heteroscedasticity (Table 2). This placed more importance on the results of our ELISA cohort, which showed neither of these imbalances.

3.4 Analyses of patients with normal creatinine levels

As renal insufficiency raises circulating SDMA plasma levels (5), we wanted to verify that intergroup differences remained statistically significant even after excluding patients with admission creatinine levels above the laboratory reference range (>100 μmol/L for men, >90 μmol/L for women, $n = 61$), or with creatinine values missing ($n = 2$). For patients with normal creatinine levels, the intergroup differences in secondary sample SDMA levels remained highly significant in both analyses: 5.68 (5.32–6.42) for IS vs. 4.93 (4.19–5.34) A.U. for HS in MS analysis ($n = 85$, $p < 0.001$), and 0.78 (0.69–0.93) for IS vs. 0.68 (0.56–0.81) nmol/mL for HS in ELISA analysis ($n = 248$, $p < 0.001$). AUCs for differentiating IS and HS patients in ELISA analysis are in Figure 3. For all patients (NIHSS ≥ 7), the discriminatory power was poor (AUC 0.681), but for patients with moderate (NIHSS > 8) and severe symptoms (NIHSS > 15), the discriminatory power was improved (AUCs 0.717 and 0.741, respectively).

Univariate analysis for categorical variables and SDMA levels and correlations between continuous variables and SDMA levels for both patient groups are shown in Tables 3, 4, respectively. In the ELISA analysis, including patients with milder symptoms, we no longer found a correlation with admission NIHSS ($\rho = 0.068$, $p = 0.372$ for IS patients; $\rho = -0.118$, $p = 0.315$ for HS). In IS patients, higher plasma SDMA levels were associated with non-smokers ($p = 0.020$) and a history of atrial fibrillation ($p = 0.029$). No significant correlation existed in HS patients between hemorrhage volume and SDMA levels ($\rho = -0.118$, $p = 0.071$).

To use plasma SDMA as a diagnostic biomarker in a potential biomarker panel, we determined, in our ELISA analysis, the optimal plasma SDMA cut-off value for detecting IS patients while reliably

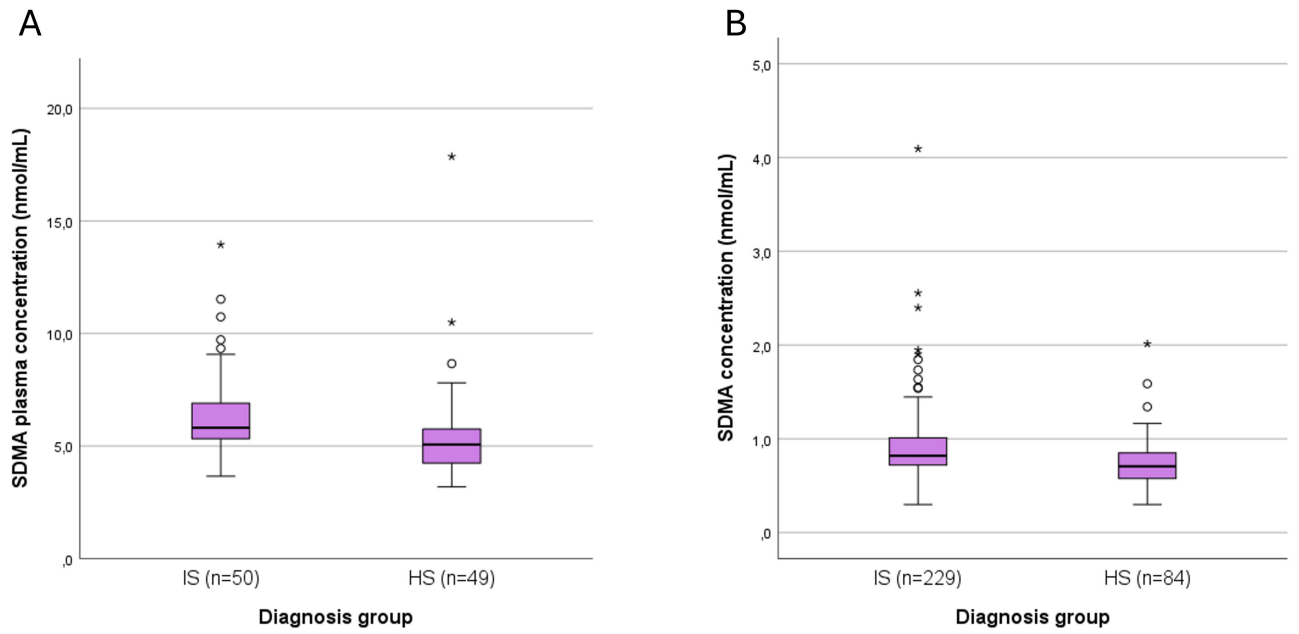

FIGURE 2
Box plots of plasma SDMA levels in the secondary samples of IS and HS patients in both **(A)** MS, and **(B)** ELISA analyses. SDMA, symmetric dimethylarginine; IS, ischemic stroke; HS, hemorrhagic stroke. Outliers are shown as small circles (>1.5 SD from mean) and stars (>3 SD from mean).

ruling out HS. With a SDMA cut-off value of 0.92 nmol/mL and with patients with normal creatinine levels included, we ruled out HS with a high specificity of 90.5% (positive predictive value of 86.5%), but with a very modest sensitivity of 25.9% (negative predictive value of 34.2%).

3.5 Stroke etiology and outcome

Of all IS patients, final stroke etiology was cardioembolic (CE) for 100 (44.1%), large-artery atherosclerosis for 42 (18.5%), small-vessel disease for 8 (3.5%), and other cause determined for 6 (2.6%). For 71 (31.3%) IS patients, stroke etiology could not be determined after adequate investigation. Compared to IS patients with another stroke etiology, patients with CE stroke had higher plasma SDMA levels: 0.84 (0.73–1.09) vs. 0.79 (0.71–0.91) nmol/mL ($p = 0.042$).

Having found SDMA to correlate with stroke severity in initial MS analysis, we analyzed whether SDMA levels could predict outcome in all IS patients. Poor outcome at 3 months (mRS 4–6) was associated with higher SDMA levels: 0.90 (0.73–1.06) vs. 0.80 (0.72–0.97) nmol/mL for mRS 0–3 ($p = 0.045$).

4 Discussion

Our study of the potential role of SDMA as a metabolite biomarker in the early differential diagnosis of acute stroke patients shows SDMA levels to be significantly higher in IS than in HS patients in the early phase of stroke, with differential diagnostic value primarily in patients with severe symptoms. Even after excluding all patients with creatinine levels above the laboratory reference rate due to SDMA's strong correlation with renal function (5), the intergroup difference remained significant. We also found higher plasma SDMA levels in IS patients to be associated with CE stroke etiology and poor outcome at 3 months.

This is, to our knowledge, the first study to compare SDMA plasma levels in the very early phase of IS and of HS. We evaluated SDMA's diagnostic performance in two phases with two complimentary measuring techniques. First, we used a screening sample of 100 cases with IS or HS for targeted SDMA measurement by mass spectrometry. A further ELISA analysis of a larger sample set of 311 patients then allowed validation of SDMA's diagnostic performance.

Our cohort consists of uniquely early stroke samples with a median delay from LKW to sampling of only 43 min for early and 83 min for secondary samples in the MS analysis, and 89 min for secondary samples in the ELISA analysis. Though no significant differences emerged in our very early prehospital samples, the timepoint of our secondary samples is highly appropriate in the light of other thrombectomy studies (25), with our results highlighting the diagnostic value of SDMA within the first few hours after stroke.

Dimethylarginines, symmetric, and asymmetric dimethylarginine (ADMA), are produced in all nucleated cells when post-translational methylation of arginine residues in proteins is followed by proteolysis (26). ADMA is an inhibitor of NO synthesis, whereas SDMA reduces NO levels indirectly by competing with cellular L-arginine uptake (27). Wide investigation of the role of ADMA has shown it to be associated in various patient populations with cardiovascular risk factors, cardiovascular events, and even death (28). The role of its structural isomer, SDMA, has attracted less study. Recent reports have shown, across

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TABLE 2 Linear regression models for early and secondary SDMA plasma levels in MS analysis, and for secondary SDMA plasma levels in ELISA analysis.

	MS analysis					
	Early samples			Secondary samples		
	Model 1 ($n = 99$)			Model 1 ($n = 99$)		
Variable	B (CI 95%)	SE B	*p*-Value	B (CI 95%)	SE B	*p*-Value
Age	0.003 (−0.007, 0.014)	0.005	0.525	0.050 (0.008–0.092)	0.021	**0.020**
Sex	0.033 (−0.203, 0.270)	0.119	0.781	−0.323 (-1.261, 0.616)	0.473	0.496
Diagnosis group	0.043 (−0.175, 0.260)	0.109	0.698	−1.046 (-1.908, −0.185)	0.434	**0.018**
Variable	Model 2 ($n = 99$)			Model 2 ($n = 99$)		
	B (CI 95%)	SE B	p-Value	B (CI 95%)	SE B	p-Value
Age	0.003 (−0.008, 0.014)	0.005	0.554	0.035 (0.008, 0.061)	0.013	**0.011**
Sex	0.055 (−0.199, 0.309)	0.128	0.670	0.665 (0.044, 1.287)	0.313	**0.036**
Diagnosis group	0.048 (−0.176, 0.272)	0.113	0.672	−0.700 (−1.249, −0.152)	0.276	**0.013**
NIHSS on admission	−0.003 (−0.030, 0.024)	0.013	0.815	0.042 (−0.024, 0.107)	0.033	0.207
Creatinine on admission	0.001 (−0.003, 0.005)	0.002	0.634	0.059 (0.049, 0069)	0.005	**<0.001**
Coronary artery disease	−0.043 (−0.553, 0.467)	0.257	0.867	−1.145 (−2.393, 0.103)	0.628	0.072
Previous myocardial infarction	0.024 (−0.642, 0.690)	0.335	0.943	0.329 (−1.300, 1.958)	0.820	0.689

	ELISA analysis		
	Secondary samples		
	Model 1 ($n = 311$)		
Variable	B (CI 95%)	SE B	*p*-Value
Age	0.008 (0.005–0.011)	0.289	**<0.001**
Sex	−0.087 (−0.165, −0.010)	−0.124	**0.028**
Diagnosis group	−0.148 (−0.232, −0.065)	−0.188	**0.001**
	Model 2 ($n = 309$)		
Variable	B (CI 95%)	SE B	*p*-Value
Age	0.003 (0.0004, 0.005)	0.102	**0.024**
Sex	0.068 (0.006, 0.131)	0.097	**0.032**
Diagnosis group	−0.089 (−0.154, −0.023)	−0.113	**0.008**
NIHSS on admission	4.29E-5 (−0.005, 0.005)	0.001	0.988
Creatinine on admission[a]	0.007 (0.006, 0.008)	0.675	**<0.001**
Coronary artery disease	−0.005 (−0.105, 0.095)	−0.006	0.916
Previous myocardial infarction	−0.023 (−0.159, 0.114)	−0.018	0.746

SDMA, symmetric dimethylarginine; B, beta coefficient; CI, confidence interval; SE, standard error; NIHSS, National Institutes of Health Stroke Scale. [a]$n = 309$. Bold indicates $p < 0.05$.

various populations, an independent link between increased SDMA concentrations and cardiovascular disease, and all-cause mortality (13). Consistent with the present results, Molnar et al. (6) has suggested that within 6 h of symptom onset, circulating levels of SDMA rise. For now, the mechanism behind this is unclear.

Our finding of significantly higher circulating SDMA levels in IS compared to those in HS patients is in line with earlier findings of elevated SDMA levels in the acute phase of IS compared to levels in patients with asymptomatic significant carotid stenosis and healthy controls (6). Additionally, levels have not been found to significantly increase in the acute phase of HS (8, 9). Among all IS patients in our ELISA analysis, when compared to other stroke etiologies, elevated SDMA levels were most associated with CE stroke ($p = 0.042$). This proved consistent with the findings of Tiedt et al. and Wanby et al. (7, 8). Notably, SDMA levels have been reported to be a potential marker of atrial fibrillation in ESUS stroke patients (29), and in our study, we report an association of atrial fibrillation and higher SDMA levels in IS patients ($p = 0.029$). Our work also supports the notion of higher SDMA levels as being associated in IS patients with poor outcome, as others also suggest (9, 10).

Promising diagnostic biomarkers differentiating IS and HS include GFAP (30, 31), RBP4 (32), PARK7 (33), and apolipoproteins ApoC-I and ApoC-III (34). However, no

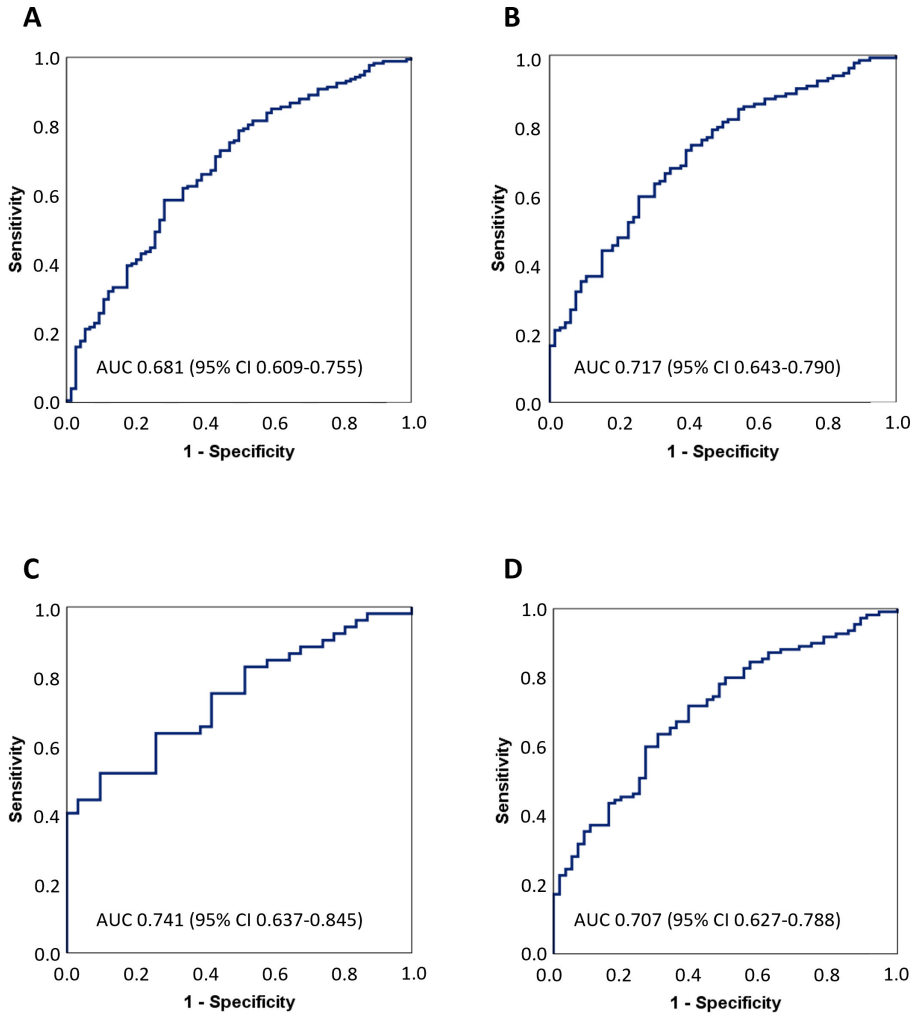

FIGURE 3
Receiver-operating characteristic curves for differentiating ischemic stroke from hemorrhage stroke patients. Patients with missing creatine values or levels above reference range were excluded from analyses. Area under the curve (AUC) analyses are shown separately for patients with **(A)** NIHSS $\geq$ 7, $n = 248$; **(B)** NIHSS > 8, $n = 201$; **(C)** NIHSS > 15, $n = 83$; and **(D)** FAST-ED $\geq$ 4 on admission, $n = 166$. CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; FAST-ED, Field Assessment Stroke Triage for Emergency Destination score.

individual biomarker has yet made it into clinical practice, and accurate out-of-hospital differential diagnosis of stroke patients may require a panel consisting of several independently established complementary biomarkers. Our results indicate that SDMA has value for the development of such diagnostic biomarker panels and is notable as a circulating biomarker that is elevated early after IS, a rare type of finding. Importantly, the best, though only fair, diagnostic value was seen for stroke code patients with severe symptoms (AUC 0.741 for patients with NIHSS > 15; AUC 0.717 for patients with NIHSS > 8; AUC 0.681 for patients with NIHSS $\geq$ 7), a significant subgroup for LVO diagnostics.

Our study has limitations. To serve as an explorative analysis to best uncover intergroup differences, we included only IS and HS patients with moderate to severe symptoms (NIHSS $\geq$ 9 for MS analysis and NIHSS $\geq$ 7 for ELISA). However, patients with suspected acute stroke include those with minor stroke, TIA and stroke mimics. Studies should thus include all patient groups with differing symptom severities. Interestingly, a recent study utilizing untargeted metabolite screening found SDMA to be a promising biomarker for differentiating IS from other conditions mimicking stroke symptoms (7). Secondly, our SDMA values come from two complimentary analyses. As we could not verify the absolute level of quantitation in the MS analysis, we report MS results as arbitrary units. Levels of our ELISA results are consistent with the reference intervals of 0.32–0.65 and 0.225–0.533 nmol/mL reported by El-Khoury et al. and Schwedhelm et al. (35, 36). Importantly, our comparisons were only within these measurement sets, not between them. Thirdly, as SDMA had only fair discriminatory power even for patients with severe symptoms and normal creatinine levels (AUC 0.741), it should be used as a part of a stroke biomarker panel. Furthermore, the strong correlation between SDMA and glomerular filtration rate might limit clinical use of SDMA. However, creatinine point-of-care testing can identify patients with normal kidney function even in a prehospital setting and could be utilized as a part of a biomarker panel to rule out cases with renal insufficiency. Finally, as all EMS units of our district participated in the study recruitment, it was not feasible to achieve consecutive recruitment of all SC patients.

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TABLE 3 Univariate analysis of categorical variables and SDMA levels of patients with normal creatinine levels in ELISA analysis.

	Diagnosis class					
	IS ($n = 174$)			HS ($n = 74$)		
	n	SDMA concentration, nmol/mL, median (IQR)	*p*-Value	*n*	SDMA concentration, nmol/mL, median (IQR)	*p*-Value
Variable						
Sex						
Male	91	0.76 (0.69–0.90)	0.309	48	0.70 (0.59–0.83)	0.167
Female	83	0.80 (0.71–0.94)		26	0.67 (0.53–0.75)	
Hypertension						
Yes	104	0.78 (0.70–0.93)	0.471	50	0.68 (0.56–0.75)	0.862
No	70	0.78 (0.68–0.90)		24	0.70 (0.55–0.83)	
Diabetes						
Yes	25	0.79 (0.72–0.88)	0.663	8	0.62 (0.54–0.74)	0.384
No	149	0.77 (0.69–0.93)		66	0.69 (0.56–0.81)	
Hyperlipidaemia						
Yes	69	0.80 (0.71–0.92)	0.425	21	0.73 (0.62–0.76)	0.483
No	105	0.76 (0.69–0.93)		53	0.68 (0.55–0.81)	
Previous IS						
Yes	24	0.78 (0.72–0.95)	0.619	5	0.73 (0.65–0.74)	0.818
No	105	0.78 (0.69–0.93)		69	0.68 (0.56–0.81)	
Atrial fibrillation						
Yes	38	0.84 (0.74–1.01)	**0.029**	13	0.68 (0.66–0.81)	0.253
No	136	0.77 (0.69–0.89)		61	0.69 (0.55–0.75)	
Congestive heart failure						
Yes	13	0.83 (0.75–0.92)	0.246	4	0.78 (0.65–1.19)	0.278
No	161	0.78 (0.69–0.93)		70	0.68 (0.56–0.81)	
Coronary artery disease						
Yes	31	0.85 (0.73–0.97)	0.067	6	0.70 (0.67–0.75)	0.513
No	143	0.76 (0.69–0.91)		68	0.68 (0.55–0.81)	
Previous myocardial infarction						
Yes	12	0.85 (0.71–1.02)	0.321	2	0.74 (0.62–0.86)	0.644
No	162	0.77 (0.69–0.91)		72	0.68 (0.56–0.80)	
Current smoking						
Yes	54	0.76 (0.60–0.84)	**0.020**	19	0.66 (0.58–0.82)	0.625
No	120	0.80 (0.72–0.94)		55	0.69 (0.55–0.81)	

SDMA, symmetric dimethylarginine; IS, ischemic stroke; HS, haemorrhagic stroke; IQR, interquartile range. Bold indicates $p < 0.05$.

5 Conclusions

Our data demonstrate that circulating levels of plasma SDMA elevate in the acute phase of severe IS and can assist in differentiation of early blood biochemical profiles between those in IS and HS during a clinically meaningful acute rime window. Our findings may thus be beneficial in development of stroke biomarker panels for detection of severe stroke when time from onset to sampling is approximately 90 min.

Importantly, our findings concerning SDMA are highly relevant in stroke biomarker discovery, since biomarker elevations have generally appeared in HS where tissue disruption and cellular constituent liberation are sudden, but appear less often in ischemia, where metabolism and biomarker release are typically downregulated before tissue disruption.

TABLE 4 Correlations of continuous variables and SDMA levels of patients with normal creatinine levels in ELISA analysis.

	Diagnosis class					
	IS ($n = 174$)			HS ($n = 74$)		
	n	ρ	*p*-Value	n	ρ	*p*-Value
Variable						
Age	174	0.194	**0.010**	74	0.124	0.292
NIHSS on admission	174	0.068	0.372	74	−0.118	0.315
Hemorrhage volume	-	-	-	73	−0.213	0.071
Time from LKW to secondary sample (min)	172	0.020	0.792	71	0.072	0.549
Creatinine levels on admission (umol/l)	174	0.161	**0.034**	74	0.271	**0.019**
HbA1c (mmol/mol)	117	0.086	0.354	47	−0.037	0.803
Total cholesterol (mmol/l)	119	−0.031	0.742	47	0.074	0.623
HDL-cholesterol (mmol/l)	119	−0.045	0.627	47	−0.201	0.175
LDL-cholesterol (mmol/l)	119	−0.058	0.530	47	0.146	0.328
Triglycerides (mmol/l)	119	0.051	0.581	50	0.078	0.591

SDMA, symmetric dimethylarginine; IS, ischemic stroke; HS, haemorrhagic stroke; NIHSS, National Institutes of Health Stroke Scale; LKW, last known well; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Bold indicates $p < 0.05$.

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 humans were approved by ethical and administrative review boards of the Hospital District of Helsinki and Uusimaa, Finland. The studies were conducted in accordance with the local legislation and institutional requirements. The 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

SP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. OM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. TN: Investigation, Methodology, Resources, Writing – review & editing. MK: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. HH-R: Investigation, Methodology, Project administration, Resources, Writing – review & editing. JR: Data curation, Methodology, Resources, Writing – review & editing. GS: Investigation, Methodology, Project administration, Writing – review & editing. SC: Investigation, Methodology, Project administration, Writing – review & editing. DS: Investigation, Methodology, Project administration, Writing – review & editing. MP: Investigation, Methodology, Project administration, Writing – review & editing. TT: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. PL: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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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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Robert G. Kowalski,
University of Colorado Anschutz Medical Campus, United States

REVIEWED BY
Marieta Peycheva,
Plovdiv Medical University, Bulgaria
Ting-Yi Lin,
Johns Hopkins Medicine, United States

*CORRESPONDENCE
Laura C. Gioia
✉ Laura.gioia@umontreal.ca

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Serum glial fibrillary acidic protein in acute stroke: feasibility to determine stroke-type, timeline and tissue-impact

Julien F. Paul[1,2,3], Célina Ducroux[1,2,3,4], Pamela Correia[1,2,3], Audrey Daigneault[3], Catherine Larochelle[1,2,3], Christian Stapf[1,2,3] and Laura C. Gioia[1,2,3]*

[1]Department of Neurosciences, University of Montreal, Montréal, QC, Canada, [2]Division of Neurology, Department of Medicine, Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, QC, Canada, [3]Neurosciences Axis, Centre de Recherche du CHUM (CRCHUM), Montréal, QC, Canada, [4]Division of Neurology, The Ottawa Hospital, Ottawa, ON, Canada

Background: Interest is emerging regarding the role of blood biomarkers in acute stroke. The aim of this pilot study was to determine the feasibility of biomarker acquisition in suspected acute stroke, using modern ultrasensitive immunoassay techniques, and explore their potential usefulness for stroke diagnosis and management.

Methods: In 62 patients with suspected acute stroke, blood samples were prospectively obtained upon arrival and prior to neuroimaging. Serum levels of glial fibrillary acidic protein (sGFAP) and neurofilament light chain (sNfL) were analyzed using a single molecule array (SIMOA®) method, according to time of symptom onset, neuroimaging, and final diagnosis.

