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Mechanisms, imaging techniques, and therapies for acute ischemic stroke and related neuroprotective strategies

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Mechanisms, imaging techniques, and therapies for acute ischemic stroke and related neuroprotective strategies

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Ischemic stroke: From pathological mechanisms to neuroprotective strategies

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Ischemic stroke (IS) has complex pathological mechanisms, and is extremely difficult to treat. At present, the treatment of IS is mainly based on intravenous thrombolysis and mechanical thrombectomy, but they are limited by a strict time window. In addition, after intravenous thrombolysis or mechanical thrombectomy, damaged neurons often fail to make ideal improvements due to microcirculation disorders. Therefore, finding suitable pathways and targets from the pathological mechanism is crucial for the development of neuroprotective agents against IS. With the hope of making contributions to the development of IS treatments, this review will introduce (1) how related targets are found in pathological mechanisms such as inflammation, excitotoxicity, oxidative stress, and complement system activation; and (2) the current status and challenges in drug development.

KEYWORDS

ischemic stroke, inflammation, excitatory toxicity, oxidative stress, complement system, thrombus

Introduction

Ischemic stroke is one of the most common cerebrovascular diseases. With the aging of society, personal underlying diseases (such as hypertension, diabetes, heart disease, and hyperhomocysteinemia), smoking, alcohol consumption, and other factors, the incidence of IS has been continuously rising. According to the World Health Organization (WHO), more than 1.1 million people die each year from IS (1), showing that IS is seriously endangering people's health. The pathological mechanism of IS is very complex, including inflammation, excitotoxicity, oxidative stress, and the complement system, which eventually cause apoptosis and necrosis of neurons in the ischemic area. In the complex pathological mechanism of IS, inflammation was undervalued in the past because the brain was for a long time considered an immune-privileged organ, but its role is now more and more appreciated. Excitotoxicity is mainly caused by the increased glutamate and the subsequent calcium overload, which transformed the field of stroke research in the 1980s (2). Oxidative stress presents quite a challenge to ischemic tissue, particularly after reperfusion. Moreover, the activation of the complement system, thrombus formation, and pericyte death are important factors in triggering IS and subsequent neuronal death.

At present, intravenous thrombolysis and mechanical thrombectomy are the main treatment methods for IS, but they all have different restrictions. Intravenous thrombolytic therapy is usually represented by alteplase. Intravenous thrombolysis can be performed with alteplase within 4.5 h after an acute stroke, with the condition of excluding coagulation disorders and controlling blood pressure below 180/105 mmHg (3). The conventional regimen of clinical antiplatelet therapy is a combination of clopidogrel and aspirin. This dual antiplatelet treatment (a loading dose of 300 mg of clopidogrel plus 300 mg of aspirin, followed by a maintenance dose of 75 mg of clopidogrel and 75 mg of aspirin during the first 21 or 90 days), is effective in preventing IS after the onset of a transient ischemic attack (TIA) (4). Tenecteplase, a genetically modified variant of alteplase with increased fibrin specificity, shows similar safety and efficacy compared with alteplase in some clinical trials (5, 6). Compared to alteplase, tenecteplase has more advantages, such as a longer half-life, greater ease of use administered as a bolus medication, lower cost in some settings, and a higher incidence of reperfusion when combined with thrombectomy (6–8). These strengths may make it more promising in the treatment of IS. Mechanical thrombectomy, performed within 6 h after the onset of stroke, is another first-line treatment strategy for patients with ischemic stroke (3). However, mechanical thrombectomy is limited to the treatment of basilar artery occlusion in hospitals with related equipment and conditions (9).

In general, there are certain deficiencies in conventional treatment regimens, because of the strict time window, difficulty in delivering drugs to the central nervous system (CNS), and the inability to reverse the neuronal death that has already occurred. Even after the thrombus is removed, there is still a blockage of capillaries attributed to the dead pericytes losing their ability to regulate blood vessels in the brain (10). Furthermore, researchers need to pay more attention to ischemia-reperfusion injury in the brain after revascularization. Therefore, to overcome these difficulties and break these traditional constraints, researchers need to find potential targets and develop new neuroprotective agents for treatment strategies based on the pathological mechanism of IS. In this review, we mainly introduce the pathological mechanisms after IS such as inflammation, excitotoxicity, oxidative stress, complement system, and microcirculation.

Inflammation

The extremely complex inflammatory response in the CNS consists of immune cells derived from the lymphatic circulation, resident microglia, monocytes, neutrophils that originate from the peripheral circulation, and cytokines secreted by various inflammatory cells after stroke. Innate immunity is rapidly involved in post-IS inflammation. Damage-associated molecular patterns (DAMPs) such as heat shock protein (HSP), high mobility group protein B1 (HMGB1), and hepatoma-derived growth factor (HDGF) can be recognized by pattern recognition receptors (PRRs) on some effector cells, thereby activating associated transcription factors and stimulating effector cells to secrete inflammatory factors. As part of the innate immune system, antigen-presenting cells (APCs) play a key role in the initiation of adaptive immunity. Dendritic cells (DCs) are one type of APC. Langerhans cells (a type of immature DCs) mature in lymphoid tissue after ingesting and processing autoantigen released by damaged tissue and necrotic neurons. Then DCs express peptide–MHC complexes along with highly expressed B7 (CD80/CD86), which generates a double stimulus for T cells, thereby mediating adaptive immunity. Inflammatory factors and chemokines are mainly produced by activated immune cells, inducing peripheral monocytes and neutrophils to migrate and infiltrate into the ischemic penumbra. In humoral immunity, B lymphocytes can differentiate into memory B cells and plasma cells that can produce antibodies. Although B cells may be involved in post-stroke pathologies, such as their ability to mediate delayed cognitive impairment following stroke (11), their effect on neuroinflammation is significantly weaker than that of T cells after acute ischemic stroke (AIS) (12). Furthermore, some experiments have shown that in the middle cerebral artery occlusion (MCAO) model, mice deficient in B lymphocytes exhibit no significant changes in cerebral infarct size and neural function compared with the wild-type (12), suggesting that B lymphocytes may not be the key to affecting neuroinflammation after acute stroke. Here, we mainly describe several inflammatory cells (Figure 1) that play important roles in the acute inflammatory response after stroke.