Results: Acute ischemic stroke (AIS) was diagnosed in 35 patients, 10 with large-vessel occlusion (LVO). The remaining were diagnosed with intracerebral hemorrhage (ICH) (n = 12), transient ischemic attack (n = 4), and stroke mimics (n = 11). Median (IQR) sGFAP levels were significantly higher in ICH (2,877.8 [1,002.1–10,402.5] pg./mL) compared to others diagnoses. In AIS, GFAP levels appear to increase with longer delays since symptom onset and were higher in patients with more extensive ischemic changes on baseline CT (ASPECTS ≤7) than those without, particularly in LVO stroke. NfL values were similar across groups.

Conclusion: In acute stroke, serum GFAP levels show potential as an adjunct tool for the distinction between ICH and AIS. Specific to AIS, GFAP may also offer insight into time from onset, and extent of ischemic tissue injury on neuroimaging, particularly in LVO stroke. These preliminary findings merit further study.

KEYWORDS

ischemic stroke, intracerebral hemorrhage, biomarkers, GFAP, large-vessel occlusion

Introduction

Current management of acute ischemic stroke (AIS) and intracerebral hemorrhage (ICH), requires (1) neuroimaging to provide a diagnosis and (2) a time of symptom onset to determine eligibility for treatment. In up to 30% of patients, however, time of symptom onset is either unknown or exceeds recommended time windows for treatment (1). Advanced neuroimaging biomarkers are now widely recognized key criteria in acute stroke management, independent of time, to identity eligible patients (2). Accordingly, a paradigm shift is evolving away from time-based decision algorithms and toward physiology-based acute stroke management strategies. By extension, brain-specific blood biomarkers may offer an innovative opportunity to provide physiology-based information and potentially improve accessibility to acute stroke treatments.

Glial fibrillary acidic protein (GFAP), an astrocytic protein found almost exclusively in the brain, is a promising biomarker of brain tissue damage in neurological conditions, including stroke (3). In the first 6 h, GFAP can discriminate between AIS and ICH (4), presumably due to acute brain damage incurred following hematoma formation and the immediate disturbance of the brain blood barrier in ICH compared to AIS where there is a slower transition between penumbral tissue into core over time in the absence of reperfusion (5). Neurofilament light chain (NfL), a novel biomarker for axonal injury, shows promise as a prognostic tool following AIS (6). However, data regarding its use in acute stroke is limited. In most studies, GFAP was measured using ELISA techniques (expressed in ng/mL) (7) or did not include stroke patients beyond the hyperacute phase (8), thus limiting information in acute stroke of unknown or delayed onset. The aim of this pilot study was to evaluate the feasibility of blood biomarker acquisition upon Emergency Department (ED) arrival in suspected acute stroke using Single Molecule Array (SIMOA), a novel ultrasensitive immunoassay. In addition, we conducted exploratory analyses to compare biomarker levels according to time of symptom onset, acute neuroimaging, and final diagnosis.

Methods

Study design

In this single-center prospective observational, descriptive pilot study, patients evaluated for a suspected acute stroke <24 h from known symptom onset or last seen well were recruited over 6 months at our comprehensive stroke center.

Sample collection and storage

Blood samples were obtained during routine blood draw upon ED arrival. After 30 min to allow for coagulation, samples were centrifuged at 1800 g for 10 min at 15–24°C to separate cells and serum. Serum was then frozen at −80°C in aliquoted cryotubes until analysis.

SIMOA analysis

Serum GFAP (sGFAP) and serum NfL (sNfL) levels were measured in duplicates with the SR-X detection system using the SIMOA Neurology 2-Plex B Kit (Quanterix, Billerica, MA, USA). Analysis was carried out according to the manufacturer's instruction. Briefly, SIMOA is a highly-sensitive multiplex technique using paramagnetic antibody-coated microbeads specific to GFAP and NfL, which emit a signal if detected in patient serum by immunofluorescence which is then converted digitally for bead (and biomarker) quantification (9).

Data collection

Clinical data was collected from a data repository of all acute stroke patients evaluated at our center, where we extracted the following variables: age, sex, relevant past medical history (previous stroke, epilepsy, or cognitive disorders), clinical stroke scales [National Institutes of Health Stroke Scale (NIHSS), modified Rankin Score (mRS)] and variables of interest including time of stroke onset, time of ED arrival, neuroimaging data presence of ICH or early ischemic changes as per the Alberta Stroke Program Early CT Score (ASPECTS), and presence of large-vessel occlusion (LVO) on CT angiography, final diagnosis. Time to blood acquisition and centrifugation were also recorded as part of study procedures.

Statistical analyses

Variables were reported as mean ± standard deviation (SD), median and interquartile range (IQR) or proportions (%) as appropriate. Due to skewed data distribution, sGFAP and sNfL values were compared across final diagnosis group using the Kruskall-Wallis test, followed by Dunn's post-hoc test for pairwise comparisons. Correlation between time of stroke onset and sGFAP values was assessed using the Spearman's rank correlation coefficient (ρ), stratified by LVO presence. Comparisons between unfavorable ($\leq$7) and favorable (8–10) ASPECTS were performed using the Wilcoxon rank-sum test. To examine potential interactions between time, ASPECTS, cognitive disorder or previous stroke and sGFAP levels, linear regression analyses were conducted, with sGFAP values log-transformed to address skewness in the data distribution.

Results

Blood samples at ED arrival were collected in 68 patients with suspected acute stroke. Of these, 6 were excluded due to delays in sample processing. Among the remaining 62 patients, median (IQR) time from ED arrival to sample acquisition was 11 (9–24) minutes and 48 (37–70) minutes from sample acquisition to centrifugation. Regarding biomarker analyses, intra-assay variabilities (coefficient of variation, CV, in %) for duplicate measures was 6.2% (sGFAP) and 6.9% (sNfL); 88% of samples had intra-assay CV <20%.

Baseline characteristics are summarized in Table 1. AIS was diagnosed in 35 patients, of which 10 (29%) had a large-vessel

TABLE 1 Characteristics of study population.

Study population	62
Age, years (mean ± SD)	68.5 ± 16.5
Female sex, *n* (%)	27 (44)
Past Medical History *n* (%)	
- Previous Stroke	14 (23)
- Intracerebral Hemorrhage	2 (3)
- Epilepsy	3 (5)
- Neurocognitive Disorder	9 (14.5)
Modified Rankin Score, median(IQR)	1 (0–1)
Initial NIHSS, median(IQR)	9 (3–17)
Sample processing delays, minutes (median(IQR))	
ED arrival to sample acquisition	11 (9–24)
Symptom onset to sample acquisition	101 (73–149)
Last seen well to sample acquisition	683 (287–917)
Sample acquisition to centrifugation	48 (37–70)
By final diagnosis	
(A) Acute Ischemic Stroke	35
- LVO Stroke	10
- ASPECTS, median(IQR)	10 (9–10)
- ASPECTS ≤7	5 (14)
- Known stroke onset, n (%)	12 (34)
- Time, stroke onset to ED arrival	63.5 (46–90)
- Time, last seen well to ED arrival	698 (282–861)
(B) Intracerebral Hemorrhage	12
- Known onset	7 (58)
- Time, onset to ED arrival	90 (66–99)
- Time, last seen well to ED arrival	329 (185–350)
(C) Transient Ischemic Attack	4
(D) Stroke Mimics	11

ASPECTS, Alberta Stroke Program Early CT Score, ED, Emergency Department, NIHSS, National Institutes of Health Stroke Scale.

occlusion (LVO). ICH was diagnosed in 12, transient ischemic attack (TIA) in 4, and stroke mimics in 11, where stroke mimics included seizures, functional neurological disorder and brain tumor (1). Median time from symptom onset to sample acquisition was 101 (73–149) minutes in the whole study population. Among the 12 AIS patients with known time of stroke onset, median time from stroke onset to sample acquisition was 91 (55–118) minutes. Median time from last seen well to sample acquisition was 738 (297–917) minutes in the 23 patients with stroke of unknown onset.

Acute stroke patients (AIS or ICH) had higher median sGFAP concentrations (384.9 [164.3–1.576.6] pg./mL) compared to those with TIA (254.2(175.4–348.1 pg./mL) or stroke mimics 106.7 (44.9–207.5 pg./mL) $p < 0.001$), with ICH showing highest median sGFAP levels (2,877.8 [1,002.1–10,402.5] pg./mL) (Figure 1A). sNfL levels did not differ between groups (ICH [24.1(16.4–54.3) pg./mL]), AIS (39.8 [20.3–78.6] pg./mL), TIA (30.3(23.2–39.3) pg./mL), stroke mimics (20.5(6.4–379.8) pg./mL) (Figure 1B).

Five out of 35 AIS patients reported a history of cognitive disorders. Linear regression analysis revealed no significant interaction between cognitive decline or age and sGFAP levels. Additionally, 6 AIS patients had a history of previous stroke. Regression analyses revealed a positive interaction exists between prior stroke and sGFAP levels. (Supplementary Table S1) However, the presence of a prior stroke was associated with lower sGFAP levels. No AIS patients had a history of epilepsy.

Among AIS patients with known time of stroke onset <4.5 h ($n = 12$), sGFAP levels appeared to increase with longer delays from symptom onset, particularly among the 5 patients with LVO stroke ($\rho = 0.9$, $p = 0.08$) (Figure 2A), but was not observed among those with stroke of unknown onset ($\rho = 0.39$, $p = 0.07$). No correlation was observed between sNfL levels with time from stroke onset, in both LVO ($R = 0.8$, $\rho = 0.13$) and non-LVO subgroups ($\rho = 0$, $p = 1$) (Figure 2C).

Regarding neuroimaging, all 12 patients with known stroke onset presented with favorable ASPECTS (8–10), and none with ASPECTS ≤7. Among the 23 patients with unknown time of stroke onset and > 4.5 h from last seen well, 5 had an ASPECTS score ≤ 7. Median sGFAP levels were higher in AIS with ASPECTS ≤7 ($n = 5$) (1,578.9(239.8–2,370.5) pg./mL) than those with ASPECTS 8–10 ($n = 30$) (246.7(135.5–444.5) pg./mL) ($p = 0.2$) (Figure 2B). Median sNfL levels were similar in ASPECTS ≤7 (24.4(21.0–91.6) pg./mL) and ASPECTS 8–10 (42.7(20.1–76.4) pg./mL) ($p = 0.87$) (Figure 2D). Regression analysis revealed no significant interaction between time from last seen well, ASPECTS, and GFAP levels in stroke of unknown onset. (Supplementary Table S1).

Of note, 3 patients had higher sGFAP levels despite favorable ASPECTS. In detail, one of these patients presented with stroke of known onset and an ASPECTS 10 on baseline non-contrast CT had a markedly elevated sGFAP level (1574.4 pg./mL). However, MRI imaging demonstrated an embolic shower of innumerable millimetric DWI lesions. (Figure 3C). Among the other 2 patients, both presented with stroke of unknown onset. Despite being classified as ASPECTS scores of 8 and 9 respectively, both patients had consolidated hypodense infarcts (in contrast to more subtle early ischemic changes) (Figures 3B,D).

Discussion

Brain-specific blood biomarker acquisition and analysis is feasible in acute stroke and shows promise as an adjunct tool in acute stroke management. In our study, sGFAP levels were markedly higher in acute ICH than AIS using a modern ultrasensitive immunoassay (SIMOA), consistent with previous published studies (7, 10). In exploratory analyses, we also found that among patients with AIS of unknown onset, median GFAP levels were higher in those with more extensive changes on baseline neuroimaging. Furthermore, among patients with known time of stroke onset, serum GFAP levels appear to increase with longer delays since symptom onset, particularly in LVO stroke.

To our knowledge, this is the first study assessing sGFAP levels in the emergency setting of acute stroke that included stroke of unknown onset beyond 4.5–6 h. In this subgroup, no clear trend was observed between time from last seen well and sGFAP levels, likely due to the heterogeneity of the population of stroke patients with unwitnessed

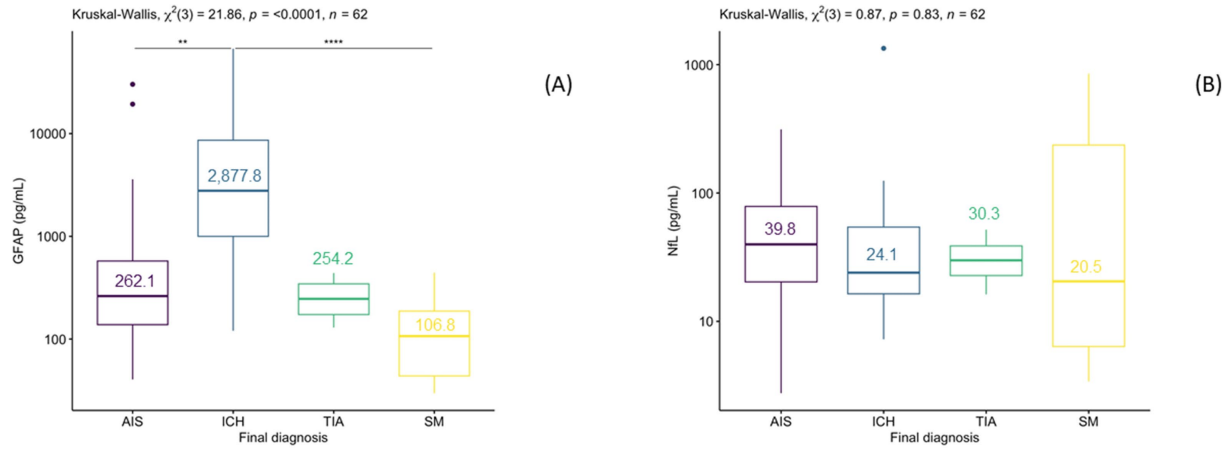

FIGURE 1
Box plot of median sGFAP values **(A)** and sNfL **(B)** values (pg/mL) in patients presenting with suspected stroke, classified according to final diagnosis.

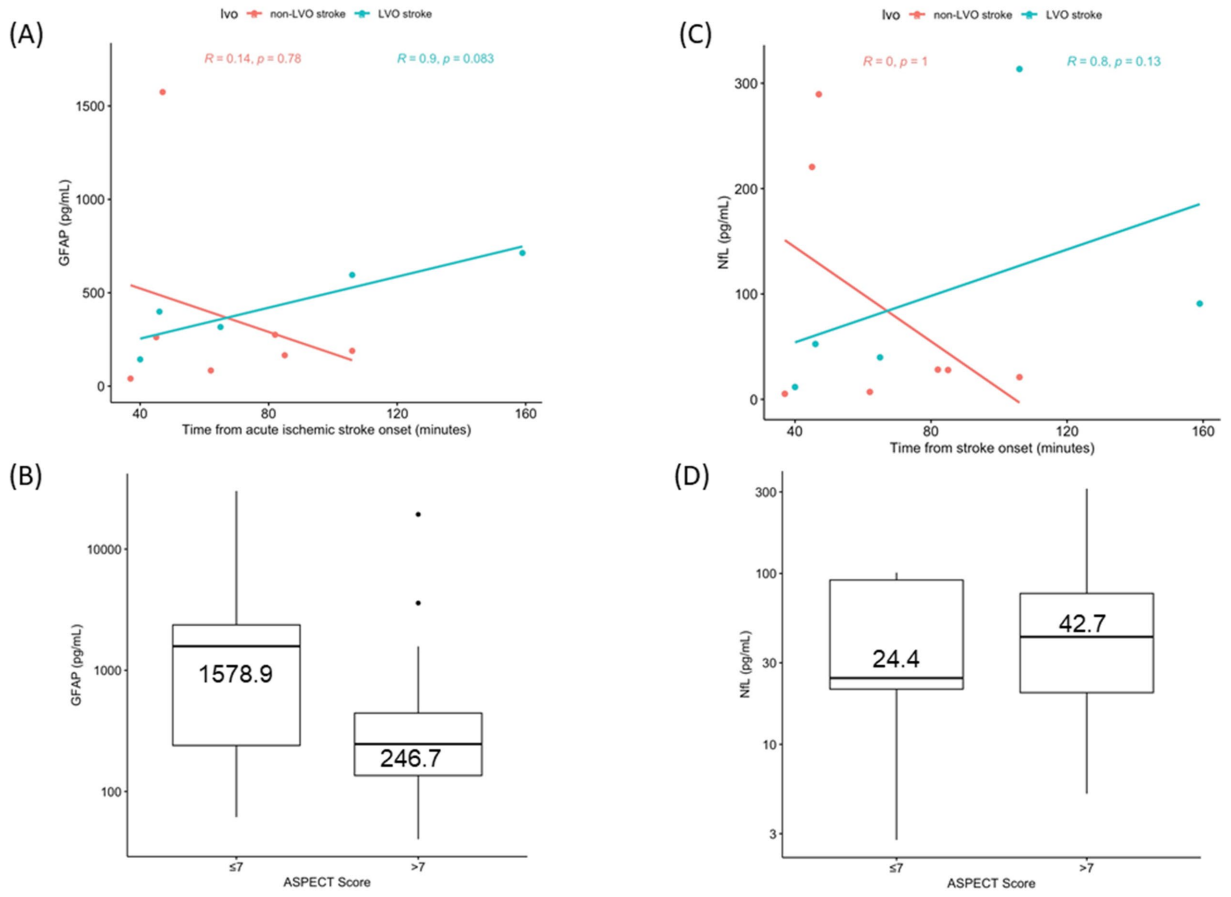

FIGURE 2
Scatterplots with regression lines of sGFAP **(A)** and sNfL **(C)** in Acute Ischemic Stroke with known onset, stratified for LVO presence. Boxplot of sGFAP **(B)** and sNfL **(D)** according to ASPECTS.

stroke onset (including both those with prolonged symptoms and those with a more recent onset). Among patients with known time of stroke onset, we observed a linear relationship between sGFAP levels and time, particularly in LVO stroke. However, given the small sample size and the absence of serial measurements of GFAP over time in each patient, these findings should be interpreted with caution and are meant to be hypothesis-generating only. Nevertheless, these findings raise hypotheses regarding the potential role of sGFAP as a surrogate

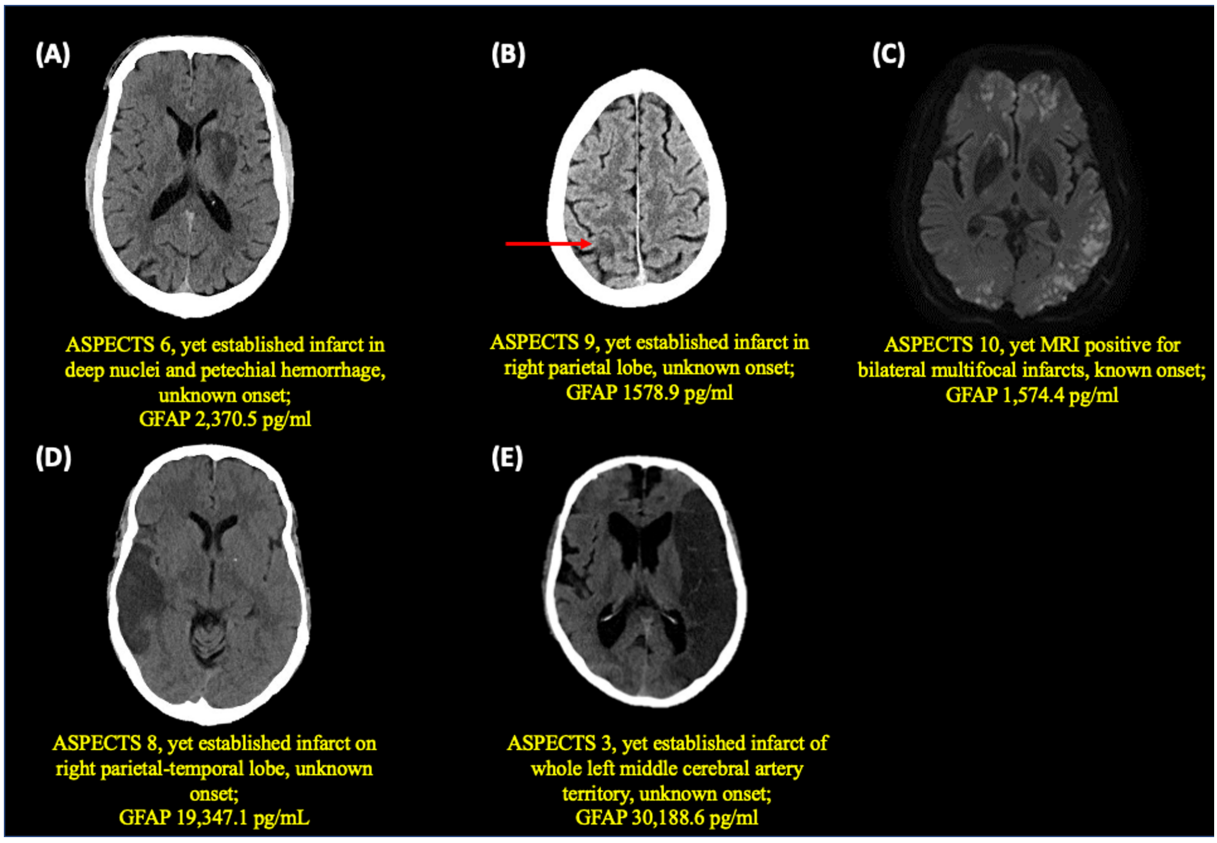

FIGURE 3
Baseline neuroimaging in 5 patients **(A–E)** with highest sGFAP values (>1,000 pg./mL).

marker for ischemic injury over time in acute stroke, particularly in the context of evolving change of ischemic core into penumbra over time in LVO stroke. A recent study of GFAP acquisition in the early phase of stroke onset also observed that blood GFAP levels increases with time (11).

Secondly, we observed that higher median sGFAP levels were associated with unfavorable imaging (ASPECTS≤7). While GFAP release patterns in acute ischemic stroke are not yet fully understood, we and others hypothesize that more extensive parenchymal damage is associated with more astrocytic damage and higher disruption of the blood–brain barrier, resulting in higher GFAP release in the bloodstream (3). While these data are preliminary, they raise the question as to whether sGFAP levels could serve as a potential surrogate marker for ischemic brain injury in stroke of unknown onset. In our study, we did not observe a significant interaction between time, ASPECTS and GFAP levels. However, the results and their interpretation are limited given that time of stroke of onset is unknown in this subgroup, as well as a risk of model overfitting on account of the small number of subjects with low ASPECTS scores in the study. Nonetheless, this concept is of interest and warrants further investigation in larger studies to better understand the relationship between time, ASPECTS, and sGFAP levels.

Notably, we also observed elevated sGFAP levels in some patients despite a favorable ASPECTS scores. Further analyses revealed that despite "higher" ASPECTS scores (which generally indicate limited brain parenchymal damage), brain imaging revealed more consolidated or established infarcts in 2 patients and diffuse multifocal small infarcts on MRI not easily discernable on non-contrast CT in 1 patient. These cases underscore some of the limitations of ASPECTS in assessing the extent and evolution of brain infarcts on CT scan (12). At the same time, they also highlight the potential of brain-specific blood biomarkers, such as GFAP, to provide insight into the extent of brain tissue damage beyond standard neuroimaging, supporting their potential role as adjunct tools in acute stroke management.

This concept is of particular interest given emerging portable point-of-care technology, such as the i-STAT® platform (13), able to measure plasma GFAP levels within minutes (14). Indeed, the need to develop portable tools to optimize acute stroke management was recently emphasized by the INTERACT-4 study assessing medical management in the ambulance in suspected yet undifferentiated stroke. In this study, intensive blood pressure reduction was associated with better outcomes in ICH yet showed harm in AIS, stressing the need to better differentiate stroke type in the ambulance (15). Current point-of care technology to assess GFAP levels requires centrifugation to obtain plasma, yet studies are ongoing to adapt this technology to the prehospital setting (16). Indeed, if rapid GFAP levels show promise as a surrogate marker for time and/or imaging in AIS of unknown onset, this could further optimize prehospital acute stroke management. Coupling GFAP levels with other inflammatory and/or cardiometabolic biomarkers could enhance detection of LVO stroke with favorable imaging, which in turn, could improve triage trajectories directly to comprehensive stroke centers with endovascular capabilities (17, 18), especially in resource-limited settings (19).

Given that elevated GFAP levels are observed across various neurological conditions, potential confounders may have influenced our results. To address this, we performed linear regression analyses examining the effects of cognitive disorder, age and prior stroke and sGFAP levels among AIS patients. Although a positive interaction was found between prior stroke and sGFAP levels, the presence of a prior stroke was associated with lower GFAP levels. This result is of uncertain significance given that the inverse association is counterintuitive and differs from previous studies (20), warranting further study in larger cohorts.

Contrary to sGFAP, sNfL has, to our knowledge, not been studied in the acute phase of undifferentiated stroke. However, in our study, we did not observe any significant associations between sNfL levels and diagnosis, time from stroke onset or neuroimaging.

Limitations

Our study has several limitations. First, as a single-center observational pilot study with small sample size and no blinding, the findings are preliminary and should be considered hypothesis-generating only, requiring validation in larger cohorts. Additionally, the sample size may limit the power to detect meaningful associations, increasing the risk of type II error, and the possibility of model overfitting. Third, because the study was not designed as a diagnostic accuracy study, we were unable to calculate sensitivity, specificity of sGFAP to diagnose ICH.

SIMOA technology has increased sensitivity to measure blood biomarker levels (in pg./mL) when compared to conventional methods (21, 22). Its low detection threshold enables quantification of very low concentration of sGFAP and sNfL (16.6 pg./mL and 1.6 pg./mL, respectively) and has been increasingly applied in various neurological diseases (23). Nonetheless, SIMOA's use in clinical practice is hindered by its high costs, complex procedures, and the need to process samples in batches, precluding its use to guide clinical management. Emerging developed point-of-care technology that can rapidly assess plasma GFAP levels could address some of the limitations of SIMOA going forward. Lastly, in our study, time from ED presentation to centrifugation was influenced by the 30-min wait required for blood coagulation. Newer assays that use plasma or ideally whole blood would eliminate this wait, and thus expediting GFAP analysis in the future.

Conclusion

GFAP levels measured in blood is increasingly recognized as a promising biomarker in neurological diseases, including acute stroke. In our study, using SIMOA technology, we found that sGFAP levels are significantly elevated in ICH compared to AIS, consistent with previous research. Additionally, we observed that sGFAP levels are higher in patients with extensive ischemic injury, particular among those with stroke of unknown onset. With developing novel technology able to measure blood GFAP levels in minutes, the potential for GFAP to serve as an adjunct tool in acute stroke management, particularly in the prehospital setting, is increasing apparent and warrants further study.

Data availability statement

Aggregated data supporting the conclusions of this article can by made available by the authors upon reasonable request and according to institutional regulations.

Ethics statement

The studies involving humans were approved by Ethics board of the Centre de recherche du centre hospitalier de l'université de Montréal. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JP: Data curation, Formal analysis, Writing – original draft. CD: Data curation, Investigation, Methodology, Writing – review & editing. PC: Data curation, Methodology, Writing – review & editing, Formal analysis. AD: Data curation, Methodology, Writing – review & editing. CL: Methodology, Writing – review & editing, Conceptualization, Formal analysis. CS: Conceptualization, Formal analysis, Methodology, Writing – review & editing. LG: Conceptualization, Formal analysis, Methodology, Writing – review & editing, Data curation, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization.

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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.2024.1470718/full#supplementary-material

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EDITED BY
Timo Uphaus,
Johannes Gutenberg University Mainz, Germany

REVIEWED BY
Wei Wei,
University Medical Center Göttingen, Germany
Hongjian Pu,
University of Pittsburgh, United States

*CORRESPONDENCE
Dianwei Liu
✉ dianwei1982@163.com
Yang Jiang
✉ yangjiang@email.sdu.edu.cn

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Characteristics of peripheral immune response induced by large-vessel occlusion in patients with acute ischemic stroke

Ling Ma[1], Bin Sun[2], Chenliu Fan[3], Juan Xiao[3], Maomao Geng[2], Jie Liu[4], Runze Jiang[5], Yang Jiang[3]* and Dianwei Liu[2]*

[1]Department of Clinical Laboratory, The Second Hospital of Shandong University, Jinan, Shandong, China, [2]Department of Stroke Center, Central Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China, [3]Hematology Department, The Second Hospital of Shandong University, Jinan, Shandong, China, [4]Department of Clinical Laboratory, Weihai Haida Hospital, Weihai, Shandong, China, [5]Jinan Biomedical Industry Academy of Shandong First Medical University, Jinan, Shandong, China

Introduction: Despite improvements in the treatment of acute ischemic stroke (AIS), some patients still suffer from functional impairments, indicating the poor understanding of pathophysiologic process of AIS. Inflammation plays an important role in the pathophysiology of AIS. The purpose of the study was to investigate the peripheral inflammation in different subtypes of AIS.

Methods: Here, retrospective data from AIS with large vessel occlusion (LVO) and small vessel occlusion (SVO), and healthy controls, were initially analyzed. Then, flow cytometry was performed to evaluate the levels of peripheral naïve and memory T-cells. Finally, we characterized the T cell receptors (TCR) repertoire using high-throughput sequencing.

Results: Elevated levels of leukocytes, neutrophils, and neutrophil-to-lymphocyte ratio (NLR), and decreased levels of lymphocytes were found in LVO group than that in SVO group, which were correlated with the severity of LVO. In addition, higher percentages of both effector memory (Tem) and central memory (Tcm) T cells, and lower percentage of naïve T cells in $CD4^+$ and $CD8^+$ T cells, were found in LVO group than that in SVO and healthy groups. Moreover, impaired TCR diversity, and different abundances of V-J gene combinations and amino acid sequences, were found in LVO as compared with healthy group, which would be potential biomarkers for LVO diagnosis.

Discussion: In conclusion, AIS with LVO can rapidly induce peripheral immune response, which provides new insight into the understanding of pathophysiology of AIS.

KEYWORDS

acute ischemic stroke, large vessel occlusion, T cell receptors repertoire, CD45, T cell

Introduction

Acute ischemic stroke (AIS) is one of the leading causes of mortality and disability worldwide (1). It occurs due to brain ischemia resulting from the thrombosis of cerebral blood vessels (2), which can be mainly caused by large artery atherosclerosis (LAA) and small artery occlusion (SAO) according to the Trial of Org 10,172 in Acute Stroke Treatment (TOAST) classification (3). Although the outcomes of AIS have dramatically improved due to the effectiveness of endovascular therapy, these treatments are highly time-dependent and only a few patients with AIS could receive effective treatment in time (4). Most importantly, several

strategies with regarding to AIS therapy have not been successfully translated into clinical application to date (5). These indicate that the pathological and physiological process contributing to neurological injury following AIS have not yet been fully understood.

Increasing evidence confirms that the activation of immune response is a crucial contributor to the pathophysiology of AIS (6, 7). Peripheral immune cells, such as neutrophils, lymphocytes, and monocytes, play important roles in the progression of AIS (8). In addition, the high neutrophil-to-lymphocyte ratio (NLR) is a potential predictor of poor functional outcome in patients with AIS (9). However, the changes of the peripheral components in different subtypes of AIS remains unclear.

The levels of lymphocytes were confirmed to be correlated with the outcome of AIS. Decreased number of lymphocytes was associated with worse pathological complete response rate of stroke (10), while increased proportion of lymphocytes had beneficial effects in AIS (11, 12). Among all the lymphocytes, T cells have been extensively studied because of their potency in both innate and adaptive immune responses (13). They are divided into $CD4^+$ helper T cells, $CD8^+$ toxic T cells, and regulatory T cells (Tregs) according to the different surface markers (14, 15), which play different regulatory roles in the pathophysiological process of AIS depending on their functional characteristics. The reduction of $CD4^+$ or $CD8^+$ T cells within 24 h after AIS leads to a decrease in the infarct size. In contrast, Tregs have protective effect on lowering infarct area and improving neurological function (16, 17). In addition, studies have shown that T cells could promote the deterioration of functional damage in the early stage but improve prognosis in the later stage of AIS, suggesting the different roles of T cell subsets in AIS (18, 19). Moreover, immune cell infiltration analysis suggested that T cell subsets with relevant genes can be identified as the diagnostic biomarkers in AIS (20). Therefore, it is essential to investigate the functions of different T cell subsets in AIS, which will provide new insights into the pathophysiological mechanisms of AIS. Recent years, a new group of T cells with CD45 surface markers has been discovered, which can be divided into two new subgroups: $CD45RA^+$ naïve T cells and $CD45RO^+$ memory T cells (21). Previous studies have confirmed the involvement of CD45 subsets in different diseases, such as sepsis and T-cell lymphoma (21, 22). However, it is still unknown whether $CD45RA^+$ and $CD45RO^+$ T cells are involved in the progression of AIS.

T cells initiate their major functions through T cell receptors (TCRs), which are produced by somatic DNA recombination of multiple gene segments (23). The diversity is generated by the random rearrangement of the variable (V), diversity (D), and joining (J) segments of TCR genes, which are central components of the adaptive immune system. TCR sequences are individual and have complex genetics due to VDJ recombination (24). Analysis of the TCR repertoire can provide a better understand of immune-mediated responses to infections, malignancies, and immunological disorders, including neuroinflammatory diseases. Based on technological advances in high-throughput sequencing (HTS), millions of TCR sequences can be used to assess clonal expansion and diversity in the peripheral blood of the multiple sclerosis (MS) patients (25, 26). The unique sequences will be valuable biomarkers for immune-mediated disease diagnosis, prognosis, and treatment response. Although a few studies have focused on TCR or characteristics of TCR repertoires in brain or peripheral blood of AIS (27–29), these studies did not distinguish the changes of TCR characteristic in the subtypes of AIS.

In the present study, we initially analyzed the circulating data retrospectively in patients with AIS, which were divided into large-vessel occlusion (LVO) and small-vessel occlusion (SVO) by imaging methods. Then, peripheral blood samples of patients with LVO and SVO were collected to detect proportional changes of $CD45RA^+$ and $CD45RO^+$ T cells. Finally, the TCR repertoire was analyzed to identify the unique immune response in AIS with LVO.

Materials and methods

Research ethics

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Jinan Central Hospital Affiliated to Shandong First Medical University (No. SZR2021-006-01) and The Second Hospital of Shandong University (No. KYLL-2021 (KJ)P-0300).

Study population

Retrospective data from 368 patients with AIS (≥18 years old), recruited from both Central Hospital Affiliated to Shandong First Medical University ($n = 312$) and The Second Hospital of Shandong University ($n = 56$) between September 2022 to December 2023, were analyzed for peripheral clinical characteristics. The AIS patients were divided into large vessel occlusion (LVO, $n = 161$) and small vessel occlusion (SVO, $n = 207$) using magnetic resonance imaging (MRI), computed tomography angiography (CTA), brain magnetic resonance angiography (MRA), and/or digital subtraction angiography (DSA) (30). Exclusion criteria: (1) only received MRI without further brain imaging; (2) had severe other disease, such as liver or kidney dysfunction, cardiac impairment; (3) had severe inflammatory conditions. The age- and sex-matched healthy participations ($n = 167$), which were confirmed to have no cerebrovascular disease or other sever conditions in the physical Examination Department of The Second Hospital of Shandong University over the same period, were included as the control group.

Clinical data collection

Venous blood samples were collected within the first 24 h of stroke onset. Blood glucose, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride (TG), and counts of leukocyte, neutrophile, and lymphocyte as well as the proportion of neutrophile and lymphocyte, were analyzed. The NLR was calculated as the ratio of the absolute neutrophile counts to the absolute lymphocyte counts. The stroke severity at onset was evaluated using National Institutes of Health Stroke Scale (NIHSS) (31).

Flow cytometry analysis

To determine the phenotype of T cells, 18 patients with LVO and 17 patients with SVO, aged from 32 to 82 years, were recruited from both Central Hospital Affiliated to Shandong First Medical University (LVO, $n = 8$) and The Second Hospital of Shandong

University (SVO, $n = 17$; LVO, $n = 10$) from May to December 2023. Peripheral anticoagulant blood samples from patients were obtained within 24 h of AIS onset at the Department of Clinical Laboratory. The samples of 22 healthy controls were collected at the same time. The whole blood of each sample was mixed gently and transferred into five groups (100 μL/tube): (1) labeled with APC-conjugated mouse anti-human CD3 (#317318, Biolegend, San Diego, CA, United States) and FITC-conjugated mouse anti-human CD56 (#304604, Biolegend) antibody; (2) labeled with APC-conjugated mouse anti-human CD3, FITC-conjugated mouse anti-human CD4 (#300506, Biolegend), and PE-conjugated mouse anti-human CD8 (#344706, Biolegend) antibody; (3) labeled with PE-conjugated mouse anti-human CD4 (#300508, Biolegend), PerCP-conjugated mouse anti-human CD45RA (#304156, Biolegend), FITC-conjugated mouse anti-human CD45RO (#304204, Biolegend), and APC-conjugated mouse anti-human CCR7 (#353214, Biolegend) antibody; (4) labeled with PE-conjugated mouse anti-human CD8, PerCP-conjugated mouse anti-human CD45RA, FITC-conjugated mouse anti-human CD45RO, and APC-conjugated mouse anti-human CCR7 antibody; (5) the isotype control tube labeled with APC/FITC/PE/PerCP rat anti-human IgG antibody (#410712, #410720, #410707, #410710, Biolegend). Five microliter of each antibody was added into the corresponding tube and incubated for 20 min. Then, 1 mL erythrocyte lysing buffer (#555899, BD Biosciences, San Jose, CA, United States) was added and incubated at 37°C for 5 min. After centrifugation for 5 min, the cells were resuspended and washed with 1 mL phosphate buffer solution (PBS). Finally, after being suspended with 0.5 mL PBS, the cells were detected by flow cytometry (FACS Aria III; BD Biosciences). The gating strategy applied for the enumeration of T cells is shown in Supplementary Figure S1. Peripheral whole blood cells, including neutrophils, lymphocytes, monocytes, and red blood cells, can be divided into different populations based on cell size and granularity, as measured by forward scatter (FSC) and side scatter (SSC) characteristics, respectively. Lymphocyte were gated on the basis of FSC and SSC characteristics for the following research. The cells were analyzed by using FlowJo VX10 software (TreeStar, Ashland, OR, United States).

HTS of TCR repertoire

Peripheral blood samples were collected into EDTA vacutainer tubes at volumes more than 2 mL. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples using Ficoll density-gradient separation lysis (LTS1077-1, TBD, Tianjin, China) according to the instructor. Total RNA was extracted from PBMCs using RNAsimple Total RNA Kit (#DP419, Tiangen Biotech, Beijing, China). RNA concentration was evaluated using a NanoDrop ND-2000 spectrophotometer (Thermo Scientific, United Kingdom). cDNA synthesis and multiplex PCR amplification of the complementary-determining region 3 (CDR3) in the TCR β-chain were performed together using the Immune Repertoire Library Preparation Kit (Geneway, Jinan, China) following a protocol described in a previous study (32). TCR libraries were sequenced on DNBSEQ-T7 platform (MGI, Shenzhen, China), generating paired-end short reads with 150 bp in length.

Sequencing data preprocessing

The sequencing data were stored in FASTQ format, in which raw reads were demultiplexed according to the sequences of index primers corresponding to different samples. The low-quality sequences were discarded for quality control. The remainders were mapped into V, D, and J gene segments of TCR β-chain using the MiXCR software (version 3.0.6) with default parameters for sequencing alignment and clonotype assembly (33). TCR reference gene data were downloaded from the IMGT database[1]. The frequency of each TCR β-clonotype was further converted into rpm (reads per million) for standardization. The diversity of samples was evaluated based on D50 Diversity index and UT index. The diversity from the cumulative 50% of the total CDR3 detected in the sample was measured using the D50 index (34). The UT index was ranged from 0 to 1, and it was calculated based on the previous study (35).

Statistical analyses

Data were analyzed using GraphPad Prism software (Version VIII, La Jolla, CA, United States) or R software (version 4.0.2). Continuous data were presented as means ± standard deviation (SD). In contrast, categorical variables were presented as numbers and percentages. In the analysis of retrospective data and flow cytometry, the one-way analysis of variance (ANOVA) or Kruskal–Wallis test was used for comparisons between more than two groups based on data distribution and homogeneity. One-way ANOVA followed by Tukey test was used when the data showed normal distribution and variance homogeneity, otherwise Kruskal–Wallis test was applied. For continuous variables in the TCR repertoire, Student's t-test was used for comparison between two groups, and the correlation between NIHSS and levels of peripheral blood cells, or between NIHSS and TCR clonotypes expression, was assessed using Pearson's test. The Chi-Square test or Fisher's exact test was used to analyze categorical variables. $p < 0.05$ was considered the threshold for statistical significance.

Results

Participation clinical characteristics

To determine the peripheral immune responses in different subtypes of AIS, we initially analyzed the retrospective data from patients with LVO, SVO, and healthy controls. The baseline demographic and clinical characteristics are shown in Table 1 and Supplementary File 1. No significant differences were found among the three groups in baseline characteristics including age and gender. As risk factors of AIS, lower levels of HDL cholesterol, and high levels of both blood glucose and triglyceride were found in patients with LVO and SVO groups than in healthy controls ($p < 0.0001$). No significant change was found in levels of low-density lipoprotein (LDL) cholesterol after AIS.

1 https://www.imgt.org/IMGTrepertoire/LocusGenes/genetable/human/geneNumber.html#TRtotal

The laboratory parameters in patients with LVO and SVO were significantly different from those of healthy controls (Figure 1 and Supplementary File 1). The leukocyte count in the patients' groups was higher than that in the healthy control group (Figure 1A, $p < 0.0001$), which was mainly due to an elevated neutrophile count (Figure 1B, $p < 0.0001$). In contrast, the lymphocyte count decreased in patients with AIS compared to the healthy control group (Figure 1C, $p < 0.0001$). An increased ratio of neutrophile to leukocyte and a decreased ratio of lymphocyte to leukocyte were also observed in patients with AIS (Figures 1D,E, $p < 0.0001$). As NLR has been reported to be a useful marker of inflammation, we also compared NLR in the three groups. Consistent with the previous study, NLR was higher in patients than that in healthy controls (Figure 1F, $p < 0.0001$). Interestingly, we found higher counts of leukocyte (Figure 1A, $p < 0.0001$) and neutrophile (Figure 1B, $p < 0.0001$), and elevated ratio of neutrophile to leukocyte (Figure 1D, $p < 0.0001$) and NLR (Figure 1F, $p < 0.0001$) in LVO group than that in SVO group. In contrast, lymphocyte count (Figure 1C, $p = 0.0003$) and ratio of lymphocyte to leukocyte (Figure 1E, $p < 0.0001$) in LVO group were decreased as compared with that in SVO group. These results suggest that AIS with LVO can rapidly induce more severe immune response in the peripheral blood.

Correlation between peripheral blood cells and NIHSS

To determine whether there was a relationship between the expression levels of peripheral blood cells and the severity of AIS, we analyzed the correlation between the peripheral laboratory data and NIHSS in LVO group and SVO group, respectively. As shown in Figure 2 and Supplementary File 1, the counts of leukocytes (Figure 2A, $r = 0.1686$, $p = 0.0331$) and neutrophiles (Figure 2B, $r = 0.2236$, $p = 0.0045$), and the percentage of neutrophiles (Figure 2D, $r = 0.2979$, $p = 0.0001$) as well as NLR (Figure 2F, $r = 0.2286$, $p = 0.0036$) were positively correlated with NIHSS in the LVO group. In contrast, both the count and percentage of lymphocytes were inversely correlated with NIHSS in the LVO group (Figure 2C, $r = -0.2207$, $p = 0.005$; Figure 2E, $r = -0.2592$, $p = 0.0009$). However, no relationship was found between the levels of peripheral blood cells and NIHSS in the SVO group (data not shown). These results suggest that the expression levels of peripheral immune cells can more specifically reflect the severity of patients with AIS caused by LVO.

Phenotype analysis of peripheral T cells

T cells play essential roles in immune response. To detect the peripheral phenotype of T cells in AIS with different subtypes, flow cytometry analysis was performed. As shown in Figure 3 and Supplementary File 2, although the total number of peripheral lymphocytes was significantly reduced in patients with AIS, the proportion of $CD4^+$ T cells was higher in the LVO group than in the SVO and healthy control groups (Figure 3A, $p < 0.001$). In contrast, the proportion of NK cells decreased in the LVO group as compared with the SVO group (Figure 3B, $p < 0.05$) and control group (Figure 3B, $p < 0.001$). No significant difference was found between the SVO group and control group. These results indicate that AIS with LVO can rapidly enhance the adaptive immune response mediated by T cells.

To further analyze the immune response after AIS, naïve, effector memory T (Tem), and central memory T (Tcm) of $CD4^+$ and $CD8^+$ T cells were, respectively, detected in LVO and SVO subtypes. As shown in Figure 4 and Supplementary File 2, the percentage of $CD45RA^+CCR7^+$ in $CD4^+$ (naïve $CD4^+$) T cells decreased (Figure 4A, $p < 0.01$), while the percentage of $CD45RO^+CCR7^+$ (Tcm, Figure 4B, $p < 0.001$) and $CD45RO^+CCR7^-$ (Tem, Figure 4B, $p < 0.001$) in $CD4^+$ T cells increased in LVO group as compared with SVO and control groups, suggesting a decrease in naïve $CD4^+$ T cells and an increase in Tcm and Tem $CD4^+$ T cells after AIS with LVO. Although no significant difference was found in the percentage of total $CD8^+$ T cells, similar changes were found in $CD45RA^+CCR7^+$ (Figure 5A, $p < 0.01$, Supplementary File 2), $CD45RO^+CCR7^+$ (Figure 5B, $p < 0.001$), and $CD45RO^+CCR7^-$ (Figure 5B, $p < 0.01$) in $CD8^+$ T cells, as in $CD4^+$ T cells of patients with LVO. No significant difference was found between the SVO and healthy control groups in naive, Tcm, and Tem cells. These results suggest that only AIS with LVO can stimulate the transformation of T cells into memory T cells.

The characteristics of TCR repertoires in AIS patients with LVO

Given the changes of T cells above, we sought to further determine the T cells' characteristics of LVO. As no changes were found in T cells between SVO group and healthy control group, PBMCs were isolated from peripheral blood and TCR repertoire sequencing analysis were performed in AIS patients with LVO and healthy controls. We assessed TCR sequences and identified V-J combinations at the transcription level. The results showed that the number of V-J combinations

TABLE 1 Baseline and clinical characteristics of patients with AIS and healthy controls.

Valuables	LVO (n = 161)	SVO (n = 207)	Healthy (n = 167)	p-value
Gender, male, n (%)	103 (63.98)	125 (60.39)	102 (61.08)	0.7668
Age, years (mean ± SD)	66.09 ± 12.29	65.11 ± 10.4	63.87 ± 7.986	0.1496
Glucose, mmol/L (mean ± SD)	7.527 ± 3.314	7.31 ± 2.703	5.175 ± 0.6554	<0.0001
HDL, mmol/L (mean ± SD)	1.105 ± 0.2735	1.108 ± 0.4093	1.407 ± 0.2397	<0.0001
LDL, mmol/L (mean ± SD)	2.429 ± 0.8143	2.67 ± 0.7394	2.596 ± 0.5713	0.0512
Triglycerides, mmol/L (mean ± SD)	1.295 ± 0.7852	1.573 ± 1.204	0.964 ± 0.4057	<0.0001

HDL, high density lipoprotein; LDL, low density lipoprotein.

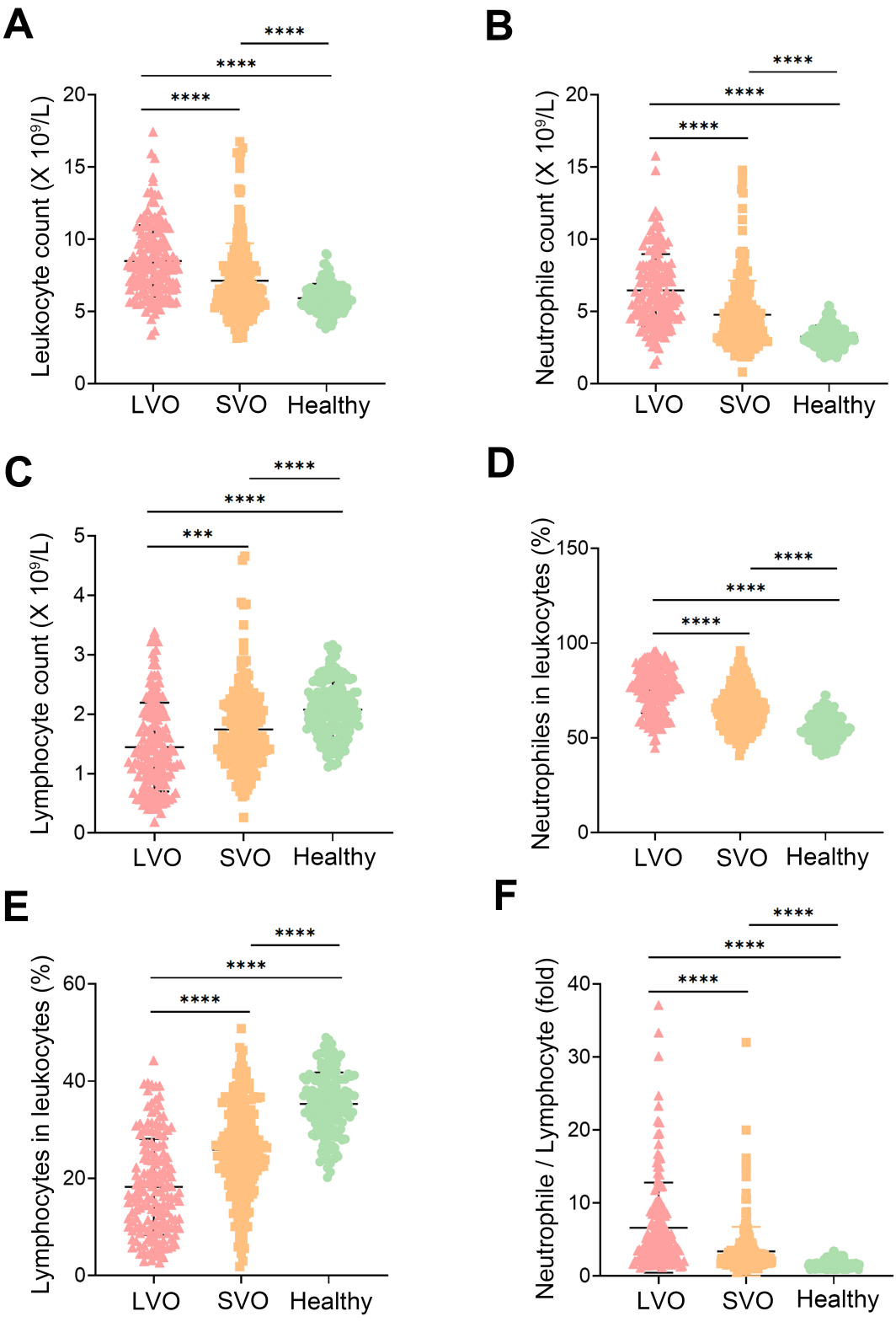

FIGURE 1
Participation characteristics. Leukocyte **(A)**, neutrophile **(B)**, and lymphocyte **(C)** counts in the peripheral blood of AIS with LVO and SVO, and healthy controls. The ratio of neutrophiles **(D)** and lymphocytes **(E)** to leukocytes in the peripheral blood of AIS with LVO and SVO, and healthy controls. **(F)** The ratio of neutrophile to lymphocyte in the peripheral blood of AIS with LVO and SVO, and healthy controls. (***$p < 0.001$, ****$p < 0.0001$).