Microglia

As a branch of the monocyte-phagocytic system, microglia are residents in the CNS, primarily responsible for immune surveillance and scavenging of pathogens and dying neurons (13). Microglia are the largest number of immune cells in the CNS, accounting for 5–10% of total brain cells (14). Microglia are involved in a variety of CNS diseases, including amyotrophic lateral sclerosis (ALS), IS, Alzheimer's disease (AD), meningeal inflammation, and schizophrenia (15–18). Microglia show contradictory functions in post-stroke inflammation (19). This may be due to their different phenotypes. Usually, microglia can be divided into three types: M0 (surveillance), M1 (pro-inflammatory), and M2 (anti-inflammatory) (19). M0 is primarily responsible for surveillance, with characteristics of low phagocytosis and inactivity (20, 21). In acute inflammation after stroke, M1 is generally considered to be activated earlier than M2. In fact, in the early stages of IS, the M2 type is the first to be activated, and its main function is to remove necrotic debris and protect brain tissue. Then M1 type mainly involved in brain tissue damage is activated (22). M1 type plays

an important role in neuroinflammation after stroke, and the polarization of microglia to M1 has attracted a lot of attention. Many stimulatory factors cause the polarization of microglia toward M1, such as INF-γ secreted by Th1 cells activating the JAK/STAT pathway (22) or lipopolysaccharide stimulating Toll-Like receptor 4 (TLR4) on microglia (23). Activated M1 type can produce a variety of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-23, IL-18, IL-12, CCL2, and CXCL10), reactive oxygen species (ROS), matrix metalloproteinase 9 (MMP9), and matrix metalloproteinase 3 (MMP3) leading to the apoptosis of neurons, the migrations of peripheral cells, the activation of immune cells, and the destruction of blood-brain barrier (BBB) (24–28). In contrast, M2 microglia mainly play an anti-inflammatory role and initiate neurogenesis, synaptogenesis, and neurovascular unit remodeling in the late stage of IS (29). In addition, the different polarization patterns of microglia may be related to the microenvironment, age, gender, temperature, and diabetes (30–34).

Promoting microglia polarization toward M2 while inhibiting M1 has emerged as a therapeutic approach for AIS. Minocycline (Table 1), as a selective inhibitor of M1 microglia, can be capable of reducing inflammation and promoting neurogenesis (35). In an open-label and evaluator-blinded study, patients with acute stroke had a significantly better outcome with minocycline treatment compared with placebo. This finding suggested a potential benefit of minocycline in AIS (36). Exendin-4 (Table 1), as a glucagon-like peptide receptor 1 agonist, in addition to its usage in blood glucose control, can promote the polarization of M2 microglia thereby providing neuroprotection and improving the prognosis of MCAO mice (37). In addition, two clinical trials on Exendin-4 treating IS are under recruitment. Hyperglycemia has been shown to activate M1 microglia (38), and the neuroprotective effects of Exendin-4 may be based on an indirect inhibition. Furthermore, the microenvironment exerting its influence on microglia is important for post-IS inflammation. For example, lacking folic acid (Table 1) will activate microglia *via* Notch/NF-kβ signaling in MCAO rats and BV-2 microglia after undergoing oxygen and glucose deprivation (OGD) (39). However, in two clinical trials of stroke, daily administration of folic acid, vitamin B6, and vitamin B12 did not seem to be more effective than a placebo in reducing the incidence of major vascular events, cognitive impairment, or cognitive decline (40, 41). In general, despite the difficulties in the transition to clinical, targeting microglia seems to be important in the treatment strategy of IS.

Macrophages

In rodents, according to the expression level of lymphocyte antigen 6 complex C1 (Ly6C) and chemokine receptors, monocytes are mainly divided into two subpopulations, namely pro-inflammatory subpopulations ($Ly6C^{high}CCR2^{+}CX3CR1^{low}$) and anti-inflammatory subpopulations ($Ly6C^{low}CCR2^{-}CX3CR1^{high}$) (42–44). The CCL2-CCR2 axis is key to driving peripheral monocytes to the infarct area (45). Targeting the CCL2-CCR2 axis appears to be an ideal anti-inflammatory regimen due to the high expression of CCR2 in pro-inflammatory subpopulations. However, anti-inflammatory subsets in CNS are mainly transformed by infiltrating pro-inflammatory subsets rather than derived from peripheral monocytes (42), which makes targeting CCL2-CCR2 unfavorable to the prognosis of patients with IS from this perspective. Microglia were previously thought to be originated from peripheral macrophages because they have the same surface markers: CD11b, F4/80, and Iba-1 (46). In addition, microglia and macrophages have similar phenotypes, resulting in difficulty to distinguish them for researchers (47). Monocytes are now thought to be derived from hematopoietic stem cells (HSCs), whereas microglia are the descendants of yolk sac erythromyeloid progenitors (EMPs) (48). Researchers have found that, after IS, CXCR4 promotes monocyte infiltration and regional restriction of infarct tissue by macrophages derived from peripheral monocytes. Conversely, CXCR4 deficiency reduces the ability of monocytes to infiltrate the ischemic brain (49).