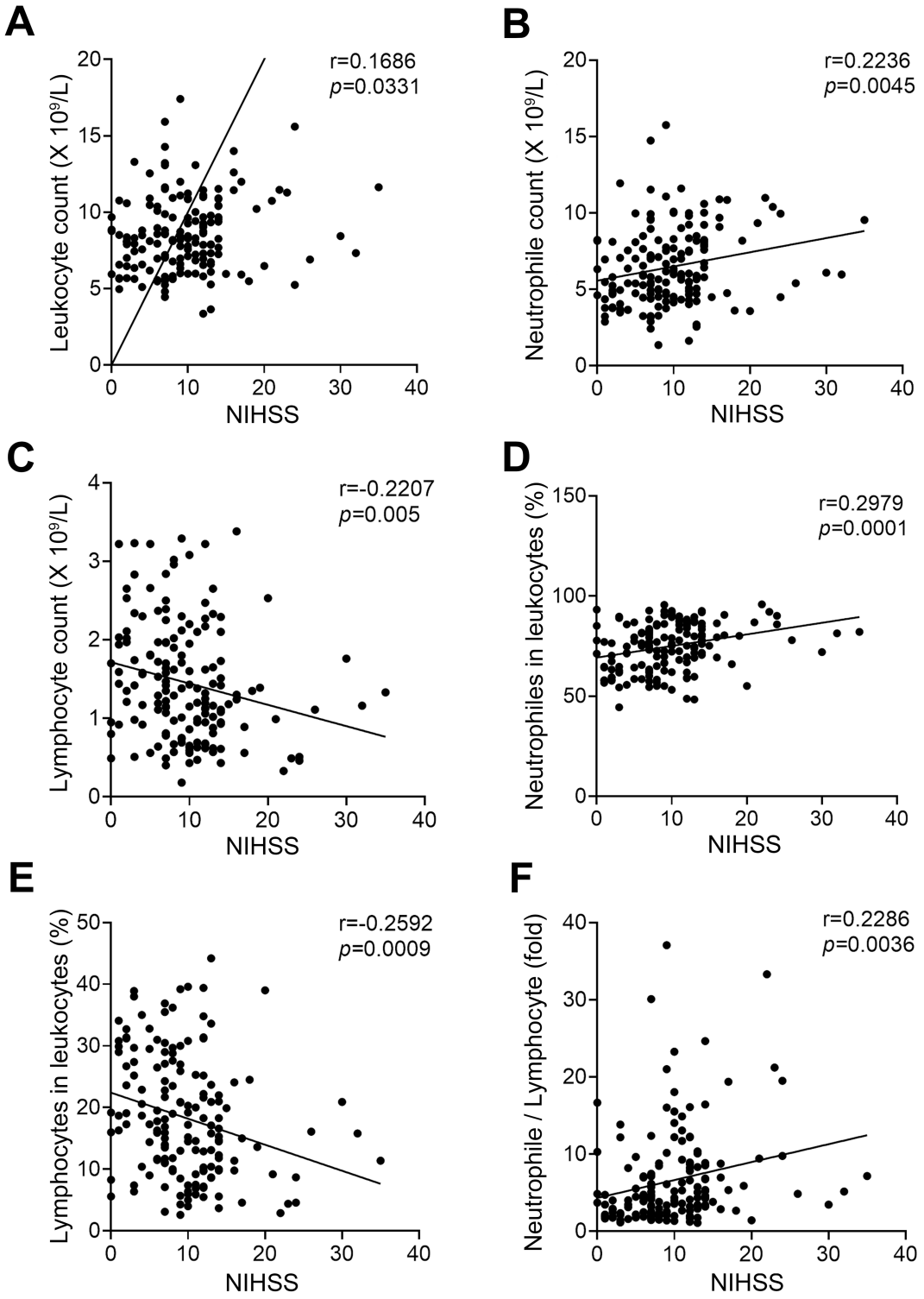

FIGURE 2
Correlation between NIHSS and circulating blood cells in patients of AIS with LVO. The correlation between NIHSS and leukocyte **(A)**, neutrophile **(B)**, and lymphocyte **(C)** counts. The correlation between NIHSS and the ratio of neutrophiles **(D)** and lymphocytes **(E)** to leukocytes. **(F)** The correlation between NIHSS and the ratio of neutrophile to lymphocyte.

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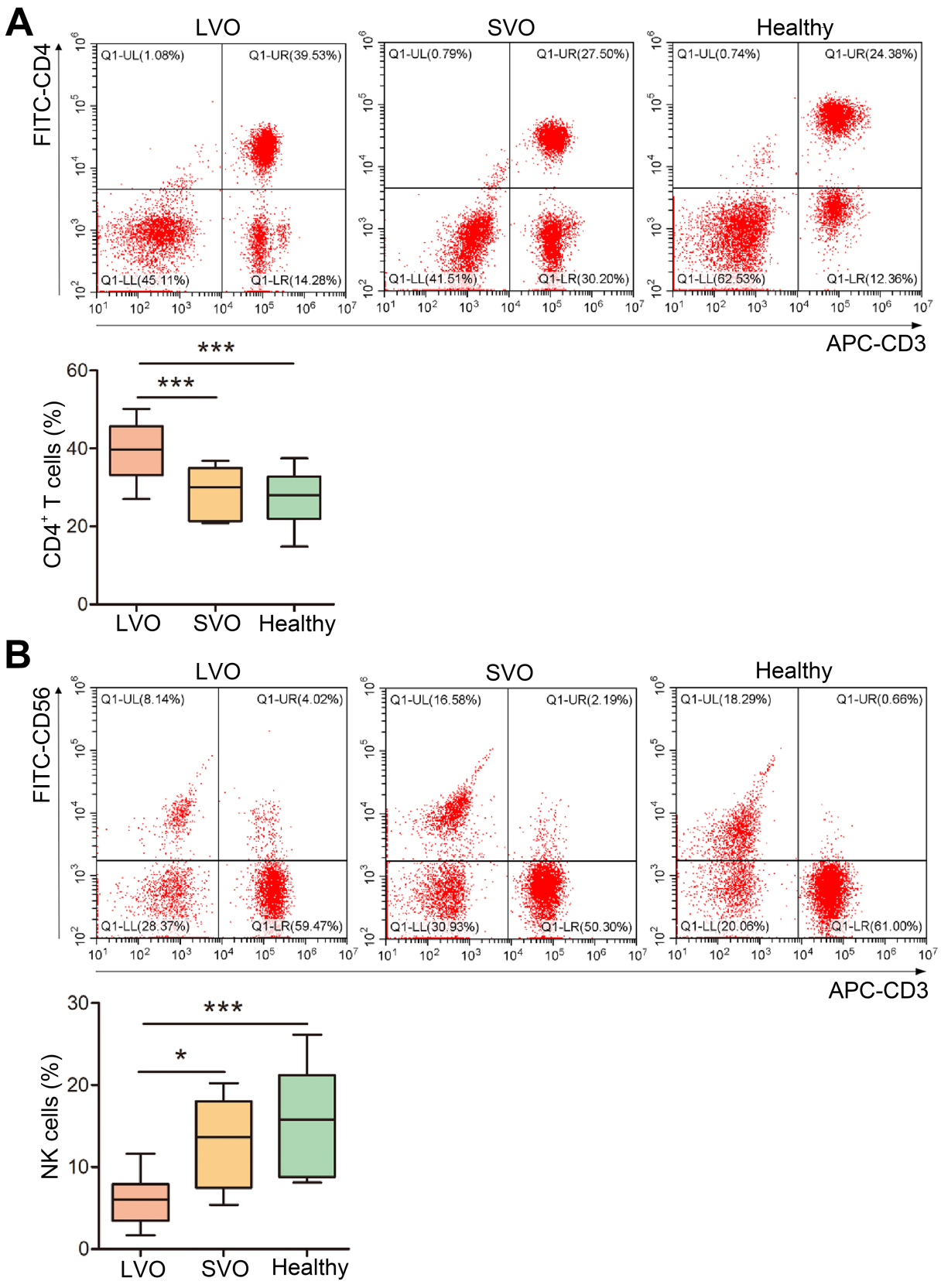

FIGURE 3
Changes in T-cell subtypes. **(A)** The percentage of $CD4^+$ T cells was determined by flow cytometry. **(B)** The percentage of NK cells was determined using flow cytometry (*$p < 0.05$, ***$p < 0.001$).

(Figure 6A, $p = 0.0028$) and the TCR sequences in CDR3 (Figure 6B, $p = 0.0018$, Supplementary File 3) were more abundant in AIS with LVO group than in control group. Notably, consistent with the increased memory T cells, these results indicate a dramatic increase in immunological activity in the number of immunological items during the pathological process of AIS with LVO.

In addition, we performed a Principal Component Analysis (PCA) on the V-J combination frequency profile. As shown in the Figure 6C and Supplementary File 3, there was a significant difference between AIS patients with LVO and healthy controls in the sample cluster. In summary, we constructed a phenotype of LVO with immunological tendencies compared to healthy controls. TCR expression profiles were subsequently analyzed to assess the systemic immune responses mediated by T cells.

Next, we estimated the diversity of TCR clonotypes in each sample by calculating the D50 Diversity and UT index, irrelevant to the variation of sample sequencing depth. A lower D50 Diversity index was observed in AIS patients with LVO than in healthy controls (Figure 6D, $p = 0.0498$). In contrast, the UT index was higher in AIS patients with LVO than in healthy controls (Figure 6E, $p = 0.02$, Supplementary File 3), indicating that AIS with LVO could decrease the diversity of TCR profiles as compared with healthy controls.

Diversity of TCR repertoires and usage frequency of V-J gene combinations in AIS patients with LVO

We further performed a characteristic analysis to reveal the specificity of TCR sequence abundance in AIS patients with LVO and healthy controls. As shown in Figures 7A,B, the whole tree-map represented the average immune status of samples based on the abundance of CDR3 sequences. Each chip represented one CDR3 sequence's abundance. The larger of the color chip, the higher abundance of this sequence. Meanwhile, large color chips led to a decrease in the quantity of chips, which indicated the reduction of diversity. We found more large-colored chips in AIS patients with LVO (Figure 7A) than in controls (Figure 7B and Supplementary File 4), which also illustrated the high-abundance sequences and poor TCR diversity in LVO patients.

As the most variable components of TCR sequence, V and J segments play a crucial role in targeting a wide range of pathogenic process and the combination of V-J segments are the primary focus of many TCR-related studies. The comparison of V-J segments could reveal their contributions to the progression of AIS, and help to explain the differences in immune status between different groups. Furthermore, Circos plots was used to show the usage of V-J gene combinations. In the Circos plots, the length of the sectors represents the relative usage frequency of the V or J genes, while the width of the links connecting the V and J genes represents the relative usage frequency of the V-J combinations. Nevertheless, patients with LVO (Figure 7C) showed the similar average frequency of the use of V-J gene combinations as healthy controls (Figure 7D and Supplementary File 4). These results further indicated that the TCR diversities in AIS patients with LVO were induced by the high abundance of VDR3 sequences.

Different abundances of V-J gene combinations in AIS patients with LVO

We then determined the different abundance of V-J gene combinations in AIS patients with LVO from healthy controls. A total of 63V and 14J gene segments were identified in all samples. Compared with the control group, a significantly lower percentage of TRBV4-1 and TRBV5-1, and a higher percentage of TRBV5-3, TRBV5-6, TRBV6-1, TRBV7-3, TRBV10-1, TRBV12-1, TRBV12-4, TRBV13, TRBV23-1, and TRBV25-1 were found in the AIS with LVO group (Figure 8A, $p < 0.05$, $p < 0.01$). Moreover, a significantly lower percentage of TRBJ1-2 and TRBJ2-2, but a higher percentage of TRBJ1-4, TRBJ2-1, and TRBJ2-6 were found in the AIS with LVO group than the healthy group (Figure 8B, $p < 0.05$, $p < 0.01$, Supplementary File 5). These results indicated that LVO induced different abundance of V-J gene combinations.

We further analyzed the abundance of CDR3 sequences between AIS with LVO and healthy groups. There were 734 upregulated and 49 downregulated amino acid clonotypes between the AIS with LVO and healthy group (Figures 8C,D). In addition, 30 differentially expressed amino acid clonotypes, were found in at least 10 samples (Figure 8E). Among these clonotypes, the expression levels of one amino acid clonotypes (CASRGQNTEAFF) was found to be positively correlated with NIHSS (Figure 8F, $r = 0.6133$, $p = 0.0068$), suggesting that the expression level of this amino acid clonotypes was related to the severity of AIS with LVO.

The prediction model for AIS with LVO

As the different abundance of TCR sequences between the AIS with LVO group and healthy group, we next want to build a diagnostic model to predict AIS with LVO. We firstly aligned the top 50 abundant CDR3 sequences with the indicated length to create a motif diagram. The results showed a significant difference in the motifs between the AIS with LVO group (Figure 9A) and the healthy group (Figure 9B). This suggests that selecting a suitable amino acid sequence can distinguish AIS patients with LVO from healthy controls.

Then, we created a model using the random forest method to predict AIS with LVO based on differences in TCR repertoire characteristics. We changed the settings from 0.2 to 0.3 to improve the accuracy, while lowering the fault tolerance rate to stabilize the classification function of the model. We then evaluated the model classification effect in predicting AIS patients with LVO. The results showed that the distribution of the ROC curve was relatively smooth, and the leave-one-out cross-validation produced an area under the curve (AUC, 95%CI: 0.519–0.981, Figure 9C). Additionally, we evaluated the V-J combinations that affected the model assessment effect and discovered 10 combinations that made the largest contributions to the model (Figure 9D). These results indicate that the model can distinguish between patients with LVO and healthy controls, which provides the possibility of developing TCR biomarkers for the early diagnosis of AIS with LVO.

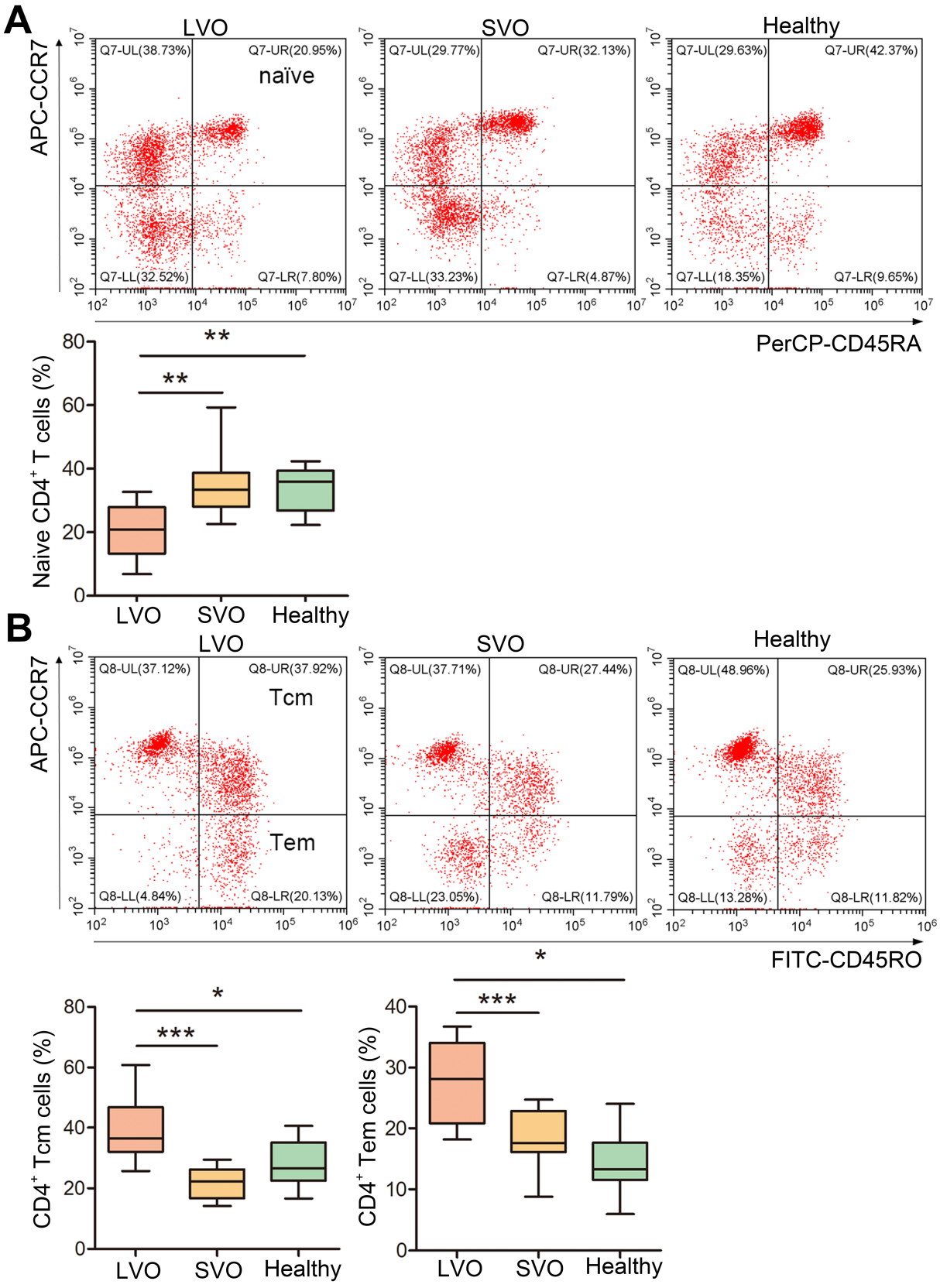

FIGURE 4
Changes in naïve, Tcm, and Tem CD4⁺ T cells. **(A)** The percentage of naïve CD4⁺ T cells was determined by detecting CD45RA⁺CCR7⁺ cells among the CD4⁺ T cells. **(B)** The percentage of Tcm CD4⁺ T cells was investigated by detecting CD45RO⁺CCR7⁺ cells among the CD4⁺ T cells. The percentage of Tem CD4⁺ T cells was investigated by detecting CD45RO⁺CCR7⁻ cells in CD4⁺ T cells. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

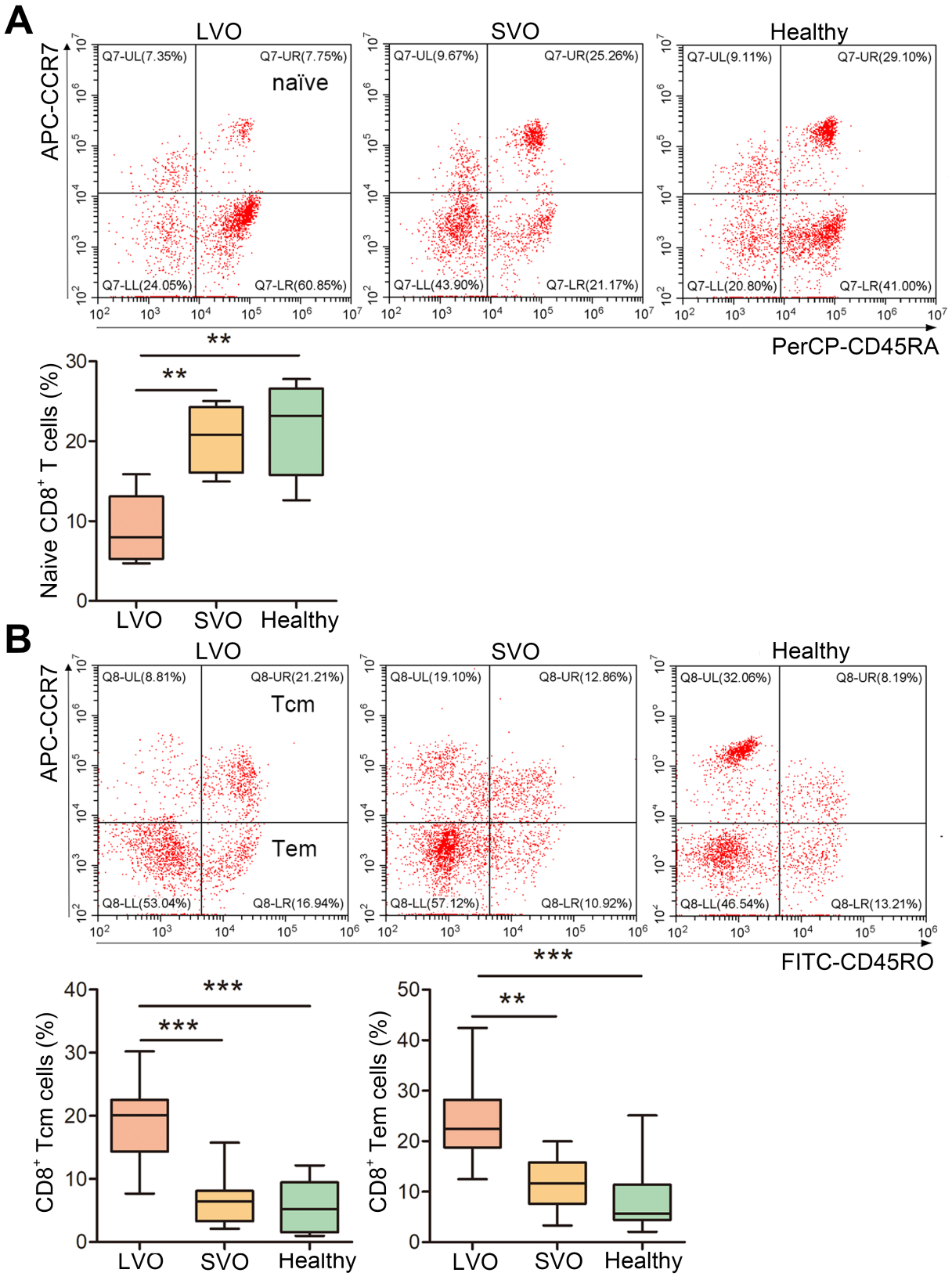

FIGURE 5
Changes in naïve, Tcm, and Tem levels in CD8⁺ T cells. **(A)** The percentage of naïve CD8⁺ T cells was investigated by detecting CD45RA⁺CCR7⁺ cells among CD8⁺ T cells. **(B)** The percentage of Tcm CD8⁺ T cells was investigated by detecting CD45RO⁺CCR7⁺ cells among CD8⁺ T cells. The percentage of Tem CD8⁺ T cells was investigated by detecting CD45RO⁺CCR7⁻ cells in CD8⁺ T cells. (**$p < 0.01$, ***$p < 0.001$).

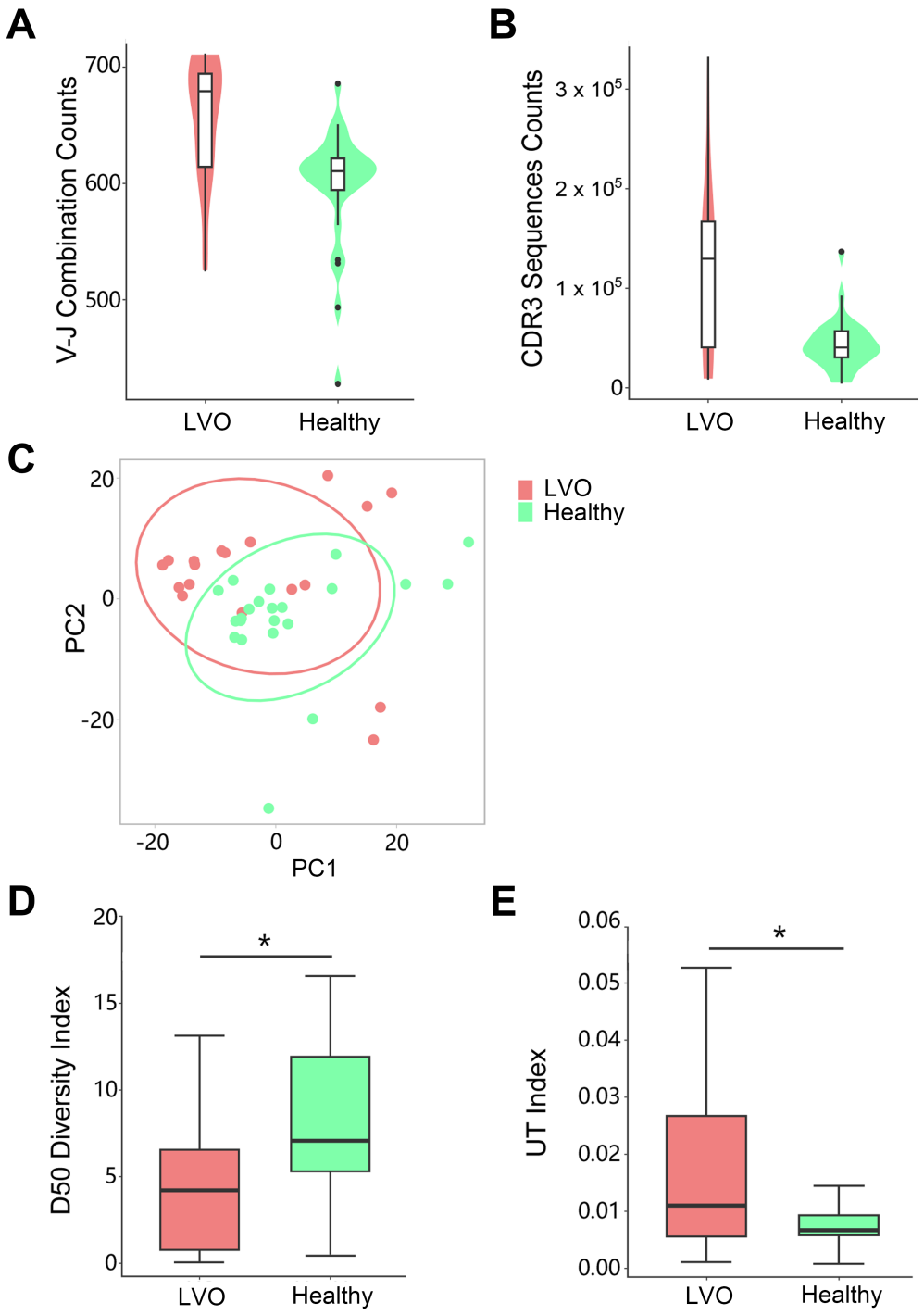

FIGURE 6
Quantity and diversity of the TCR repertoire: the number of unique V-J combinations **(A)** and counts of unique CDR3 sequences **(B)** were determined. **(C)** Principal component analysis of the AIS with LVO (red) and healthy groups (green). *X*-axis and *Y*-axis represent principal component 1 (PC1) and principal component 2 (PC2), respectively. The D50 Diversity Index **(D)** and UT Index **(E)** show the diversity of the TCR repertoire in the AIS with LVO and healthy groups. The violin chart and box plot show the data distribution with the minimum, first quartile, median, third quartile, and maximum (*$p < 0.05$).

Discussion

Although endovascular therapy is effective for AIS, some patients still suffer from permanent disability. From the retrospective data, we firstly found that the changes of peripheral blood cells were correlated with the severity of AIS with LVO but not SVO. Using flow cytometry, we found that AIS with LVO enhanced the peripheral adaptive immune response by increasing the percentage of Tcm and Tem cells. Furthermore, TCR repertoire sequencing analysis showed that TCR diversity was impaired in patients with LVO, although the

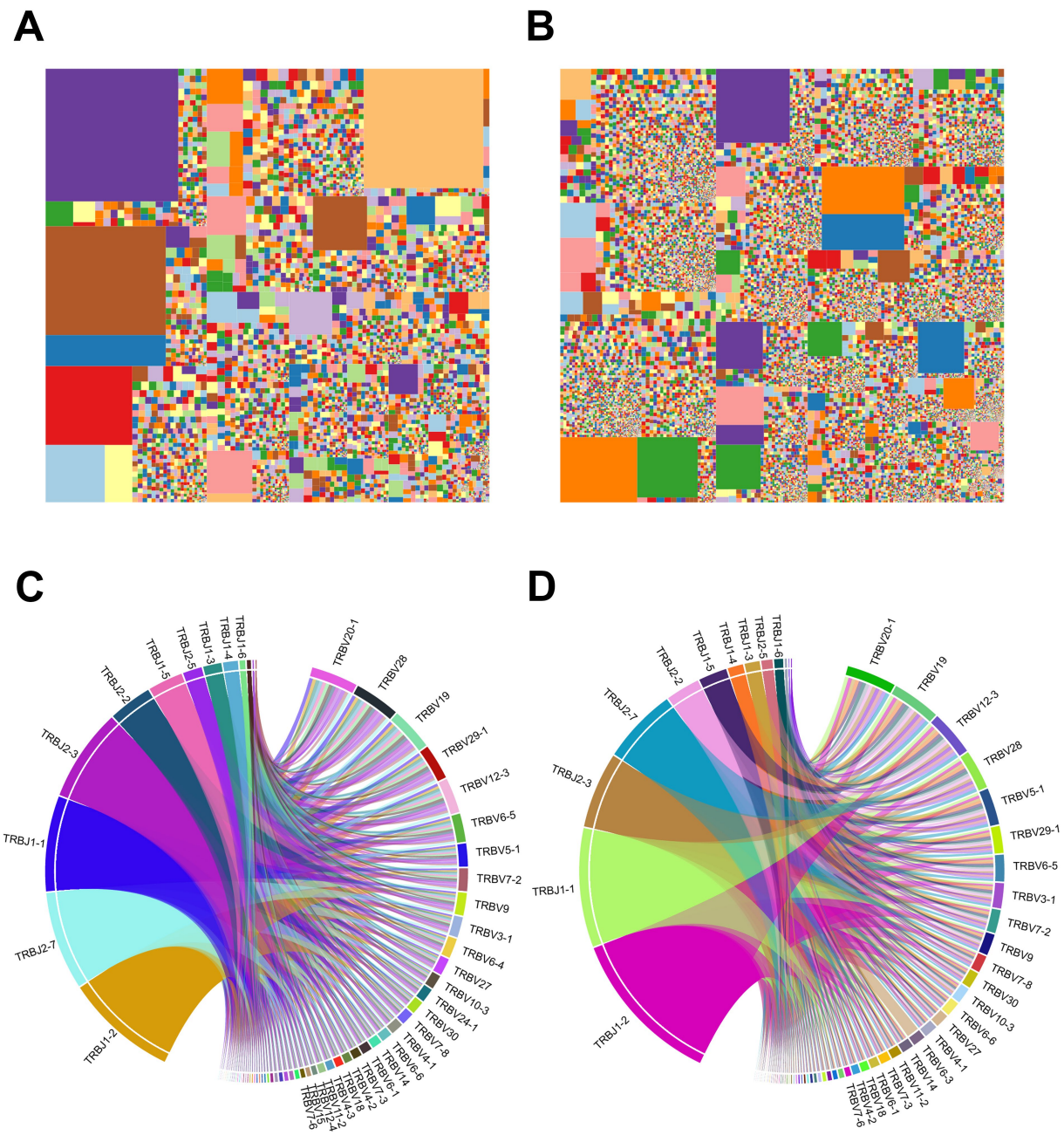

FIGURE 7
Samples' immunological characteristics. **(A,B)** TCR sequence abundance and usage frequency of V-J combinations in patients with LVO. Tree-map of module CDR3 sequence abundance in samples from the AIS with LVO group **(A)** and healthy group **(B)**. **(C,D)** Circos plots of the V-J gene combination usage frequency in samples from the AIS with LVO group **(C)** and healthy group **(D)**. The left half-circle indicates the J gene and the right half-circle indicates the V gene. The length of sectors represents the relative usage frequency of the V genes or the J genes.

number of V-J combinations and CDR3 sequences increased. Together with the flow cytometry results, these findings suggest that AIS with LVO could induce an adaptive immune response, accompanied by a lack of comprehensive immunological activity, owing to the specific immune response to disease. Importantly, we found different abundances of V-J gene combinations and amino acid clonotypes between the AIS with LVO and control groups, which could be used as diagnostic biomarkers for AIS. This study will provide new insights into the pathophysiological process of AIS.

Human and animal studies have confirmed that AIS can lead to immediate activation of local immune cells and prompt mobilization of peripheral immune cells in the first hours and up to days after stroke (8, 36). Although studies have confirmed that the peripheral neutrophiles and NLR are closely related to the prognosis of AIS, few studies have focused on their roles on different subtypes of AIS. Our results confirmed that the changes of peripheral immune cells were more obvious in the LVO group than that in SVO group. In addition, these changes have a correlation with NIHSS in the LVO but not SVO

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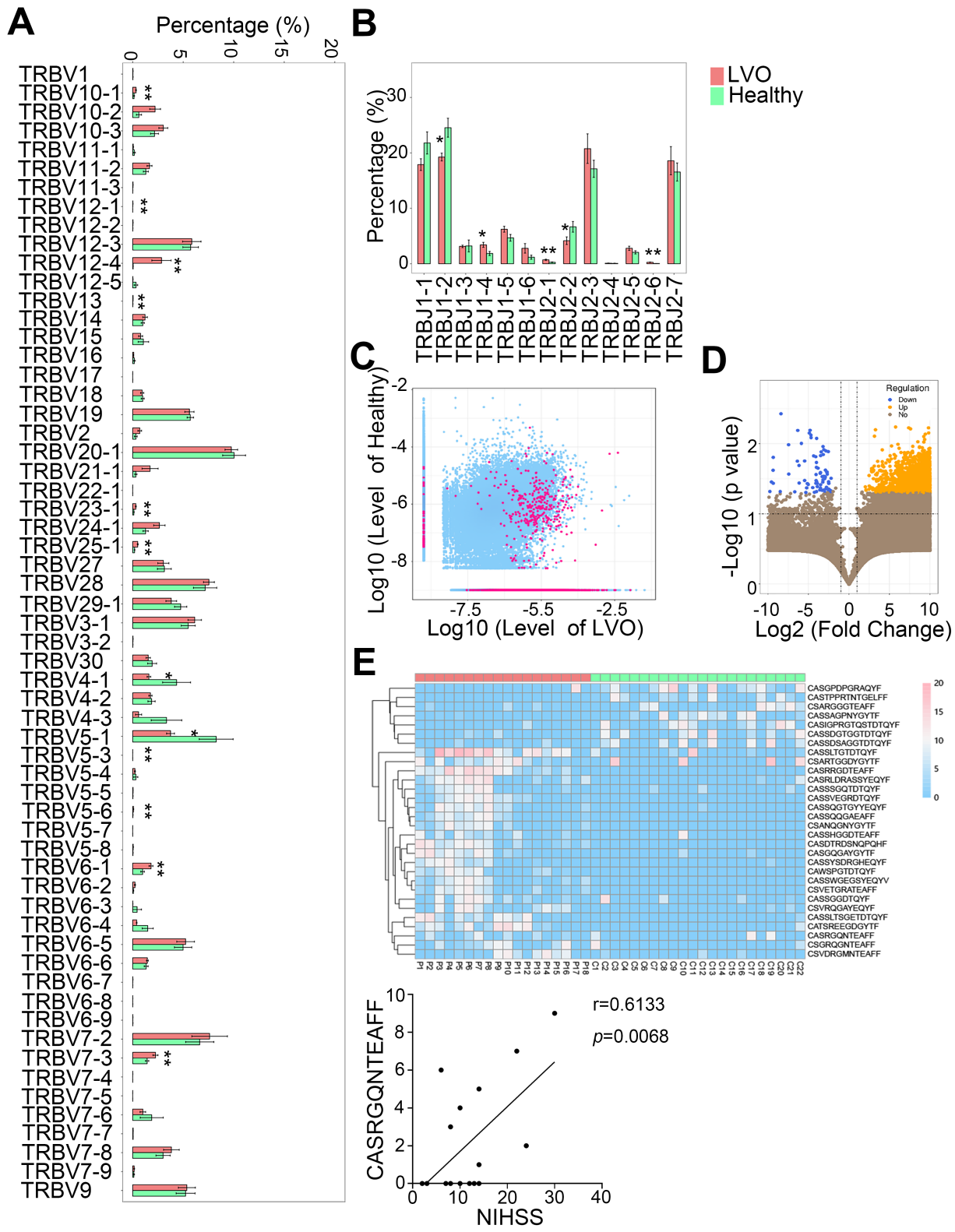

FIGURE 8
Differential abundances of the V and J gene segments and CDR3 sequences between the AIS with LVO and healthy groups. The relative abundance of V gene **(A)** and J gene **(B)** in the two groups. **(C)** Scatter plot showing differential abundance of CDR3 sequences in LVO and healthy groups (red, different CDR3 sequences; blue, CDR3 sequences with no significant difference). The *X*- and *Y*-axis represents the log-transformed mean of the relative abundance of the healthy and LVO group, respectively. **(D)** The volcano map shows the different clones between the LVO and healthy groups (yellow with increased abundance and blue with decreased abundance). The *X*- and *Y*-axes represent the log transformed *p*-value and fold changes, respectively. **(E)** Thirty differentially expressed amino acid clonotypes are shown as a heatmap. The *X*- and *Y*-axis represent samples and expression levels of CDR3 sequences, respectively. **(F)** The correlation between NIHSS and the unique amino acid clonotype (*$p < 0.05$, **$p < 0.01$).

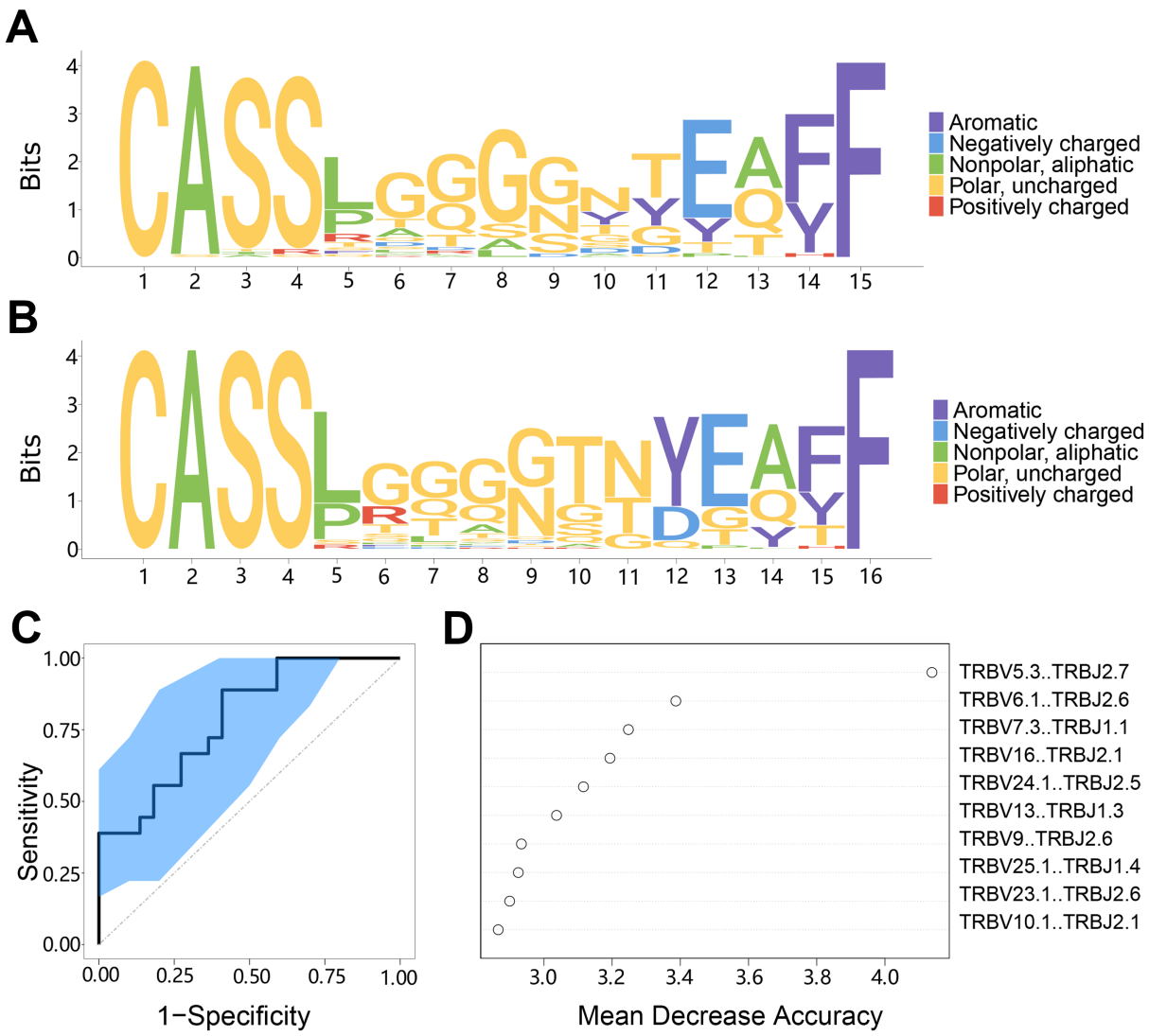

FIGURE 9
The motif specificity of TCR repertoires and prediction model system for AIS with LVO. **(A)** Motif diagram of the CDR3 sequences in AIS with LVO group. **(B)** Motif diagram of the CDR3 sequences in the healthy group. **(C)** ROC curve showing the classification effect of the LVO prediction model. The ordinate is the true-positive rate (sensitivity), and the abscissa is the false-positive rate (1-specificity). **(D)** The top 10 segments of V-J combinations that influenced the model effect. The mean decrease in accuracy is a rating index, and its value positively correlates with the effect on the model.

group, suggesting that the peripheral immune changes can more specifically related to the severity of AIS patients with LVO. To our knowledge, this is the first study showing the relationship between the peripheral components and different AIS subtypes, which will be useful for the understanding of the roles of peripheral immune response in different AIS subtypes.

Studies have shown that innate immune cells were initially activated, followed by T cells activation after AIS (37). In addition, the relative levels of $CD45RA^+$ and $CD45RO^+$ T cells can reveal the systematic immune response and are associated with the pathophysiology and prognosis of multiple disease, such as pancreatic and non-small cell lung cancer (21, 38). In our study, although the lymphocyte count decreased in patients with AIS, the reduced ratio of $CD45RA^+CCR7^+$ (naïve) T cells, and increased ratio of $CD45RO^+CCR7^+$ T (Tcm) and $CD45^+CCR7^-$ T (Tem) cells, further confirmed that the adaptive immune response could be rapidly stimulated in patients with LVO by stimulating the transformation of T cells into memory T cells. Together with the high NLR, the percentage of naïve, Tcm, and Tem can more specifically reflect the immunological condition after AIS with LVO. This is the first study to evaluate naïve, Tcm, and Tem of $CD4^+$ and $CD8^+$ T cells in peripheral blood of patients with different AIS subtypes.

Considering the critical roles of the immune response, we performed an analysis to quantify and compare the TCR repertoire in PBMCs samples. Analysis of TCR repertoire has been used to characterize various diseases. For instance, the impaired TCR diversity and significant differences in V-J segments in systemic lupus erythematosus (SLE) make the TCR repertoire profile a potential biomarker of SLE (32, 39). Here, we clearly demonstrated that AIS with LVO induced rapid impairment of TCR diversity and the enrichment or reduction of specific V-J combinations in the PBMCs. As the TCR repertoire investigates CDR3, and each CDR3 sequence

is a unique label, it can track T cell composition (40). Together with the varied percentage of T cell subsets, the decreased percentage of naïve T cells and segments of TRBV4-1, TRBV5-1, TRBJ1-2, and TRBJ2-2 sequences, as well as the increased percentage of Tcm and Tem cells and segments of TRBV5-3, TRBV6-1, TRBV7-3, TRBV10-1, TRBV13, TRBV23-1, TRBV25-1, TRBJ2-1, and TRBJ2-6 might indicate changes in these sequences in the relevant T cell subsets. In addition, we also found the correlation between amino acid sequence and the severity of AIS with LVO. The combined application of the percentage of Tcm/Tem cells with different abundances of V-J gene combinations and specific amino acid clonotypes could better reflect the body's immune status in the patients with LVO.

We also found a range of amino acid clonotypes which can be used as a signature for the trained prediction model due to the altered TCR profile. Despite the limited sample size, our model efficiently discriminated AIS patients with LVO from healthy controls, indicating its potential as a biomarker for the diagnosis of AIS with LVO. Currently, the diagnosis of AIS relies mainly on the evaluations of clinical and neuroimaging features, including computed tomography (CT), MRI, and digital DSA (41). However, in the early stage of infarction, mild or no abnormal changes can be found on CT and MRI, because of the low sensitivity of these imaging modalities (42). Although DSA is the gold standard for diagnosing AIS, the expensive cost and invasive operation make its universal application impossible. In addition, all the examinations above require radiation exposure and are not feasible for patients with special circumstances, such as those with a pacemaker or emotional instability. Most importantly, these treatments take a long time and can easily delay the optimal treatment time. Therefore, several studies have been conducted to investigate the rapid diagnostic biomarkers of AIS, including glucose, iron, ferritin, homocysteine, insulin, P-selectin, matrix metalloproteinase-9, high-density lipoprotein cholesterol, platelets, glial fibrillary acidic protein, TNF-α, and proenkephalin-A (43–48). However, these biomarkers are not widely used for diagnosing AIS, because of significant individual differences. Moreover, the inflammation-related biomarkers, such as C-reactive protein and interleukin (IL)-6, play a crucial role in predicting AIS (49). Still, they were limited to be used as diagnostic tools because of their similar changes in other inflammatory and infectious processes (50). Taken together, as the rapid changes of TCR repertoire sequences in AIS patients with LVO and the correlation between the CDR3 sequence and LVO severity, our study will provide important assistance for the diagnosis of AIS with LVO. These changes of unique amino acids may be the potential biomarkers for the rapid diagnosis of AIS with LVO.

Conclusion

In this study, we provided evidence of a change in the peripheral blood cells, the percentage of Tcm/Tem cells, and a predictive role of the TCR repertoire in the AIS with LVO. We found that the LVO group had increased leukocytes, neutrophils and NLR, and decreased lymphocytes as compared to the SVO group, which correlated with the severity of LVO. TCR diversity was impaired in the LVO group, with unique V-J gene combinations, indicating potential biomarkers for LVO diagnosis. Overall, AIS with LVO rapidly triggers a peripheral immune response and our findings will help further understanding of the pathophysiological mechanism of AIS with LVO.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found: https://www.ncbi.nlm.nih.gov/, PRJNA1171875.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Jinan Central Hospital Affiliated to Shandong First Medical University (No. SZR2021-006-01) and The Second Hospital of Shandong University (No. KYLL-2021(KJ)P-0300). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LM: Data curation, Funding acquisition, Investigation, Writing – original draft. BS: Data curation, Investigation, Writing – review & editing. CF: Investigation, Writing – review & editing. JX: Investigation, Writing – review & editing. MG: Investigation, Writing – review & editing. JL: Investigation, Writing – review & editing. RJ: Investigation, Writing – review & editing. YJ: Data curation, Investigation, Writing – review & editing. DL: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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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.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

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The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2024.1512720/full#supplementary-material

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EDITED BY
Joachim Eduard Weber,
Charité University Medicine Berlin, Germany

REVIEWED BY
Natasza Blek,
Maria Sklodowska-Curie Medical Academy, Poland
Alina Sacarescu,
Grigore T. Popa University of Medicine and Pharmacy, Romania

*CORRESPONDENCE
Antonia Ioana Vasile
✉ antoniaioana97.vasile@gmail.com

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Copeptin as biomarker for acute ischemic stroke prognosis and revascularization treatment efficacy

Antonia Ioana Vasile[1,2]*, Cristina Tiu[1,2] and Corin Badiu[2,3]

[1]Neurology Department, University Emergency Hospital of Bucharest, Bucharest, Romania, [2]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, [3]Endocrinology IV, C.I. Parhon National Institute of Endocrinology, Bucharest, Romania

Introduction: Pro-arginine vasopressin consists of three peptides: *arginine-vasopressin, neurophysin II, and copeptin*. AVP is released by the neurohypophysis in response to increased plasma osmolality, decreased blood volume and stress. Copeptin has the advantage of being stable ex vivo and easy to measure. New data show the importance of copeptin in ischemic stroke and its complications.

Methods: We present a literature review that highlights the importance of copeptin as a biological marker for stroke. We searched the Pubmed and Scopus databases for papers with the following keywords: "stroke AND copeptin." PRISMA criteria were used.

Results: We identified 332 papers that met the criteria. We excluded analyzed reviews, systematic reviews and meta-analyses. 31 articles resulted. The number of patients included in the analyzed studies varied between 18 and 4,302. Copeptin is a marker that associated with clinical stroke severity, infarct volume, short-term and long-term functionality and mortality and adds prognostic value to the previously used scales. It may reflect the effectiveness of revascularization therapy. Copeptin is a biomarker that can help predict post-stroke complications such as: cerebral edema and hemorrhagic transformation.