Macrophages are mainly derived from circulation, intestine, spleen, etc. In the early stage of IS, macrophages are induced to express triggering receptors expressed in myeloid cells 1 (TREM1), amplifying the inflammatory effects along with PRR (50). Interestingly, macrophages also contribute to neurogenesis. Mohle et al. believe that the reduction of neurons in the hippocampus is strongly correlated with the reduction of peripheral monocytes after oral antibiotics (51, 52). This suggests that macrophages may play a different role after a stroke. Macrophages derived from the spleen also get into CNS after IS. On the first day after cerebral infarction, the number of macrophages infiltrating CNS was significantly reduced in MCAO mice without spleen compared with the model group (53). Therefore, blocking the source of macrophages and preventing the differentiation of pro-inflammatory phenotype may be a strategy for the treatment of IS.

Neutrophils

Neutrophils are the first leukocytes to infiltrate the ischemic brain after stroke, peaking at 48–72 h in CNS (54). The role of neutrophils in IS mainly includes the following aspects. The first role is secretory effect. There are many active substances such as MMP9, ROS, RNS, chemokines, and pro-inflammatory factors secreted by neutrophils, which mediate inflammation and the disruption of the BBB (55). Second, promoting thrombosis and blocking cerebrovascular, then leading to a no-reflow phenomenon after stroke (56). The third aspect is the neutrophil extracellular traps (NETs). The primary role of NETs is to

TABLE 1 Clinical trials and pre-clinical studies of drugs.

Generic name	Target or signaling pathways	Pre-clinical references	Applications	Clinical trial	Phase	Results/status of the clinical trial
Anti-inflammatory						
Natalizumab	Integrin α4β1	(158)	Mice	NCT02730455	2	Completed
Minocycline	microglia	(159)	Mice	NCT00930020	4	Terminated
Exendin-4	Microglia	(37)	Mice	NCT03287076	2	Active not recruiting
Folic acid/B12/B6	Microglia	(39)	Rat	NCT00354081	3	Completed
i6-FP	MAIT	(89)	Mice	—	—	—
Tak242	TLR4	(160)	Rat/*in vitro*	—	—	—
Isoquercetin	TLR4	(161)	Animals/*in vitro*	—	—	—
Dexmedetomidine	HMGB1/TLR4/NF-kB	(162)	Rat	NCT04916197	4	Recruiting
Anti-excitotoxicity						
NA-1	GluN2B-PSD95-NOS	(163)	Animals/neuronal Cultures	—	—	—
ZL006	GluN2B-PSD95-NOS	(164)	Mice/rat	—	—	—
IC87201	GluN2B-PSD95-NOS	(165)	*In vitro*	—	—	—
β-lactam antibiotics	GLT-1	(103)	Mice/glial cell (gestation day 14–16 CD1 mice)	NCT05375240	2	Not yet recruiting
Memantine	Extrasynaptic NMDARs	(125, 126)	Rat/mice	NCT02535611/ NCT02144584	3/0	Completed/active not recruiting
Ketamine	NMDARs	(166)	Mice	NCT03223220	2, 3	Unknown
Geniposide	GluN2A/AKT/ERK	(167)	Rat	—	—	—
Pseudoginsenoside-F11	Akt-Creb	(168)	Rat		—	—
Anti-oxidative stress						
Acetylcysteine	TNF-α, iNOS, GSH, System x(c)-	(112, 113)	Rat	NCT04918719/ NCT04920448	2/2	Not yet recruiting/ Not yet recruiting
Edaravone	ROS, RNS	(169)	Mice	NCT02430350	3	Completed
Uric acid	ROS	(170)	Mice	NCT00860366	2, 3	Completed
Melatonin	ROS, MDA, TNF-α	(171)	OGD/R-induced neuron	NCT05247125	4	Recruiting
tBHQ	Nrf2/ARE	(172)	Rat	—	—	—
Trans sodium crocetinate (TSC)	SIRT3/FOXO3a/SOD2	(173)	Rat	NCT03763929	2	Terminated
Genipin	UCP2-SIRT3	(174)	Mice	—	—	—
Complement system						
Human C1-esterase inhibitor	C1s, MASP-2	(157)	Mice	NCT01694381	0	Terminated
B4cry	IgM	(149, 150)	Mice	—	—	—
polyman2	MBL, C1s	(156)	Rats	—	—	—

capture and neutralize invading microorganisms (57), yet it is involved in the formation and stabilization of thrombus after stroke, which can lead to persistent ischemia in the brain (58). The fourth aspect is the phagocytosis of neutrophils. Neutrophils contribute to the clearance of necrotic tissue (59). The fifth aspect is the neurorestorative function. There is growing evidence that reparative neutrophil subsets and their products can be deployed to improve neurological outcomes (60, 61).

Similar to microglia and macrophages, conflicting phenotypes are also present in neutrophils. Neutrophils affected by tumor microenvironments can differentiate into two

subtypes: N1 (anti-tumor) and N2 (pro-tumor) (62, 63). This contradictory phenotype also occurs in patients with stroke (55, 64). Targeting the neutrophil phenotype may also serve as an alternative to anti-neuroinflammation, and the ability of neutrophils to infiltrate the CNS also makes it a potential target for assisting drugs in getting into brain tissue (65).

T cells

T cells develop, differentiate, and mature in the thymus, undergoing processes such as TCR development, positive selection, and negative selection. Then the vast majority of T cells transform into single-positive T cells ($TCR\alpha\beta^+CD4^+CD8^-$ or $TCR\alpha\beta^+CD4^-CD8^+$) mainly involved in adaptive immune, others mainly differentiate into $TCR\gamma\delta^+CD4^-CD8^-$ T cells involved in innate immune. T cells in the spleen are involved in the pathological process of IS, resulting in reduced spleen volume and histomorphological changes (66). The involvement of T cells is often thought to aggravate brain damage but in some experiments the performance of T cells is contradictory. In mice with splenectomy, the neurological function is improved at the early stages of IS, but long-term neurological recovery is detrimental (67). In addition, studies have found that neurogenesis in the hippocampus is significantly reduced in mice lacking a complete immune system, especially those lacking $CD4^+$T cells (68).

According to the leukocyte differentiation antigens, T cells are roughly divided into $CD4^+$ T cells and $CD8^+$ T cells, as well as mostly double-negative γδT ($CD4^-$ $CD8^-$) cells.

$CD4^+$ T cells

$CD4^+$T cells mainly include Th1, Th2, Th17, Th9, Th22, TFH, Treg, and other subpopulations. Th1 cells release the pro-inflammatory cytokines IFN-γ, IL-2, and TNF-α/β, and induce microglia and macrophage polarization toward M1 (69), which aggravates neuroinflammation after stroke. Contrary to Th1, Th2 promotes the polarization of microglia and macrophages toward the M2 type (70). Th1 and Th2 differ significantly in downstream cytokine lineages, and the Th1/Th2 mold can affect the outcomes of stroke (71). IL-33, as a member of the IL-1 family, improves MCAO mice's neurological deficit scores and reduces infarction volume by reducing IFN-γ^+T cells and increasing $Foxp3^+$ T cells in the spleen, thereby shifting Th1/Th2 mode to Th2 immune deviation and exerting a neuroprotective effect (70, 72). Similar to Th1, Th17 aggravates brain injury after a stroke. Intestinal Th17 are activated and then migrate into the meninges attributing to the CCL20-CCR6 axis after stroke (73). Furthermore, the CCR6-CCL20 axis can inhibit Treg differentiation and direct Tregs toward the pathogenic Th17-lineage (74). Targeting CCL20-CCR6 may be an ideal strategy for treating IS. Pioglitazone as a drug for the treatment of diabetes can reduce peripheral CCL20. Some animal experiments have shown that PG can reduce the inflammatory response after traumatic brain injury (TBI) (75), but whether PG can reduce neuroinflammation after stroke remains unclear. Treg is a special subset of Th2 that has been shown to negatively regulate neuroinflammation after stroke (76, 77). $CD4^+CD25^+Foxp3^+$ Treg can inhibit neuroinflammation by producing the inhibitory cytokine TGF-β, IL-10, and IL-35 (78, 79). Treg cells have been a key topic in dealing with neuroinflammation after acute ischemic stroke in recent years.

$CD8^+$ T cells

$CD8^+$T cells are mainly cytotoxic T lymphocytes (CTL), which are the key to the occurrence of neuroinflammation after stroke. CTL mainly mediates cellular immunity and exerts cytotoxic effects on target cells. The function process of CTL is as follows: first, CTL cells bind target cells. CTL cells are activated by identifying the peptide-MHC-I complexes on target cells. Then the active substances in CTL are transferred to the immune synapse which is structured with a ternary structure (TCR–MHC–peptide) and surrounding adhesion molecules. Finally, CTL launches a lethal attack and mediates apoptosis through the perforin-granzyme pathway, Fas/Fasl pathway, and TNF-α pathway. Some studies have shown that consuming $CD8^+$T cells show a better neuroprotective effect than consuming $CD4^+$T cells, indicating that $CD8^+$ T cells are more active than $CD4^+$T cells in post-IS neuroinflammation (70, 80, 81). In addition, after peripheral $CD8^+$ T cells were depleted, the infiltration of macrophages, neutrophils, and $CD4^+$ T cells into the infarcted brain tissue in transient middle cerebral artery occlusion (TMCAO) mice was correspondingly reduced (82). The toxic effects of $CD8^+$T cells can be achieved through the FASL-PDPK1 pathway and inhibition of PDPK1 can effectively improve neural function after stroke (83). Compared to $CD4^+$T cells, targeted therapy for $CD8^+$T cells may achieve a better outcome in acute IS.

Other T cells

After IS, non-specific immune T cells are also involved in the inflammatory response in the ischemic brain. γδT cells are distributed to the skin, intestines, airways, and other tissues after maturity in the thymus (84, 85), exerting an innate immune effect. Nasal-associated lymphoid tissue (NALT) may be one of the sources of γδT cells in the ischemic brain due to distance, but NALT ablation does not improve infarct size in stroke animals (84). After the proposal of the microbiome-gut-brain axis, γδT cells in the intestine are considered to be capable of migrating to the meninges after stroke, and the state of the microbiome in the gut can affect Treg/γδT cells ratio which is highly correlated with stroke outcomes (86). γδT cells predominantly secrete IL-17, mediating chronic inflammation after stroke, and promote

the migration of neutrophils and monocytes to the ischemic brain, exacerbating stroke outcomes (86–88). Compared to other T cells, mucosal-associated invariant T (MAIT) cells were involved earlier in neuroinflammation after stroke. TMCAO mice with a MAIT deficiency or MAIT inhibitory ligand drugs (isobutyryl 6-formylpterin, i6-FP, in Table 1) showed a smaller infarct size compared to the model group (89). NKT cells are also part of non-specific immune T cells. Although NKT cells in cancer, hepatitis, pneumonia, and sepsis are increasingly valued (90–92), their involvement in neuroinflammation after IS requires further investigation.