Discussion: Copeptin is a novel and promising biomarker for evaluating cerebrovascular diseases. Because it is considered a non-specific biomarker, it is not yet used routinely and it cannot replace the clinical examination. However, combined with other clinical or paraclinical parameters, it can increase the accuracy of the diagnosis.

KEYWORDS

copeptin, acute ischemic stroke, stroke prognosis, stroke biomarkers, treatment efficiency in stroke patients

1 Introduction

Stroke is globally recognized as an important cause for mortality and disability (1). Ischemic stroke is the second leading cause of death in elderly patients over 60 years old and the fifth leading cause of death in patients aged 15 to 59 years (2). It is the leading cause of long-term physical and cognitive disability (3).

Survivors often require long-term care and are at a higher risk of recurrent stroke, compared to normal controls (4). This is why there is a need for rapidly measurable biomarkers that can predict stroke prognosis in order to optimize care and allocation of healthcare resources.

Although much progress has been made in the clinical and imaging management of stroke patients, there is still a lack of biological markers that can be used in the diagnosis and prognosis of these patients. Blek et al. (5) mention several markers associated with the prognosis of these patients, among which are: markers of *inflammation* (procalcitonin and mannose binding lectin), markers of *atherogenesis* (adipocyte fatty acid-binding protein), markers that demonstrate *stress response* (copeptin and cortisol) and natriuretic peptide. Other biomarkers used to evaluate functional outcome such as: mid-regional pro-atrial natriuretic peptide (MRproANP), mid-regional proadrenomedullin (MRproADM), C-terminal proendothelin, interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), lipopolysaccharide-binding protein (LBP), human leukocyte antigen-DR isotype (HLA-DR) (1, 6), C-reactive protein (CRP) (7–9), matrix metalloproteinase-9 (MMP-9) (10), fibrinogen (FBG) (11) and brain natriuretic peptide (12).

Thrombolysis and mechanical thrombectomy are the only effective treatments for patients with acute ischemic stroke that arrive at the hospital in the early stages (13, 14). However, the efficacy of both treatments are time-dependent, so the fast diagnosis of ischemic stroke is required (15). Clinical diagnosis of stroke in the emergency room can be challenging and, most of the time, requires brain imaging. Computer tomography (CT) or magnetic resonance imaging (MRI) are the only paraclinical evaluations that could rule out hemorrhagic stroke or other pathologies that mimic stroke. But there are some situation (such as lack of radiology department availability) when the time-critical decision to start treatment is based only on the clinical presentation (15). In these situation, biomarkers that diagnose or differentiate stroke may be helpful. One of the biomarkers that gained attention recently is copeptin (5, 15–19).

Pro-arginine vasopressin (pro-AVP) is produced by the supraoptic and paraventricular nuclei in the hypothalamus and acts as a precursor for 3 peptides: *arginine-vasopressin, neurophysin II, and copeptin* (20). Arginine-vasopressin (AVP) or antidiuretic hormone (ADH) is released by the neurohypophysis in response to stress, increased plasma osmolality or decreased blood volume (21). While AVP receptor antagonists are studied for their cerebral protective effects, they are not typically administered as standard treatment in stroke but may hold promise in managing cerebral edema or related complications (22).

Copeptin can be identified in circulation in equimolar amounts with AVP (23–25). Measuring the blood concentration of AVP is complicated given that it is an *unstable* peptide (plasma half-life of 5–15 min) and is *bound to platelets* (26, 27). On the other hand, copeptin is a *stable* molecule and *easy to measure* (28). Copeptin may be a useful biomarker in the Emergency Room because the assay can be available within 60 min only (29). Median copeptin levels for healthy controls range from 3.7 to 4.2 pmol/L (24).

Botros et al. (30) evaluated copeptin levels at 64 patients who presented to the emergency room for acute illness such as: chronic liver disease, chronic obstructive pulmonary disease (COPD), stroke or decompensated heart failure in comparison to healthy controls. They reported that there was a significant difference between survivors and non-survivors of stroke patients. The researchers also demonstrated that a high copeptin level is associated to longer hospital stay and a poor outcome (30). Also, it was demonstrated that copeptin levels were lower in non-stressed healthy controls in comparison with hospitalized patients and with patients that went under significant surgical interventions associated with a high level of stress, this result reflecting that copeptin is influenced by the individual stress level (31).

Studies have shown that elevated copeptin levels correlate with prognosis and may help in the differential diagnosis prior to imaging in several cardiovascular pathologies such as: acute myocardial infarction, congestive heart failure, ischemic stroke, aneurysmal subarachnoidal hemorrhage and head trauma (32–36).

AVP has an important role in the development of cerebral edema (37), which increases the severity of a stroke (38). Kozniewska & Romaniuk (39) explained that vasopressin prevents brain cell adaptation to hyponatremia and participates in vasogenic edema and cellular ballooning after stroke. Vakili et al. (40) demonstrated that *vasopressin administration exacerbates acute ischemic cerebral edema*. Moreover, it was shown that *treatment with vasopressin receptor blocker drugs reduces cerebral edema* (41). Thus, it was postulated that *measuring copeptin can have prognostic significance in the development of cerebral edema post-stroke* (42).

While there are several studies that demonstrated the prognostic value of copeptin in acute ischemic stroke patients, there are only 4 studies that took into consideration recanalization therapies (4, 37, 43, 44). The efficacy of recanalization therapies are time-dependent and change the prognostic of patients, so fast diagnosis of ischemic stroke is needed (15).

This review summarizes and discusses the value of copeptin in ischemic stroke *diagnosis*, short-term and long-term *prognosis* of ischemic stroke and its importance in relation with *treatment options*. The present study presents a review of recent studies that assessed: (1) diagnostic and prognostic value of plasma copeptin concentration in acute ischemic stroke patients; (2) prognostic value of plasma copeptin concentration in acute ischemic stroke patients who were eligible for recanalisation therapies.

2 Methodology

We performed a review of the literature analyzing PubMed and Scopus databases. We used "stroke" AND "copeptin" as keywords. The search was limited to articles in English language. We applied the search filter of publications between the years 2010–2024. We analyzed only available full-text articles, such as clinical trials and randomized clinical trials, excluding reviews, systematic reviews and meta-analyses. PRISMA criteria were used.

Thus, the PubMed database revealed 141 results. We excluded articles that were reviews, systematic reviews or meta-analysis ($n = 32$). We excluded studies performed *exclusively on transient ischemic attack* ($n = 4$). We excluded studies performed *exclusively on subarachnoid hemorrhage* ($n = 5$). We excluded studies performed *exclusively on intracerebral hemorrhage* ($n = 4$). We excluded articles that focused on copeptin as a *marker for differential diagnosis* (*with vertigo, with stroke mimics, between stroke subtypes;* $n = 6$). We excluded studies that evaluated *exclusively other biomarkers, not copeptin* ($n = 3$). We excluded studies that evaluated patients with diagnoses other than stroke or symptoms suggestive of another condition (end-stage renal disease, chronic renal disease, adult polycystic kidney disease, chest pain, acute myocardial infarction, heart failure, stable coronary disease, atrial fibrillation, coronary artery ectasia, pulmonary embolism, pregnancy, cirrhosis, craniopharyngioma, chronic insomnia, post-stroke depression,

post-stroke infections, hyponatriemia/hypernatriemia; $n = 50$). We excluded studies that were performed on non-acute stroke patients (patients recovering from stroke, patients undergoing non-cardiac surgery, patients after coronary surgery, patients after elective carotid endarterectomy, recreational marathon runners, healthy people, elderly people, dialysis patients; $n = 9$). We excluded nursing practice guidelines ($n = 1$). We excluded one more article that was in Chinese language ($n = 1$). In the end, PubMed database revealed 26 results.

The Scopus database revealed 191 results. After applying the criteria (article types, English language only), 131 relevant articles were identified. We excluded articles that were reviews or meta-analysis ($n = 9$). We excluded studies performed *exclusively on transient ischemic attack* ($n = 3$). We excluded studies performed *exclusively on subarahnoid hemorrhage* ($n = 3$). We excluded studies performed *exclusively on intracerebral hemorrhage* ($n = 4$). We excluded articles that focused on copeptin as a *marker for differential diagnostic* (*with vertigo, with stroke mimics, between stroke subtypes;* $n = 6$). We excluded studies that evaluated *other biomarkers*, but not copeptin ($n = 3$). We excluded studies that evaluated other biomarkers, but not copeptin ($n = 2$). We excluded studies that evaluated patients with other diagnosis than stroke (patients with acute illnesses, hyperthyroidism, acute myocardial infarction, cardiogenic shock, pulmonary embolism, heart failure with preserved ejection fraction, acute coronary syndrome, atrial fibrillation, heart failure with reduced ejection fraction, preeclampsia, chronic heart failure, coronary artery ectasia, chronic obstructive pulmonary disease, chronic insomnia, chest pain, cirrhosis, polycystic kidney disease, post stroke depression, post-stroke infections, post-stroke fever, pregnancy, resynchronization therapy, hypernatriemia/hyponatriemia, acute illness, acute mental stress; $n = 55$). We excluded studies that were performed on non-acute stroke patients (patients who recovered from stroke, patients after coronary surgery, patients after cardiac surgery, patients undergoing non-cardiac surgery, patient undergoing elective carotid endarterectomy, recreational marathon runners, healthy people, elderly people, dialysis patients; $n = 9$). We excluded articles that were only protocols or nursing practice guidelines ($n = 1$). We excluded a study performed on animals ($n = 1$). In the end, Scopus database revealed 29 results.

After excluding duplicate articles between the two databases ($n = 24$), 31 articles resulted for study. The number of patients included in the analyzed studies varied between 18 and 4,302.

The PRISMA flow diagram that summarized the screening process can be seen in Figure 1.

3 Results

3.1 Copeptin as a prognostic biomarker in acute ischemic stroke

Jihad et al. (45) evaluated renalase, copeptin, N-terminal pro B-type natriuretic peptide (NT-proBNP) and MMP-9 concentrations in 42 ischemic stroke patients in comparison to 40 healthy individuals. They demonstrated a significant increase in the levels of copeptin, NT-proBNP and MMP-9 for stroke patients compared to healthy controls. Moreover, they also found a significant difference according to severity for stroke patients for copeptin (0.0012), renalase (0.0069) and MMP-9 (0.0094): for severe ischemic stroke (National Institutes of Health Stroke Scale (NIHSS) score > 14) compared to moderate ischemic stroke (NIHSS score < 14).

The results of a study by Keshk et al. (46) suggest that copeptin, thrombomodulin, and alarmin signaling pathways play an important role in the chronic inflammatory state of obese patients with ischemic stroke. The study included 30 ischemic stroke patients (15 overweight and 15 normal weight) and compared them to 20 healthy controls (10 overweight and 10 normal weight). Their result suggested that copeptin level was significantly increased in overweight ischemic stroke patients with NIHSS scores over 7 points. They found increased copeptin levels to all overweight stroke patients. Also, copeptin levels correlated with anthropometric measurements, severity scores and vascular damage scores. The authors hypothesized that increased inflammation and endogenous stress in obesity and after an ischemic stroke stimulate the same hypothalamo-hypophysis-vasoppresin axis (46). Similar to previous studies, they suggested that higher copeptin levels are linked to obesity, metabolic syndrome and insulin resistance (47).

Wannamethee et al. (48) reported that copeptin is independently associated with an increased risk of stroke and cardiovascular mortality in men with diabetes. They conducted a prospective study on 3,536 men aged between 60 and 79 years old who were followed to evaluate the occurrence of myocardial infarction, stroke events and cardiovascular disease deaths. They demonstrated that there is no association between copeptin and incident stroke in men *without* diabetes, while *elevated copeptin levels were associated with increased risk of stroke* and cardiovascular disease mortality in men *with* diabetes. Their result suggested that targeting the AVP system might have beneficial effects on cardiovascular disease mortality and stroke risk in older men with diabetes (48). Their findings demonstrate that *copeptin is associated with the development of stroke, even in diabetic patients without prevalent stroke*. It is still uncertain if there is a causal relationship between copeptin and stroke risk in patients with diabetes, but it was not explained by conventional risk factors such as stroke, insulin resistance or level of NT-proBNP. Studies have shown that copeptin is strongly associated with microalbuminuria, suggesting the role of AVP system in producing albuminuria, which has been linked to incident stroke in diabetic patients (49, 50). This may be the consequence of the antidiuretic effects of vasopressin, leading to increased excretion of albumin (51). Therefore, the relationship between copeptin levels and incident stroke may be linked to the development of albuminuria (48). This conclusion is important in relation to treatment options, such as empagliflozin. This molecule was added to the standard treatment protocol for reducing morbidity and mortality in patients with type 2 diabetes at high cardiovascular risk (48). Empagliflozin is a sodium glucose cotransporter (SGLT)-2 inhibitor that stimulates the excretion of glucose leading to osmotic diuresis and reduced blood pressure.

Fenske et al. (52) analyzed data from hemodialysis patients with type 2 diabetes and reported that copeptin levels strongly associated with an increased risk of stroke, sudden death, cardiovascular events and mortality.

Katan et al. (53) evaluated several blood markers in stroke patients: procalcitonin, copeptin and MRproANP. They evaluated 3,298 healthy individuals who were followed-up. During the follow-up period, 172 individuals developed acute ischemic stroke. The authors compared their results with 344 healthy controls. In their unadjusted analysis, individuals

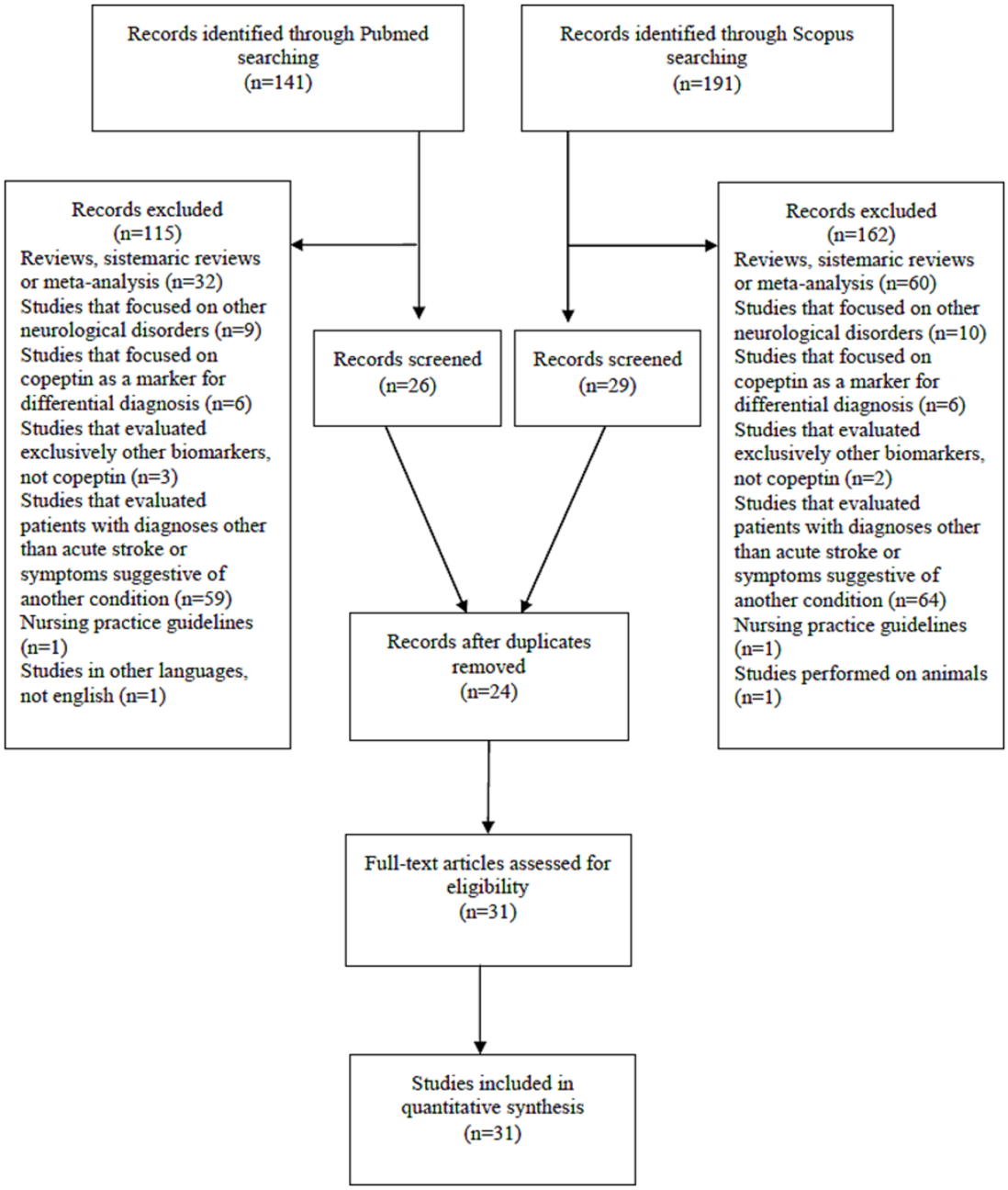

FIGURE 1
PRISMA flow diagram.

who were in *the top copeptin quartile had an increased risk of ischemic stroke compared to those in the lowest quartile*. However, they did not report any significant association between copeptin and stroke etiology. Katan et al. (53) support the association between ischemic stroke and procalcitonin and MRproANP. The associations of procalcitonin and MRproANP differed by stroke etiology based on TOAST classification. Procalcitonin levels in the top quartile were associated more with small vessel stroke and MRproANP levels were associated with cardioembolic stroke.

Perovic et al. (54) assessed differences in resistin and copeptin concentration between 112 ischemic stroke patients and 63 healthy controls, but also assessed whether these markers have any prognostic value for *ischemic volume*, *stroke severity* (measured by NIHSS score) and *patients' functionality* (measured by Barthel Index). Resistin is an adipocytokine produced by adipocytes that has been shown to be a promising prognostic marker in lacunar ischemic stroke (55). Perovic et al. (54) mentioned that the increase in resistin levels in the acute phase after stroke may be explained by an early inflammatory response to acute tissue injury. However, studies are contradictory regarding the role of resistin in stroke. Weikert et al. (56) reported that elevated levels of resistin are associated with a higher risk of myocardial infarction but not with stroke risk. Perovic et al. (54) demonstrated that resistin levels were significantly higher in stroke patients compared to healthy controls (3.2 mg/L versus 2.5 mg/L), but found no correlations between resistin

level and NIHSS score, Barthel index or ischemic volume. On the other hand, copeptin concentration did not differ between healthy controls and stroke patients. However, *among stroke patients, copeptin levels differentiated those with good functionality from those with poor functionality* (Barthel index below 60), with higher levels observed in patients with a lower index. This result suggests that copeptin can predict the functionality of patients. The researchers did not identify differences in copeptin levels between groups based on NIHSS severity score. They also disproved the hypothesis that resistin and copeptin levels vary with infarction volume. The conclusion of Perovic et al. (54) was that resistin, but not copeptin, is elevated in ischemic stroke patients compared to age and sex matched healthy controls.

Hotter et al. (1) demonstrated an association between NIHSS score and IL-6, as well as an association between 90-day functional outcomes (measured with the mRS) and levels of copeptin, MR-proADM, IL-6, and HLA-DR in their evaluation of 91 stroke patients. IL-6 was the only biomarker that correlated with some radiological features (acute volume of DWI, extent of perfusion imaging (PI), DWI-PI-mismatch, final lesion volume, cortical infarcts). Their conclusion support the idea that IL-6 is an inflammatory marker of cerebral parenchymal damage and that copeptin correlated with functionality at 3 months (1).

Oraby et al. (43) evaluated 45 patients with first ever acute ischemic stroke and 45 healthy controls in order to investigate the relationship between copeptin and short-term prognosis of acute ischemic stroke after 3 months (measured by mRS). They demonstrated that copeptin levels were higher in stroke patients (mean of copeptin 120.52 pg./mL), compared to healthy controls (mean of copeptin 76.51 pg./mL). In addition, patients with severe clinical presentation (NIHSS>16 points) had a higher level of copeptin (mean of copeptin 139.45 pg./mL) in comparison to those with mild-to-moderate stroke (NIHSS 0–15 points; mean of copeptin 95.47 pg./mL). Patients with unfavorable outcome (mRS > 3) had a higher level of copeptin (mean of copeptin 142.45 pg./mL) in comparison to those with favorable outcome (mRS 0–2 points; mean of copeptin 93.47 pg./mL). Another important finding was that copeptin was significantly lower in patients who were eligible for revascularization with recombinant tissue plasminogen activator (rTPA; mean of copeptin 99.55 pg./mL compared to 127.31 pg./mL). They did not identify any significant differences between copeptin levels and ischemic stroke subtypes according to TOAST classification. However, they reported that patients with diabetes mellitus, hypertension and dyslipidemia had a higher levels of copeptin. Other significant correlation identified were with: volume of infarction measured by MRI, NIHSS score, clinical outcome at 3 month follow-up.

Hotter et al. (6) analyzed 573 stroke patients and evaluated several biomarkers (procalcitonin, copeptin, C-terminal pro-endothelin and midregional pro-adrenomedullin) and demonstrated an association between all of them and functional outcome at 3 months and death. Another interesting result was that cardioembolic stroke was associated with higher levels for all biomarkers, especially for MRproANP. However, none of the biomarkers improve the prediction of death or functional outcome in regression models. The multivariate model for predicting death at 90 days retained only age and NIHSS as predictors.

In a study evaluating stroke in patients with type 2 diabetes, researchers evaluated the prognostic value of copeptin on patients' functionality and mortality at 3 months (57). They evaluated 247 patients, and the median copeptin value was 14.3 pmol/L. Copeptin level was strongly associated with unfavorable functional outcome. Copeptin levels increased with stroke severity, as defined by the NIHSS score. In the group of patients who underwent cerebral MRI, copeptin levels were associated with lesion size on MRI: for small lesions (<10 mL), the copeptin level was 6.9 pmol/L; for medium-sized lesions (10–100 mL), the level was 13.6 pmol/L; and for large lesions (>100 mL), the level was 18.1 pmol/L. At 3 months, 86 patients (34.8%) had favorable functionality. Copeptin level in patients with unfavorable functionality was significantly higher compared to those with favorable functionality (16.2 pmol/L compared to 12.4 pmol/L). In addition, among the 41 patients who died, *copeptin levels were almost twice as high compared to those who survived* (20.9 pmol/L vs. 12.4 pmol/L). Their main finding was that copeptin predicts the development of unfavorable functional outcome independent of NIHSS score, FBG or CRP. Thus, the conclusion of Wang et al. (57) was that an increased level of copeptin plays an important role in the progression of stroke in patients with comorbid type 2 diabetes.

De Marchis et al. (37) evaluated 783 patients with acute ischemic stroke and demonstrated that copeptin independently predicts unfavorable outcome [modified Rankin Score (mRS) > 3 points] within 90 days, mortality within 90 days and complications (symptomatic intracerebral hemorrhage, space-occupying cerebral edema, pneumonia, seizures or mortality within 10 days from stroke onset). They concluded that copeptin is a validated blood marker that adds predictive value for outcome and mortality at 3-month follow-up beyond stroke severity and age.

De Marchis et al. (58) developed a score (CoRisk score) that can predict the 3 month poor prognosis of patients with ischemic stroke (measured by mRS). The study was conducted on 1,102 patients. The score is calculated based on copeptin level, age, NIHSS score, and recanalization therapy. The advantage of CoRisk score is that it adds the recanalization therapy, because the most prominent current prognostic scales are lacking variables for acute treatment (59). Moreover, evidence shows that eligibility for acute treatment improves outcomes for stroke patients, and the importance of including it in prognosis scores is well-established (14). They demonstrated that age, NIHSS score, and copeptin were independent variables associated with 3-month prognosis and mortality. Then, the researchers evaluated two prediction models: the first predicted functional prognosis using the mRS score; the second predicted death versus survival at 3 months post-stroke. An unfavorable prognosis was identified in 436 (40%) of the patients, and the CoRisk score correctly classified 75% of patients. The CoRisk score demonstrated a sensitivity of 67% and a specificity of 80% in predicting prognosis. For predicting death versus survival, their model was not well calibrated. De Marchis et al. (58) acknowledged that a limitation of their study was testing in a limited geographic area and emphasized the importance of validating the CoRisk score in other regions. Second limitation of their study was the lack of validation for mortality prediction. However, the main strength of CoRisk score was that it has a high prognostic accuracy despite having only 4 variables, all easily accessible in the emergency room, only using blood sampling (58).