Inflammation has been increasingly studied in IS since researchers moved away from the dogma that the brain is an immune-privileged organ. Additionally, this theory of immune privilege may rest on the low permeability of the BBB. However, the integrity of the BBB is disrupted after stroke, which facilitates the migration of peripheral inflammatory cells to the CNS (93, 94). In fact, a damaged BBB is not the only way inflammatory cells enter the ischemic brain. Currently, more investigators tend to support the theory that the choroid plexus is the main route of peripheral lymphocytes getting into the ischemic brain (95). Chemokines and chemokine receptors play a key role in the migration of inflammatory cells to the ischemic brain, such as CCR2-CCL2 related to most T cells (95), CCR6-CCL20 related to 17^+ T cells (74), and CXCL1/CXCR2 related to neutrophils. But not all chemokines and chemokine receptors exacerbate neuroinflammation. For example, the CXCR3/CXCL10 axis, which served as the brain-homing mechanism for $CD8^+CD122^+CD49d^{lo}$ T regulatory-like cells, can provide neuroprotection in MCAO mice (96). Therefore, regulating peripheral inflammatory cells or regulatory-like cell migration could serve as a strategy for treating IS. In addition, it is important to regulate the activation of inflammatory cells in the CNS. Although the emergence of the brain-gut axis theory may make the gut microbiota and pathogen-associated molecular patterns (PAMPs), the initiators of neuroinflammation, sterile inflammation triggered by DAMPs remain the main type after the onset of IS. DAMPs internalization was largely mediated by the class A scavenger receptors MSR1 that was regulated by the transcription factor Mafb (97). MSR1 and Mafb may be promising targets for treating IS. Finally, targeting inflammatory cells themselves or the inflammatory factors they produce can provide enlightenment for future drug development for IS.

Excitatory toxicity

Excitatory toxicity is mainly caused by the increased glutamate (Glu) in the ischemic brain, which leads to neuronal necrosis and apoptosis by a series of biochemical cascades. After an acute stroke, mitochondrial ATP production decreases due to cerebral ischemia and hypoxia. Then intracellular and extracellular ion disorders (intracellular: Na^+, Ca^{2+}, Cl^- increase; extracellular: K^+ increase) caused by the dysfunctional ATP-dependent ion pump will eventually lead to an increase in glutamate in the extracellular or synaptic cleft. For example, intracellular transport of glutamate dependent on a normal Na^+ gradient (extracellular Na^+ are more than intracellular) is regulated by an ATP-dependent Na^+ pump. The normal Na^+ gradient is reversed (intracellular Na^+ are more than extracellular) when ATP synthesis is reduced and the ATP-dependent Na^+ pump is deactivated, which ultimately increases glutamate in the extracellular or synaptic cleft. As glutamate-mediated excitotoxicity severely affects prognosis in patients with stroke, studying the production and metabolism of glutamate and the downstream pathways mediated by glutamate receptors have great potential for the development of neuroprotective drugs against IS.

Production and metabolism of glutamate

Glutamate in the brain originates from multiple pathways (Figure 2). Glutamate in the periphery does not enter the brain under physiological conditions due to BBB. However, studies have shown that glutamate levels in the brain can be reduced by peritoneal dialysis after stroke (98). The main reasons for this phenomenon may be as follows. First, disrupted BBB. After the stroke, a high concentration of glutamate in brain tissue and a low concentration of glutamate in blood will form a gradient (99, 100). Disrupted BBB may facilitate glutamate to enter the periphery. Second, glutamate in the brain generates glutamine, which can directly cross BBB into the periphery. Then peripheral glutamate regenerated by glutamine is cleared by peritoneal dialysis. There is a glutamate-glutamine cycle in neurons and their neighboring astrocytes (Figure 2) (101, 102) and many targets in this cycle are important for extracellular glutamate production. Glutamate transporter GLT-1 is a member of excitatory amino acid transporters (EAATs), and it is capable of removing glutamate from the synaptic cleft. β-lactam antibiotics (Table 1) can stimulate the activity of GLT-1 in mice glial cells after OGD (103). Glutamine synthase (GS) is the speed limit of the glutamate-glutamine cycle (104) and can be degraded by reactive oxygen species (ROS) after stroke, resulting in the accumulation of glutamate (105). Targeting GS may be an ideal strategy for treating IS.