Urwyler et al. (60) demonstrated on 362 patients that copeptin was an independent predictor of functional outcome after 1 year (measured by mRs) and all-cause mortality. In the 146 patients with poor functional outcome, copeptin levels were higher compared to the patients with good functional outcome (19.3 pmol/L compared to 8.12 pmol/L). In their regression analysis, the authors demonstrated that copeptin had a better prediction for prognosis than white blood cell count, CRP and glucose, but a similar prediction rate to NIHSS score. In terms of analyzing mortality rate, copeptin levels in nonsurvivors were higher compared to survivors (28.10 pmol/L compared to 9.34 pmol/L). In their regression

analysis, the authors demonstrated that copeptin had a similar prediction rate to NIHSS score in predicting mortality, but better results in comparison to white blood cell count, CRP or glucose. Urwyler et al. (60) concluded that copeptin may be a reliable and independent marker for predicting long-term outcome in ischemic stroke patients. They demonstrated that copeptin is a better prognostic marker compared to other markers, because it improves the prognostic value of the NIHSS score for long-term mortality and functional outcome at 1-year of follow-up. Compared to other brain markers, copeptin mirrors intracerebral processes and is released directly into circulation, bypassing the blood–brain barrier (60).

Zhang et al. (27) evaluated 245 ischemic stroke patients and 100 healthy volunteers in order to investigate the relationship between copeptin levels and functional outcome (mRS) and mortality within 1 year. Patients with stroke had a higher level of copeptin compared to control cases (12.4 pmol/mL compare to 3.9 pmol/mL). Patients with unfavorable outcome and increased levels of copeptin at admission compared to those with favorable outcome (21.9 pmol/mL versus 10.3 pmol/mL). Copeptin levels increased with the increase on the NIHSS scale: the mean copeptin for patients with NIHSS 0–6 points was 8.3 pmol/mL, for patients with NIHSS 7–15 points was 14.3 pmol/mL, for patients with NIHSS >15 points was 27.2 pmol/mL. Copeptin levels increased with the increased lesion size on MRI: the mean copeptin for patients with small lesions was 6.2 pmol/mL, for patients with medium lesions was 13.9 pmol/mL, for patients with large lesions was 17.9 pmol/mL. Also, non-survivors had a higher level of copeptin at admission (mean copeptin for patients who died was 36.2 pmol/mL versus 10.6 pmol/mL for patients who survived). Their multivariate regression showed that copeptin was an independent predictor for functional outcome and survival.

Tu et al. (61) conducted a multicenter observational study of 4,125 patients, where they demonstrated that copeptin and NT-proBNP levels in patients with ischemic stroke can predict all-cause mortality and cardiovascular mortality at 1 year. In the follow-up year, 906 patients (20.1%) died, of which 589 died from cardiovascular causes (13.1%). They revealed a significant correlation of copeptin level with age, glomerular filtration rate, NIHSS score and NT-proBNP level. For patients in whom brain MRI was available, the *volume of the infarction correlated with copeptin levels*. Also, patients with *previously known type 2 diabetes were those with higher levels of copeptin*. During 1 year follow-up, Tu et al. (61) demonstrated that both NT-proBNP and copeptin are independent prognostic biomarkers for assessment of prognosis of stroke. Tu et al. (61) demonstrated an association between the level of the two biomarkers and mortality, managing to stratify the research group into 3 risk groups: those with low risk (those with the two biomarkers below average), those with intermediate risk (with one of the two biomarkers above average) and those at increased risk (with both markers above average). Tu et al. (61) speculated on the possible mechanisms underlying their results: AVP secretion may be stimulated through the brainstem and limbic system, triggered by stress factors. Thus, the concentration variation of copeptin may be useful for evaluating the severity of damage independently of lesion dimensions, age, sex and clinical impairment at the moment of admission. Another mechanism speculated was that ischemic neuronal injury stimulates adrenocorticotropic hormone that leads to hypercortisolism and may influence the level of copeptin/AVP (61).

Tang et al. (4) evaluated 316 patients with ischemic stroke and wanted to determine copeptin levels and its association with stroke recurrence during the first year of follow-up. Patients were followed at 3, 6 and 12 months after the stroke. Recurrence of stroke was defined as the sudden functional deterioration of the neurological status demonstrated by an increase in the NIHSS score by at least 4 points or by having a new focal neurological deficit lasting more than 4 h. One of the results of the study was that 54 patients (17.1%) had a *stroke recurrence* and in these patients copeptin levels were *higher compared to those without recurrence* (28.9 pmol/L versus 21.0 pmol/L). 135 patients in their study had a *minor stroke* (NIHSS score below 5 points) and they had a *lower copeptin level compared to those with more severe forms* (19.5 pmol/L versus 25.4 pmol/L). The researchers demonstrated that copeptin levels above 31.8 pmol/L were associated with a NIHSS score above 6 points. The positive correlation between copeptin levels and moderate-to-severe clinical severity demonstrates that *copeptin mirrors the stress associated with extensive stroke*. They demonstrated positive correlations between copeptin and cortisol levels, infarcted brain volume, body mass index, age, systolic blood pressure (BP), diastolic BP and NIHSS score. One of the most important conclusions of the study was that copeptin, age, NIHSS score, infarct volume, stroke etiology, revascularization treatment, personal history of atrial fibrillation, CRP, homocysteine and cortisol levels are *predictors of stroke recurrence*. In addition, they demonstrated that copeptin has a higher predictive value for stroke recurrence than CRP, homocysteine, cortisol, or NIHSS score. Another interesting result of their study was that the median copeptin levels were higher for atherothrombotic stroke subtype compared to other stroke subtypes. The researchers concluded that an increased level of copeptin plays an important role in the progression of ischemic stroke. Tang et al. (4) concluded that copeptin is an important and independent marker that predicts 1-year stroke recurrence in patients with ischemic stroke. Moreover, they demonstrated that copeptin can improve the prognostic value of NIHSS score.

Greisenegger et al. (23) reported that, after adjusting for age, sex and risk factors, copeptin predicted recurrent vascular events, recurrent ischemic stroke and death, particularly after cardioembolic TIA/stroke. Accurate prediction of recurrent vascular event is extremely important, because patients at high risk should benefit from more aggressive secondary preventive strategies and should be included in trials of new treatments (23). Interestingly, in their study, levels of copeptin did not differ statistically significant between first sampling and 1-year follow-up with a median of 6.0 pmol/L and 5.4 pmol/L, respectively. Moreover, the researchers detected a significant association between plasma copeptin levels and etiology of stroke and a borderline interaction with sex: the predictive value of copeptin was more pronounced in male patients and in patients with cardioembolic stroke. Another important aspect reported by Greisenegger et al. (23) was *comparing* the predictive value of copeptin for recurrent vascular events *with other standard biomakers related to: inflammation* (CRP, IL-6, Neutrophil gelatinase associated lipocalin, Tumor necrosis factor receptor 1), *thrombosis* (von Willenbrand factor, D-dimer, P-selectin, FBG, Thrombomodulin, protein-Z), *cardiac or neuronal function or injury* (Heart-type fatty acid binding protein, NT proBNP, Neuron specific enolase, Brain derived neurotrophic factor). They demonstrated that copeptin had the highest predictive value in the prognostic of recurrent vascular events and exceeded all other biomarkers (23). Their conclusion was that copeptin might help with better risk stratification of patients with stroke.

Zeng et al. (62) evaluated 4,302 ischemic stroke patients in a multicenter cohort study to determine whether plasma copeptin and

NT-proBNP levels correlated with stroke recurrence within 3 months of the initial event. In their receiver-operating characteristic analysis of stroke recurrence, the authors demonstrated an increase from 0.80 to 0.83 when adding NT-proBNP to clinical scores and an increase from 0.83 to 0.86 when adding both NT-proBNP and copeptin levels to clinical scores. They demonstrated that copeptin levels may predict stroke recurrence, especially for patients with higher than median NT-proBNP levels. Another interesting idea suggested by Zeng et al. (62) was that the fact that copeptin and NT-proBNP have complementary power may suggest that these two markers are stimulated by different aspects of cardiovascular homeostasis. Their complementary power also explain that both markers together may be useful for risk stratification in stroke patients, more than each marker alone (62).

Wang et al. (63) evaluated the correlation between copeptin levels and 1 year mortality on 275 ischemic stroke patients which were recruited within 24 h after onset. Copeptin levels were significantly higher in acute ischemic stroke patient compared to healthy controls. They found a significant correlation between copeptin levels and NIHSS score. Infarction lesions on MRI correlated with copeptin levels (for small lesions 6.8 pmol/L, for medium lesions 15.9 pmol/L, and for large lesions 20.2 pmol/L). Moreover, the authors found that copeptin levels were higher in patient with atherosclerosis subtype of ischemic stroke. The authors observed that copeptin levels were significantly higher in non-survivors compared to survivors. They also found copeptin as an independent stroke mortality predictor, which an area under ROC curve of 0.882, demonstrating a sensitivity of 90.7% and specificity of 84.5% (63). The cut off value for copeptin as an indicator or mortality was around 20.5 pmol/L. In their multivariate analysis, the predictors for death were: copeptin levels, NIHSS score, age, CRP, D-dimer.

Similar to Wang et al. (63), Katan et al. (64) analyzed 362 patients with ischemic stroke and demonstrated that copeptin levels positively associated with unfavorable outcome and mortality within 90 days. Copeptin levels positively correlated with NIHSS scores and mRS scores and paralleled infarct lesion sizes on MRI. Copeptin levels were more than 3 times greater in patients who died compared to survivors. In their univariate analysis for predicting functional outcome, the significant variables were: copeptin, CRP, age, female sex, NIHSS, hypertension, atrial fibrillation, total anterior circulation syndrome, posterior circulation syndrome and small vessel occlusive. In their univariate analysis for predicting mortality within 90 days, the significant variables were: copeptin, CRP, glucose, age, NIHSS, atrial fibrillation, coronary heart disease, total anterior circulation syndrome and small-vessel occlusive. The authors demonstrated that the predictive value of copeptin was comparable with the predictive value of NIHSS score, but superior to CRP and glucose (64). Their conclusion was that copeptin had a prognostic accuracy that is superior to other commonly used laboratory parameters or clinical variables.

In contrast, Richard et al. (65) evaluated several biomarkers (4 cell adhesion molecules, CRP, IL-6, NT-proBNP, troponin, copeptin and S100 calcium binding protein B) on patients presenting with ischemic stroke, hemorrhagic stroke and transient ischemic attack. They did not find any association with copeptin levels.

Table 1 presents a summary of findings on copeptin as a prognostic biomarker in acute ischemic stroke, highlighting the number and types of patients included, assessed biomarkers, evaluated outcomes (stroke severity, stroke occurrence, short-term and long-term prognosis, scales used, stroke recurrence, and mortality), findings (correlations and predictions), and practical applications.

3.2 Copeptin in acute ischemic stroke depending on revascularization strategies

Several studies have suggested that prognostic scores for ischemic stroke patients need to consider intravenous or endovascular recanalization therapies (14). Many studies proved the relation between copeptin and functional outcome and mortality in ischemic stroke patients, but Spagnolello et al. (44) is the first one that added the relation to acute intervention and complications of stroke (cerebral edema and hemorrhagic transformation).

Spagnolello et al. (44) evaluated the temporal profile of copeptin levels in revascularized ischemic stroke patients and sought to identify correlations with the development of cerebral edema and hemorrhagic transformation. Researchers evaluated 34 patients at the time of admission (T0), 24 h post-recanalization procedure (T1), and 3–5 days after admission (T2) with imaging and serologic evaluations.

They demonstrated a mean copeptin concentration of 50.71 pmol/L at time T0; 18.31 pmol/L at time T1; and 10.92 pmol/L at time T2. The researchers found no correlation between copeptin levels and the length of time since the onset of symptoms. Copeptin levels at the *time of admission (T0) were higher in those with NIHSS score above 10 points*. In addition, although not statistically significant, *higher* levels of copeptin were identified in patients who were *hospitalized in less than 6 h* after the onset of symptoms. Copeptin levels at T0 correlated with a worse functional outcome at 1-year follow-up. Copeptin levels at T1 correlated with worse functional outcome at 1-year follow-up and mortality at 1-year follow-up. Copeptin levels at T2 were higher at patients with mRS over 3 points or died at the 1-year follow-up (44).

When analyzing the complications of stroke, the authors demonstrated that copeptin level at time T1 was more elevated in patients who, subsequently, at time T2, had moderate–severe brain edema. In addition, patients with hemorrhagic transformation at T1 and T2 were shown to have increased levels of copeptin at T1 (44).

When analyzing the relation to revascularization therapy, the authors highlighted that: the difference in copeptin level between T1 and T0 was significantly greater in patients who received revascularization therapy, especially in those who received *both* thrombolysis and thrombectomy compared with those who received only conservative management. Differences in copeptin between T1 and T0 were: −4.50 pmol/L in patients who received only thrombolysis; −37.84 pmol/L in patients who received only thrombectomy; −129.34 pmol/L in patients who received thrombolysis and thrombectomy. In addition, the researchers also demonstrated a positive correlation between the *difference in copeptin levels between T1 and T0* with the *Thrombolysis in Cerebral Infarction (TICI) score* (44).

The first conclusion of their study was that copeptin is associated with resolution of cerebral edema and hemorrhagic transformation in ischemic stroke. The second conclusion of their study was that the decrease in copeptin dynamics was more significant in patients who received dual reperfusion therapy (thrombolysis and thrombectomy) compared to those who received single reperfusion therapy (thrombolysis or thrombectomy) and those who received conservative treatment. Thus, the dynamic decrease in the level of copeptin at 24 h after the recanalization intervention may mirror the efficiency of the revascularization therapy. The main limitation of the study was the small sample of patients (34 patients) (44).

TABLE 1 Studies that evaluated copeptin as a prognostic biomarker in acute ischemic stroke.

Study	Participants	Assessed biomarkers	Consequence	Finding (associations and predictions)	Practical application
Jihad et al. (45)	42 stroke patients versus 40 healthy controls	Copeptin Renalase MMP-9 NT-proBNP	Stroke severity	Elevated copeptin levels correlate with stroke severity (NIHSS)	
Zhou et al. (68)	70 stroke patients versus 40 healthy controls	Copeptin	Stroke severity	Copeptin levels were higher in patients with cerebral infarction and intracerebral hemorrhage compared to healthy subjects Copeptin levels positively correlated with NISS score and mRS score	Copeptin level has a certain value in the clinical diagnosis and prognosis of stroke
Keshk et al. (46)	50 ischemic stroke patients (15 overweight and 15 normal weight) versus 20 healthy controls (10 overweight and 10 normal weight)	Copeptin Thrombomodulin High mobility group box1 Lipocalin 2	Stroke severity	Copeptin level was significantly increased in overweight ischemic stroke patients with NIHSS scores over 7 points	Copeptin is an acute damage marker for ischemic stroke patients Copeptin may play a role in central adiposity that leads to metabolic syndrome
Wannamethee et al. (48)	3,536 men with diabetes	Copeptin	Stroke occurrence during follow-up period (approx. 13 years)	Elevated copeptin levels were associated with increased risk of stroke and cardiovascular disease only for men with diabetes	Copeptin is associated with development of stroke, even in diabetic patients without prevalent stroke Need to control diabetic risk factors Treatment options such as empagliflozin for patients with high cardiovascular risk
Katan et al. (53)	172 acute ischemic stroke patients versus 344 healthy controls	Copeptin Procalcitonin MR-proANP	Stroke occurrence in the follow-up period (approx. 13 years)	Individuals who were in the top copeptin quartile had an increased risk of ischemic stroke compared to those in the lowest quartile	Other markers (procalcitonin and MR-proANP) are associated with ischemic stroke risk
Perovic et al. (54)	112 acute ischemic stroke patients versus 63 healthy controls	Copeptin Resitin	Functionality at dischange (Barthel index)	Copeptin made no difference between stroke patients and healthy controls Copeptin was higher among patient with poor functionality (Barthel index<60) No differences in copeptin in terms of NIHSS or lesion size	
Dong et al. (69)	125 acute ischemic stroke patients	Copeptin	Functionality at 90 days (mRS)	Copeptin positively correlated with NIHSS score Copeptin levels were higher in patients that had a poor functional outcome, compared to those with favorable outcome Copeptin levels were almost double for patients who died compared to those who survived Copeptin was an independent predictor for poor functional outcome and mortality	
Hotter et al. (1)	91 acute ischemic stroke patients	Copeptin MR-proADM IL-6 HLA-DR	Functionality at 90 days (mRS) Radiologic features of the stroke lesion	There is an association between copeptin and functionality at 90 days Only IL-6 correlated with some radiological features of stroke lesion	No association between copeptin and radiologic features of the lesion

(Continued)

TABLE 1 (Continued)

Study	Participants	Assessed biomarkers	Consequence	Finding (associations and predictions)	Practical application
Oraby et al. (43)	45 acute ischemic stroke patients versus 45 healthy controls	Copeptin	Functionality at 3 months (mRS)	Copeptin levels were higher among stroke patients compared to healthy controls Patients with severe clinical presentation had a higher level of copeptin compared to those with mild-to-moderate stroke Patients with unfavorable outcome (mRS > 3) had a higher level of copeptin compared to those with favorable outcome (mRS 0–2 points) Copeptin was significantly lower in patients who were eligible for revascularization with rTPA No differences in copeptin levels according to TOAST classification Patients with diabetes, HTA and dyslipidemia had higher levels of copeptin Copeptin correlated with lesion size of MRI	
Hotter et al. (6)	573 acute ischemic stroke patients	Copeptin Procalcitonine C-terminal pro-endothelin Midregional pro-adrenomedullin	Functionality at 3 months Mortality at 3 months	All biomarkers were associated with functional outcome and mortality Cardioembolic strokes were associated with higher levels of all biomarkers None of the biomarkers did not improve the prediction of death or functional outcome, the only predictors remaining age and NIHSS	
Wang et al. (57)	247 acute ischemic stroke patients with diabetes	Copeptin Fibrinogen CRP	Functionality at 3 months (mRS) Mortality at 3 months	Copeptin levels in patients with poor functionality was higher compared to those with good functionality Copeptin correlates with NIHSS, lesion size Copeptin levels were 2 times higher in patients who died, compared to those who survived	Copeptin predict a poor prognosis intendent of NIHSS, FBG or CRP Copeptin plays an important role in the progression of stroke patients with type 2 diabetes comorbidity
De Marchis et al. (37)	783 acute ischemic stroke patients	Copeptin	Functionality at 3 months (mRS) Mortality at 3 months Complications	Copeptin independently predicts poor outcome within 3 months, mortality within 3 months and complications (symptomatic intracerebral hemorrhage, space-occupying cerebral edema, pneumonia, seizures or mortality within 10 days from stroke onset)	
De Marhis et al. (58)	1,102 acute ischemic stroke patients	Copeptin	Functionality at 3 months (mRS)	Age, NIHSS and copeptin levels were independent variables associated with 3-month functionality	CoRisk score that consist of: copeptin, age, NIHH, recanalisation therapy
Urwyler et al. (60)	362 ischemic stroke patients	Copeptin	Functionality at 1 year (mRS) All-cause mortality	Patients with poot functionality had higher levels of copeptin Copeptin in non-survivors were higher than in survivors Copeptin predicted better than WBC, CRP and glucose, but similar to NIHSS	Copeptin is a reliable marker for long-term prognosis
Zhang et al. (27)	245 acute ischemic stroke patients versus 100 healthy controls	Copeptin	Functionality at 1 year (mRS) Mortality at 1 year	Stroke patients has higher levels of copeptin compared to healthy controls Patients with poor functionality had higher levels of copeptin compared to those with good functionality Copeptin levels increased with the increase of NIHSS Non-survivors had a higher level of copeptin compared to survivors	

(Continued)

TABLE 1 (Continued)

Study	Participants	Assessed biomarkers	Consequence	Finding (associations and predictions)	Practical application
Wang et al. (63)	275 ischemic stroke patients versus 100 healthy controls	Copeptin	Mortality at 1 year	COPEPTIN levels were significantly higher in acute ischemic stroke patient compared to healthy controls Significant correlation between copeptin levels and NIHSS score INFARCTION lesions on MRI correlated with copeptin levels—copeptin levels were higher in patient with atherosclerosis subtype of ischemic stroke. Copeptin levels were significantly higher in non-survivors compared to survivors. Copeptin is an independent stroke mortality predictor, demonstrating a sensitivity of 90.7% and specificity of 84.5% at a cut-off value of 20.5 pmoL/L Predictors for 1 year mortality were: copeptin, NIHSS score, age, CRP and D-dimer	
Tu et al. (61)	4,125 acute ischemic stroke patients	Copeptin NT-proBNP	All-cause mortality at 1 year Cardiovascular mortality at 1 year Risk stratification	Copeptin correlated with age, glomerular filtration rate, NIHSS, NT-proBNP level and lesion size Higher levels of copeptin occured at diabetic patients Both copeptin and NT-proBNP are independent prognostic biomarkers for prognosis	Stratify stroke patients into 3 risk group according to levels of copeptin and NT-proBNP: low risk (both biomarkers below average), those with intermediate risk (with one of the two biomarkers above average) and those at increased risk (both markers above average)
Zeng et al. (62)	4,302 acute ischemic stroke patients	Copeptin NT-proBNP	Stroke recurrence during 3 months follow-up	Copeptin levels predict stroke reccurence, especially for patients with higher than median NT-proBNP levels	Copeptin alongside NT-proBNP may help for risk stratification in acute ischemic stroke patients, more than each marker alone
Tang et al. (4)	316 acute ischemic stroke patients	Copeptin Cortisol Homocysteine	Stroke recurrence during 1 year follow-up	Patients who had a stroke recurrence were the ones with higher levels of copeptin Patients with more severe stroke forms (NIHSS>5) had higher levels of copeptin Median copeptin levels were higher for atherosclerosis stroke subtype Copeptin, age, NIHSS, infarct volume, stroke etiology, revascularisation treatment, personal history of atrial fibrillation, CRP, homocysteine and cortisol levels are predictors of stroke recurrence.	Copeptin mirrors the stress associated with extensive stroke

(Continued)

TABLE 1 (Continued)

Study	Participants	Assessed biomarkers	Consequence	Finding (associations and predictions)	Practical application
Greisenegger et al. (23)	1,076 patients with acute ischemic stroke or TIA	Copeptin CRP IL-6 Tumor necrosis factor receptor 1 Neutrophil gelatinase associated lipocalin von Willenbrand factor D-dimer P-selectin Fibrinogen Thrombomodulin protein-Z Heart-type fatty acid binding protein NTproBNP Neuron specific enolase Brain derived neurotrophic factor	Reccurence of vascular event during the follow-up period (approx. 12 years)	The predictive value of copeptin was more pronounced in male patients and in patients with cardioembolic stroke Copeptin has the highest predictive value in predicting recurrent vascular events	Copeptin may help for a better risk stratification in terms of reccurence Patients at high risk for recurrence should profit from more aggressive secondary preventive strategies

4 Discussion

Abnormalities in endocrine functions have been reported in stroke, activation of the hypothalamo-pituitary–adrenal axis being one of the first measurable physiological responses to cerebral ischemia (27). AVP is an indicator that reflects the activation of hypothalamic–pituitary–adrenal axis and it was shown to correlate with the state and prognosis of cerebral infarction (29).

Firstly, we assessed copeptin as a prognostic biomarker in acute ischemic stroke. The prospective studies that we analyzed on healthy patients who were followed-up for stroke occurrence showed *contradictory results*. On one hand, research has shown that men with diabetes and higher copeptin levels have a greater risk of developing stroke compared to those with lower copeptin levels (48). Furthermore, some studies have demonstrated an association between elevated copeptin levels and stroke risk, such as Fenske et al. (52), who observed a strong link in hemodialysis patients with type 2 diabetes. On the other hand, other studies did not find this connection; for example, Katan et al. (53) reported that markers like procalcitonin and MR-proANP were associated with a higher risk of stroke, but not copeptin. When evaluating for short-term outcome, long-term outcome (measured on mRS scale or with Barthel index) and mortality, most studies have shown that higher copeptin levels associate with: poor functionality (at discharge, at 3 months and at 1 year) and death. Other studies have shown a correlation between copeptin levels and ischemic lesion size, NIHSS score, age, stroke subtypes, personal previous pathologies, but the *results are inconsistent* (1, 6, 27, 37, 43, 54, 57, 60, 61). When evaluating for stroke recurrence, some predictive models were developed based on copeptin levels (4) and it was shown that copeptin may help for a better risk stratification for a recurrent cardiovascular event (23).

Secondly, we assessed copeptin in acute ischemic stroke patients depending on revascularization strategies. We found in the literature only one study that assessed the copeptin levels in consecutive moments of time in revascularized ischemic stroke patients (44). Their first conclusion was that copeptin level may mirror the efficiency of the revascularization therapy. Their second conclusion was that copeptin levels correlate with development of cerebral edema and hemorrhagic transformation. Both conclusions help in prognosis of revascularized ischemic stroke patients, but *the results should be replicated* on higher number of patients.

However, another limitation of dosing copeptin in ischemic stroke patients is that this biomarker can be significantly higher in many fluid disorders and stress-associated disorders (66).

Copeptin is secreted in equimolecular amounts with AVP and is considered a seric surrogate of AVP. Copeptin has the advantage of being easier to measure, stable *ex-vivo* and the result may be ready in approximately 1 h. Blek et al. (5) stated that measurement of copeptin is still not routinely performed in stroke patients despite many years of studies elucidating its association with stroke prognosis. This is due to the limitations given by previous studies: being carried out on small target groups and mostly in the eastern population.

The limitations of this review are primarily determined by the limitations of the included studies. The main limitations encountered in the literature were:

- Only few studies that took into account revascularization therapy (thrombolysis, endovascular treatment) and its relation to copeptin levels
- Only few studies took into account the time of onset of symptoms, the time until the patient presented to the emergency department, door-to-needle time, door-to neurologist time and their relation to copeptin levels
- Most studies were performed in rural areas and Eastern population

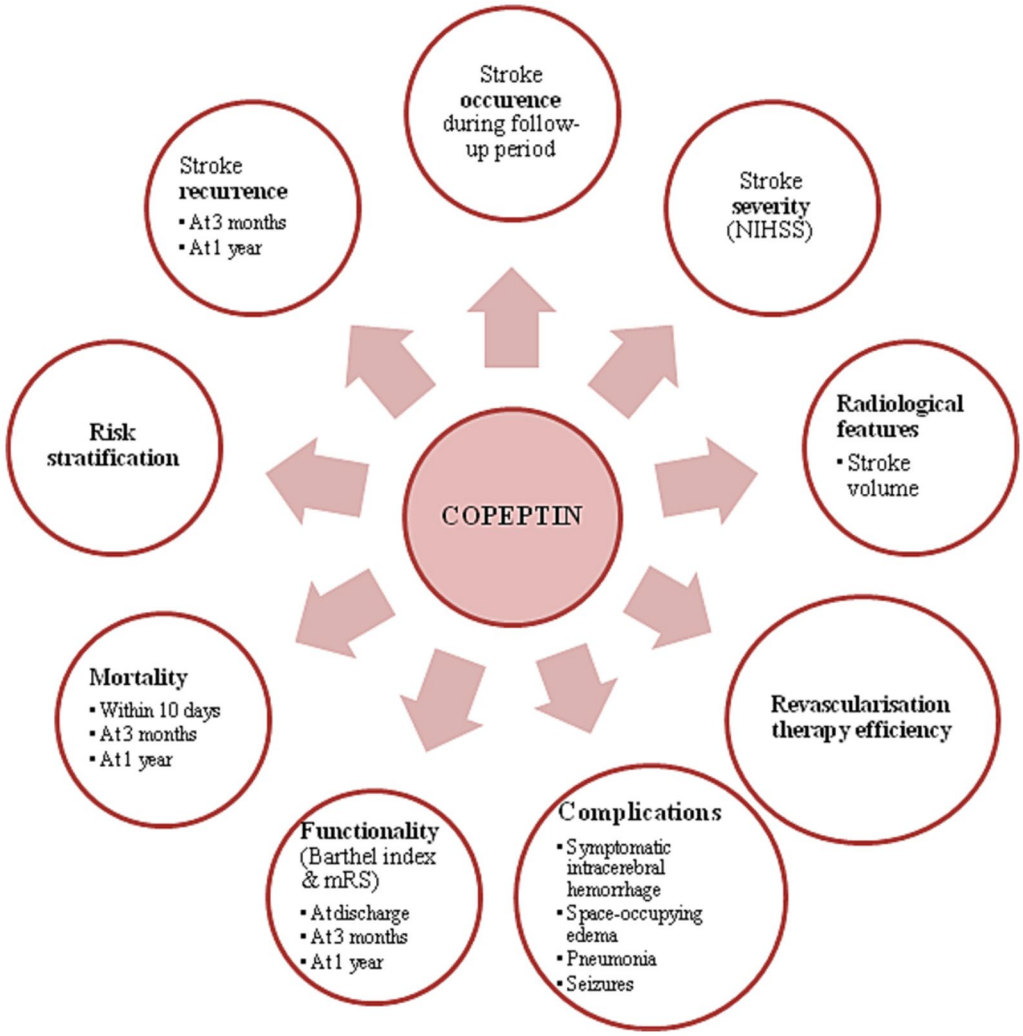

FIGURE 2
Benefits of copeptin use in ischemic stroke patients.

- Most studies were performed on stroke patients and further subgrouped depending on their imaging diagnosis (intracerebral hemorrhage, cerebral infarction, subarachnoid hemorrhage, others): further studies should focus on more targeted patients, with more strict inclusion criteria (such as: only ischemic stroke patients with cardioembolic mechanism)
- Only one study assessed stroke related complications such as brain edema and hemorrhagic transformation and their relation to copeptin levels: further studies should focus on finding "a troponin for brain" that could explain to physicians that a certain copeptin level at the moment of presentation may put the patient at risk for stroke-related complications, so they should target a more extensive therapy.
- There are only few studies that mention the use of vasopressin receptor blocker drugs that may reduce cerebral edema (41)

5 Conclusion

Prediction of long-term outcome at stroke onset only based by clinical deficits is difficult. This explains the need for useful blood biomarkers. Copeptin is a novel and promising biomarker for evaluating cerebrovascular diseases, even though multiple pathologies can lead to increased copeptin and it can be considered non-specific. However, if it is combined with other clinical or paraclinical parameters, the diagnostic accuracy may increase (67).

An early risk evaluation with an early estimation of the severity of stroke and prognostic of stroke is pivotal for individualized management, triage decisions, acute therapeutic management, optimized care and allocation of healthcare resources (27).

The above review explains that monitoring copeptin levels may help in several key moments for an ischemic stroke patient: prevention (copeptin predicting stroke occurrence among healthy individuals based on their comorbidities), moment of presentation (copeptin correlating with stroke severity and infarcted stroke volume), treatment efficacy, post-stroke complications, prognosis (short-term and long-term), mortality, risk stratification and stroke recurrence. A summary of the benefits of dosing copeptin in neurological practice are presented in Figure 2. In conclusion, copeptin may be a useful marker in ischemic stroke from a continuous point of view: *primary prevention* and *secondary prevention* (focusing on the moment of presentation in the *present*, but also focusing on the *future* of the patients).

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

AV: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. CT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. CB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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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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EDITED BY
Timo Uphaus,
Johannes Gutenberg University Mainz, Germany

REVIEWED BY
Gerrit M. Grosse,
University Hospital of Basel, Switzerland

*CORRESPONDENCE
Franziska Lieschke
✉ Lieschke@med.uni-frankfurt.de

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Perspective: Use of protein S100B as a quality assurance marker for endovascular therapy in acute ischemic stroke

Franziska Lieschke[1]* and Christian Foerch[1,2]

[1]Department of Neurology, University Hospital, Goethe University Frankfurt, Frankfurt/Main, Germany, [2]Department of Neurology, RKH klinikum Ludwigsburg, Ludwigsburg, Germany

Mechanical thrombectomy (MT) is a highly effective treatment for ischemic stroke associated with large vessel occlusion. Given its complexity, this procedure is widely used throughout the world in hospitals with different levels of experience. Therefore, practical quality assurance is advised to ensure a high standard of care across the board. In this perspective article, we propose the implementation of measuring serum S100B after MT as a surrogate outcome parameter for the extent of tissue damage as an additional quality indicator for internal and external benchmarking in endovascular therapy. We focus on the analysis of patients, in whom there is a discrepancy between the expected (e.g., based on favorable preconditions) and the actual biomarker outcome. We aim to illustrate the advantages and drawbacks of measuring S100B after MT, reliably depicting the procedure's quality and its use for comparison and identification of "outlier" patients in MT patient cohorts for further process and single-case analysis.

KEYWORDS

quality assurance, biomarker, S100B, acute ischemic stroke, mechanical thrombectomy

Introduction

Mechanical thrombectomy (MT) is a highly effective treatment for ischemic stroke associated with large vessel occlusions (LVOs) (1–4). However, it is a resource-intensive procedure from a logistical and technical point of view, as it frequently requires careful patient selection with advanced imaging techniques, inter-hospital transfers, and post-procedural care in the neurological intensive care unit. Furthermore, trained interventionalists within a multidisciplinary team and appropriate devices are needed. Simple, practicable quality assurance is important to maintain high standards within and across different centers. In particular, differentiation between directly associated complications (i.e., unsuccessful MT or reperfusion injury) and indirectly associated complications (i.e., pneumonia after endotracheal intubation, status epilepticus, and cardiac complications) is warranted. At present, the "modified thrombolysis in cerebral infarction" (mTICI) score as a measure for recanalization success during angiography and the short-term case fatality rate are often used as benchmarks (5, 6), but these have their limitations, particularly with regard to differentiating the above-mentioned causes of poor outcomes.

Serum protein S100B levels in acute ischemic stroke

We recently demonstrated that serum levels of the astroglial protein S100B reliably indicate the extent of ischemic tissue damage after MT and suggested its use as a surrogate outcome parameter, providing added value in clinical routine and interventional trials (7). S100B concentrations can be rapidly assessed using electrochemiluminescence immunoassay techniques (8). Elevated S100B serum concentrations measured 48–72 h after symptom onset are highly correlated with final infarct volume (the infarct core) and functional outcome (9–11). In contrast, large perfusion deficits that do not transition into a demarcated infarction (e.g., due to successful MT) do not cause the release of S100B into the serum (12). Any complication resulting in additional brain tissue damage likely causes S100B release (e.g., intracerebral or subarachnoid hemorrhage, arterial re-occlusion, and hypoxia due to extracerebral complications) (10, 13–16).

Proposed use of serum S100B as a quality assurance indicator

We previously observed that especially "successful" recanalization (mTICI >2) resulted in both low and high S100B levels. There are various possible explanations for this, which is why the implementation of S100B in a quality monitoring system is of particular interest.

The determination of S100B after MT can be used for performance feedback to healthcare professionals, in addition to data obtained from regular quality registries. As such, it can be easily integrated as an outcome indicator into dashboards [as currently being investigated in the PERFEQTOS performance feedback trial (17)]. This approach can be incorporated into existing treatment concepts without requiring additional resources (Figure 1).

To assess the utility of S100B as a quality indicator for endovascular therapy, we measured S100B levels in patients' serum on the second day after MT for over a year and analyzed the values together with our existing data (7). Detailed information on the methods can be found in the Supplementary material.

We examined 183 patients with acute middle cerebral artery infarction associated with LVO, of whom 44% were female. The mean age of the patients was 69.2 ± 13.6 years. The median Alberta Stroke Program Early CT Score (ASPECTS) on the first CT scan was 8 (IQR 7–10). The median S100B level in the entire cohort was 0.15 µg/L (IQR 0.09–0.32 µg/L), the S100 B values in the first tertile were below 0.103 µg/L, the values in the second tertile ranged between 0.103 and 0.229 µg/L, and the values in the third tertile were above 0.229 µg/L. S100B levels showed a high correlation with infarct size (Spearman $r = 0.74$, $p < 0.001$). Successful recanalization (mTICI ≥2) was achieved in 95% of cases in the first S100B tertile, 92% in the second S100B tertile, and 73% in the third S100B tertile. A good functional outcome at discharge (mRS ≤ 2) was achieved in 72% of patients in the first tertile, 49% in the second tertile, and 18% in the third tertile. Patients with successful recanalization (mTICI ≥2b, $n = 159$) showed on average significantly smaller infarcts (37.9 mL ± 65.4 mL vs. 137.2 mL ±132.4 mL $p < 0.001$) and lower S100B concentrations (median 0.13 µg/L vs. 0.44 µg/L $p < 0.001$) than patients in whom recanalization was unsuccessful (mTICI<2b, $n = 19$).

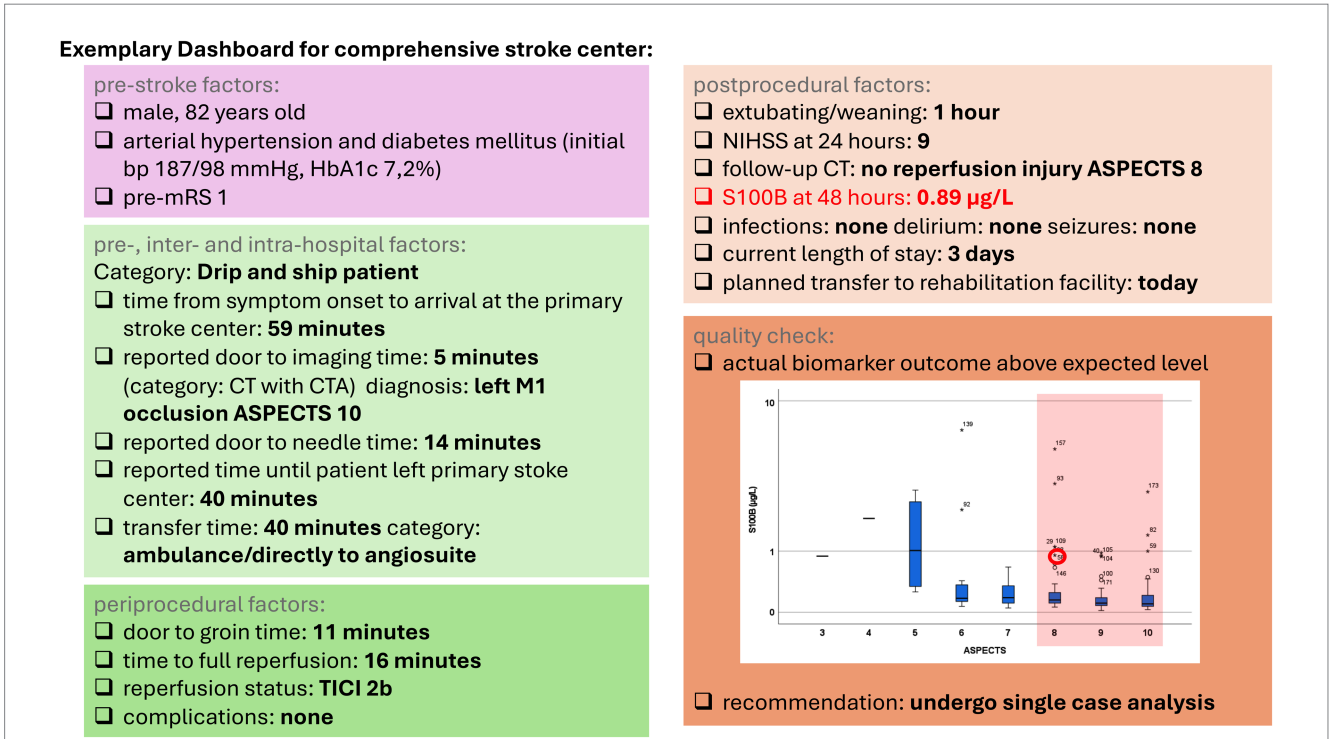

FIGURE 1
Exemplary dashboard for a comprehensive stroke center. Boxplots depict the distribution of the S100B values stratified according to the Alberta Stroke Program Early CT Score (ASPECTS). Highlighted S100B outliers and extreme values were used for the following analysis. mRS, modified Rankin Scale; CT, computed tomography; CTA, computed tomography angiography; ASPECTS, Alberta Stroke Program Early CT Score; TICI, thrombolysis in cerebral infarction; NIHSS, National Institutes of Health Stroke Scale.

Example 1: high S100B levels after MT despite high ASPECTS

We further analyzed patients who showed a discrepancy between the expected (based on given conditions) and the actual biomarker outcome. All patients with S100B upper outliers (1.5–3x the IQR) and upper extreme values (>3x the IQR) indicating significant brain tissue destruction despite an ASPECTS of 8–10 at admission were analyzed for underlying factors (e.g., futile recanalization and complicative intracranial hemorrhage). We identified 129 patients with this favorable precondition, and the median S100B value in this subgroup was 0.12 µg/L (0.08–0.23 µg/L). For this group, an acceptable ('expected') biomarker outcome would range from the lower level of detection up to a maximum of 0.345 µg/L. We further selected 16 patients with S100B values above this limit to undergo single-case analysis. Futile recanalization was found in three patients with mTICI 0 and two patients with mTICI 2a (due to pre-existing stenosis of the middle cerebral artery (MCA) and calcified emboli, respectively). One patient hereof developed a malignant infarction. Eight patients had partially successful recanalization (mTICI 2b) with peripheral artery branch occlusions described in three patients, hemorrhagic transformation in four patients (including three patients with parenchymal hemorrhage), and development of a malignant infarction in one patient. Two patients were treated beyond a 6-h time window. Three patients had complete recanalization (mTICI 3) but developed significant infarcts and one with HT. When comparing the 16 "outlier" patients to all other patients with an initial ASPECTS of 8–10, recanalization rates differed significantly toward a higher percentage of futile MT (mTICI 0 in 19% vs. 4%) and lower rates of successful recanalization (mTICI 3 or 2b in 19% vs. 38, 50% vs. 56% $p = 0.007$). Accordingly, "outlier" patients yielded significantly increased National Institutes of Health Stroke Scale (NIHSS) scores at 24 h (median of 24 vs. 6 points, $p < 0.001$) and infarct volumes (mean 146.2 ± 105.2 mL vs. 14.5 ± 22.6 mL, $p < 0.001$). The hemorrhagic transformation occurred in 38% of the outliers, whereas only 24% of all other patients with an initial ASPECTS of 8–10 developed HT.

Example 2: unfavorable outcomes despite a low S100B

In the second example, we looked at patients having an unfavorable clinical outcome (modified Rankin Scale "mRS" 4–6) at discharge despite very low S100B levels (i.e., little tissue damage). We identified 60 patients with serum S100B values below the upper normal range of 0.105 µg/L, of whom 11 still had an unfavorable clinical outcome. In addition, we aimed to characterize the responsible factors (e.g., extracerebral complications after successful MT). In all of these patients, recanalization was successful (mTICI 2b, 2c, 3), and only small infarct volumes were measured (mean 5.7 ± 7.8 mL vs. 100.3 ± 111.1 mL in all other patients with mRS 4–6). In three patients, CT hyperdensities were described; however, differentiation of iodine contrast staining vs. hemorrhagic transformation was not possible. Ten of eleven patients developed pneumonia, five of whom were refractory to treatment after failing multiple antibiotic therapy regimes. The infection resulted in death in three of these patients. Furthermore, we found frequent constellations of high age and (pre-) existing comorbidities, which were acutely exacerbated. As such, four patients developed acute cardiac complications based on previous chronic heart disease. Altogether, we identified respiratory and weaning failures as one of the main reasons for unfavorable outcomes despite successful MT ($n = 6$). Two patients suffered early recurrent strokes, which occurred after day 2 (and therewith after the first follow-up imaging used for evaluation of the infarct volume and the S100B collection on day 2), of whom one patient suffered additional epileptic seizures. Three patients failed to achieve a symptomatic recovery in the absence of relevant tissue damage.

Discussion

To identify emerging shortcomings in the quality of care early ahead, the practice of quality assurance, especially in hospitals with limited experience, is of utmost relevance. In our opinion, protein S100B as a measure of ischemic brain tissue damage (i.e., infarct volume) is a feasible quality assurance marker. By introducing S100B measurements into our quality monitoring system, we identified futile recanalization and hemorrhagic complications as the main reasons for noticeably increased S100B levels despite successful recanalization in our MT cohort. A frequent reason for poor functional outcome at discharge despite low S100B values was post-stroke complications such as infections, especially aspiration pneumonia after endotracheal intubation for MT, or exacerbation of pre-existing disorders.

Determination of S100B is objective and readily available, thereby reliably indicating brain tissue outcomes after MT. In contrast to the mTICI score measuring the recanalization success during angiography, no further special expertise is necessary for its application. Moreover, mTICI is determined during angiography, and re-occlusions that occur after the completion of the intervention—contributing to infarct development despite initially restored flow—cannot be captured by this measure. Using the short-term case fatality rate for monitoring and benchmarking the quality of MT can be misleading, as this metric does not account for lethal complications that may occur despite a successful MT procedure. In this study, the distinction of whether these complications are related to the intervention can be easily made by determining S100B, which highlights its particular importance for quality assessment. Establishing the monitoring of MT by S100B at a center does not require any special resources other than those available at an average hospital of standard care. The determination of S100B can be easily integrated into the routine care of a stroke unit, with a single blood draw 48–72 h after MT (10) in all MT patients. By looking at the individual S100B value distribution during 1 year and single-case analysis of outliers as well as comparison of the distribution by benchmark with other hospitals and previous years, changes in complication rates, unusually high mortality rates (not directly associated with the stroke or MT), and trends toward improvement and deterioration of procedure quality can be detected early. We confirmed that S100B levels in our cohort correlated highly significantly with infarct demarcation on follow-up imaging. The non-invasive determination by blood test, due to the lack of radiation exposure, lower costs, and its point of care (POC) character, may represent a decisive advantage over follow-up imaging, especially in situations of limited resources.

Particularly after general anesthesia is required for MT, the clinical assessment of the stroke severity may be clouded by concomitant extracerebral diseases and complications. Thus, S100B may offer additional diagnostic value, especially in cases where neuroimaging is not immediately conclusive or in the hyperacute stage before significant radiographic changes are visible. Across multiple studies, adding S100B

to clinical and imaging assessments results in improved AUROCs, indicating a moderate but real enhancement in predictive accuracy (14, 18–20). In this regard, the study of Honegger et al. is of particular interest, where using serum S100B added to the pre-defined prediction models led to an increase in the AUC and reclassification indices, showing its value beyond the established risk factors (20).

Other brain-specific biomarkers used following stroke include neuron-specific enolase (NSE) (21), tau (22, 23), neurofilament light chain (NfL) (24, 25), glial fibrillary acidic protein (GFAP) (26–28), and ubiquitin C-terminal hydrolase L1 (UCH-L1) (27, 28). Despite showing great potential in stroke diagnostics and prognosis, they are not yet widely used in routine clinical practice (29). Their benefits are obvious. As such, they can help differentiate between ischemic and hemorrhagic strokes (especially GFAP), provide early detection of neuronal or glial damage, and offer insights into patient prognosis (7, 14, 30, 31). Second, they can be used to select and adapt therapies in terms of personalized medicine, reducing unnecessary, expensive tests and procedures (12). In addition, they can be used to assess the risk of developing certain complications, which can influence the decision to take preventive measures (13, 20). However, challenges remain. A lack of standardized protocols and threshold values limits their broader use. Some lack specificity and can be elevated in other neurological or systemic conditions, leading to potential misinterpretation (32). Alarmins and cell-free DNA (cfDNA) have also emerged as promising candidates. Frequent reviews here raise the question of whether cfDNA is merely a marker of severe brain injury released from damaged cells or an active contributor to brain injury or stroke-associated infection. In this study, the particular source of cfDNA may be important to consider as they may have different functional properties and whether it is released from necrotic lysed cells or actively expelled from cells (33–35).

There are several limitations to our study. First, this study does not capture patients who have experienced time delays when being transferred from primary stroke centers, received repeated imaging on arrival to our center (usually MR-guided, bearing the risk for further time delays), and were excluded from MT based on an extending ischemic demarcation evident on the very same imaging. Second, to ensure the proper identification of explanatory factors and to determine the most likely applicable causalities, prior experience in the evaluation of medical findings must be available. This requires either a certain level of training for the evaluating person from quality assurance or the use of, e.g., computer-assisted analysis, which in turn requires advanced and precise data collection from the clinics. However, this should not add to the already existing, in part extremely extensive, documentation obligations of medical and nursing staff.

We demonstrate the feasibility and limitations of using S100B in the quality analysis after MT. Measuring S100B after MT can reliably depict the procedure's quality, allowing comparison and identification of "outlier" patients in MT patient cohorts for further process evaluation and single-case analysis.

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 humans were approved by the Ethics Committee of the University Hospital of the Goethe-University Frankfurt (No. 242-17). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

FL: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. CF: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Supervision, Writing – review & editing.

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Conflict of interest

CF holds the patent for using GFAP for identification of intracerebral hemorrhage (US20150247867) and further reports speaker honoraria and honoraria for participating in advisory boards from Alexion, Bristol Myers Squibb, Novartis, Teva, Merck, Sanofi Genzyme, and Roche.

The remaining author declares 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.2025.1488018/full#supplementary-material

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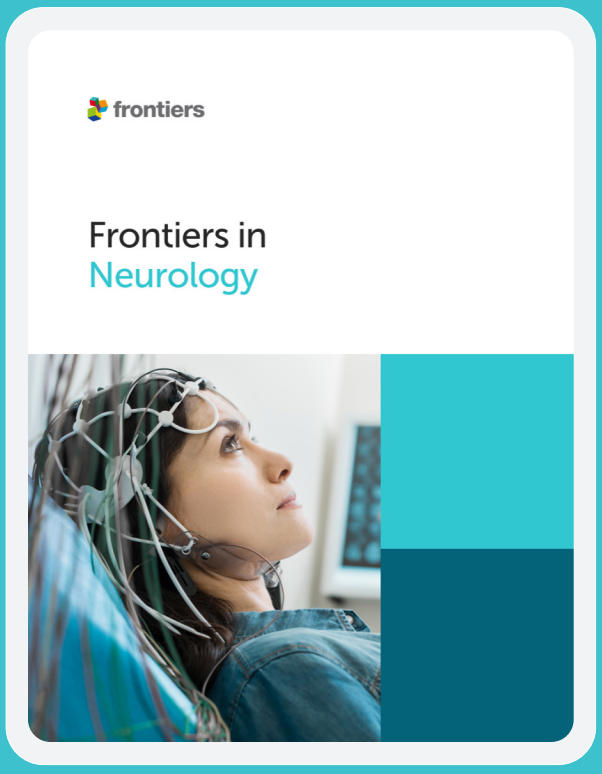

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