The glutamate/cystine antiporter system x(c)- transports cystine into cells in exchange for neurotransmitter glutamate at a ratio of 1:1 (106, 107). Cystine ingested into the cell produces cysteine, which is used as a raw material for the synthesis of glutathione (GSH) and participates in the scavenging of intracellular free radicals (106, 107). System x(c)- relies on a gradient of glutamate, and when

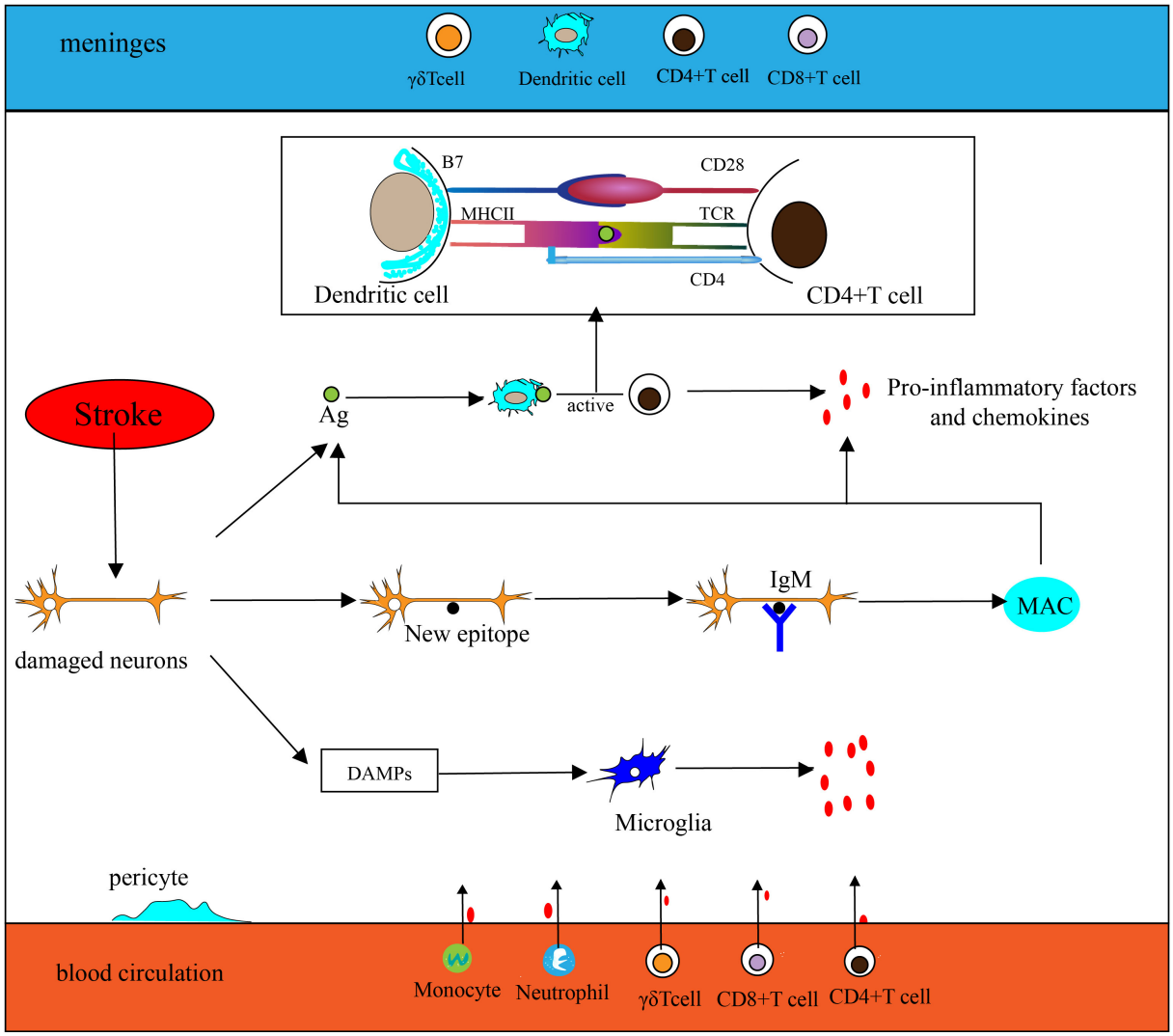

FIGURE 1
Inflammation in the ischemic brain. (1) Antigen (Ag) released by damaged neurons is ingested and processed by dendritic cells (DCs), then stimulates T cell activation (take $CD4^+$ T cells as an example, CD4 T cells are activated by MHC II–peptide–TCR and CD28-B7). Activated T cells secrete pro-inflammatory factors and chemokines to participate in the inflammatory response. (2) Neurons are exposed to new epitopes after necrosis, which can be recognized by natural immunoglobulin IgM in brain tissue, thereby activating the classical pathway of the complement system and forming a membrane attack complex (MAC) eventually. Then cellular contents are released extracellularly by MAC, which exacerbates the inflammatory response. (3) Injured neurons release DAMPs to activate microglia and participate in the inflammatory response. (4) Chemokines secreted by microglia and other inflammatory cells promote peripheral phagocytes, neutrophils, $CD4^+$T cells, $CD8^+$T cells, and γδT cells to migrate toward CNS.

extracellular glutamate concentration increases, the transfer of cystine into cells decreases, resulting in oxidative free radical damage (107–109). Acetylcysteine (Table 1) contributes to the scavenging of this oxidative free radical by stimulating glutamate/cystine antiporter system x(c) and promoting the generation of GSH (110, 111). Acetylcysteine protects against injury in a rat model of focal cerebral ischemia and ischemia/reperfusion, respectively (112, 113). In addition, two clinical trials registered with acetylcysteine treating IS, are still ongoing (114).

Glutamate and glutamate receptors

Glutamate exerts excitotoxic effects through glutamate receptors (NMDARs). NMDARs have a dual role in neurons, which may depend on the subtype of NMDARs (115). NMDARs consist of two NR1 subunits and one other subunit (NR2A/B/C/D or NR3A/B). NMDARs containing NR2A subunits mainly promote neuronal survival, while NMDARs containing NR2B subunits mainly mediate neuronal excitotoxicity and promote apoptosis (116). Another argument

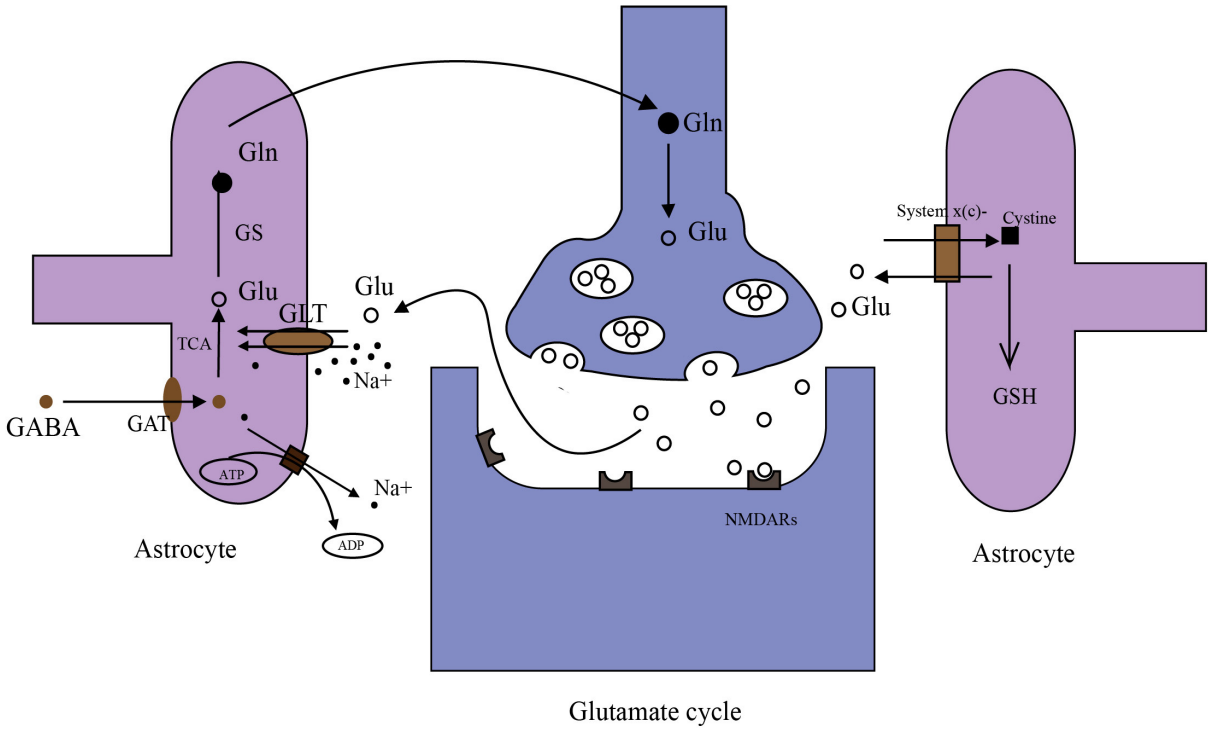

FIGURE 2
Metabolism of glutamate in the brain. (1) Glutamate (Glu)–glutamine (Gln) cycle: when nerve impulses are transmitted to the postsynaptic membrane, the neurotransmitter glutamate (Glu) stored in synaptic vesicles is released into the synaptic cleft and binds with glutamate receptors (NMDARs). Glutamate transporters (GLT-1 shown in the figure) on astrocyte transport Glu together with Na^+ into astrocytes. Na^+ in astrocytes is transferred to extracellular by an ATP-dependent Na^+ pump. Glu in astrocytes is converted to Gln by GS, then Gln is taken up by synapses and converted to Glu in synapses. Glu is stored in synaptic vesicles again. (2) γ aminobutyric acid (GABA) is taken up by GABA transporters (GATs) in astrocytes. Then GABA can be converted to Glu through the tricarboxylic acid cycle (TAC). (3) System x(c)- transports cystine into cells in exchange for the Glu. Cystine synthesizes glutathione (GSH) in astrocytes.

for the dual role of NMDARs has to do with their location. Studies have shown that there are two distinct subtypes of NMDARs: extrasynaptic NMDARs and intrasynaptic NMDARs (117). Stimulation of intrasynaptic NMDARs can activate CREBs in a variety of ways (115, 118). Activated CREB can enhance mitochondrial tolerance to cellular stress (119) and inhibit the pro-death transcription factor by promoting the expression of brain-derived neurotrophic factor (BDNF) (115), exerting an anti-apoptotic effect. Conversely, stimulating extrasynaptic NMDARs can dephosphorylate CREBs by inhibiting Ros/ERK1/2 pathway, which activates pro-apoptotic genes in the bcl-2 family, and thus induces apoptosis (120, 121).

In short, extrasynaptic NMDARs and intrasynaptic NMDARs play opposite roles. Various subtypes have different affinities with glutamate, and their positional relationships can both serve as explanations for glutamate excitotoxicity. In other words, physiological glutamate levels fail to activate extrasynaptic NMDARs (low affinity or low level of glutamate), but they can activate intrasynaptic NMDARs (high affinity or high level of glutamate) exerting a pro-survival signal. Extrasynaptic NMDARs can only be activated when the glutamate concentration rises above a certain threshold to exert a pro-death signal. Blocking NMDARs has been a potential target for inhibiting glutamate excitatory toxicity, particularly blocking extrasynaptic NMDARs and NMDARs containing NR2B subunit. Compared with intrasynaptic NMDARs, memantine (Table 1) can inhibit extrasynaptic receptors more effectively (122), and it has been clinically used in the treatment of AD in the US. However, memantine only shows efficacy in patients with severe and moderate AD (123). For mild AD, a meta-analysis found no difference between memantine and placebo in cognition, activities of daily living, or behavior (124). In a preclinical study of IS, memantine blunted the noxious effects of delayed thrombolysis on lesion volumes and neurological deficits in MCAO mice (125) and exerted synergistic neuroprotective effects with clenbuterol in MCAO rats (126). However, *in vivo* experiment of Trotman et al., higher doses of memantine (20 mg/kg/day) significantly increased injury. Similar results were also found in their *in vitro* experiments. Therefore, a proper dosage of memantine is significant in future clinical trials (127). Ifenprodil is capable of binding to NMDARs containing NR2B subunit with a high affinity (128, 129). Although clinical trials of ifenprodil are currently only conducted in idiopathic pulmonary fibrosis

(IPF)/corona virus disease 2019 (COVID-19)/post-traumatic stress disorder (PTSD), using ifenprodil in treatment against IS remains promising.

Glutamate toxicity

When glutamate binds with the receptor, an action potential is formed. After excitotoxicity occurs, glutamate continues to excite receptors and keeps Na^+ channels open. Then intracellular osmotic pressure increases due to this persistently opened Na^+ channel, resulting in acute neuronal death. When neurons are in a resting state, Ca^{2+} channels are blocked by Mg^{2+}. When glutamate binds to NMDARs, Mg^{2+} is removed and Ca^{2+} channels are opened by the depolarized postsynaptic membrane. Intracellular Ca^{2+} is elevated by these opened Ca^{2+} channels, which contributes to the activation of a ternary structure (PSD95-NR2B-NOS) (130). The activated ternary structure then releases nitric oxide synthase (NOS) leading to the production of reactive nitrogen species (RNS). Cell *in vitro* experiments and animal experiments have demonstrated that disrupting this ternary structure can reduce glutamate-mediated excitotoxicity and improve neuronal tolerance to glutamate (131, 132). Furthermore, mitochondria are disturbed by calcium overload to release a large number of oxidative free radicals, which can promote neuronal apoptosis and aggravate calcium overload again through activated transient receptor potential melastatin-subfamily member 7 (TRPM7) and transient receptor potential melastatin-subfamily member 2 (TRPM2) (115). Recently, Zong et al. discovered that TRPM2 directly interacts with GluN2a/b of extrasynaptic NMDARs through the unique EE3 motif in its N-tail and the KKR motifs in the C-tail of GluN2a/b. This coupling mechanism plays an important role in the excitotoxicity of ischemic brain injury in mice (133). Therefore, in downstream of glutamate-mediated excitotoxicity, some ion channels that mediate calcium overload, some complexes that mediate oxidative stress, and the coupling of TRPM2 and extrasynaptic NMDARs are expected to be targets for the treatment of IS.

NMDAR-mediated excitotoxicity has been extensively studied in stroke; however, NMDAR antagonists face challenges now in the treatment of ischemic stroke in human patients. This may be attributed to some key targets and structures that have not been fully studied. Additionally, we hope to provide some inspiration for future drug development through the above summary of excitotoxicity.

Oxidative stress

Generation of oxidative free radicals

Physiologically, there is stable oxidation and antioxidant system in the body. In the process of reperfusion of the ischemic brain, glucose, and oxygen enter the brain again. Oxidative glycolysis of glucose produces a large amount of reduced NADH-H+ and FADH2 along with superoxide anion generated in the process of electron transfer, resulting in excessive ROS and RNS, as well as damage to the ischemic brain (134). It is currently believed that there are two oxidative respiratory chains (Figure 3) in mitochondria, one is NADH oxidation respiratory chain (NADH-complex I-CoQ-complex III-Cytc-complex IV-O_2) and the other is the FADH2 oxidation respiratory chain, also called succinate oxidation respiratory chain (succinate -complex II-CoQ-complex III-Cytc-complex IV-O2). Studies have shown that FADH2 accumulation can be attributed to the reversal of succinate dehydrogenase (SDH) after stroke. In the early stage of perfusion, the accumulated FADH2 activates SDH and drives mitochondria to produce a large amount of ROS *via* reverse electron transfer (RET) (105). Excessive ROS and RNS promote lipid peroxidation, mitochondrial and DNA damage, protein nitrification and oxidation, and depletion of antioxidants (135). In addition to this, ROS and RNS lead to the overexpression of inflammatory genes, inflammatory and chemokine production, BBB disruption, leukocyte recruitment, and cerebral edema (136).

Removing ROS and RNS is a major strategy to treat IS and protect neurons. Edaravone (Table 1) is clinically used for the treatment of IS, which can scavenge oxidative free radicals and achieve the purpose of protecting ischemic neurons. Uric acid (Table 1) contributes to the scavenging of ROS, including nitrite, which can reduce cerebral infarct volume after stroke and improve neurological outcomes after transient or permanent cerebral ischemia in rodents (137–139).

Antioxidant enzyme system

There are various antioxidant enzymes and small molecule antioxidants (such as vitamin C/E, ubiquinone, β-carotene) in our body, which together constitute the antioxidant system. Superoxide dismutase (SOD) is widely distributed in the human body, responsible for catalyzing.O_2- and converting it to oxygen and hydrogen peroxide (H_2O_2), and it is an important part of the cellular antioxidant system (140). Catalase (CAT) has a strong catalytic ability to H_2O_2. However, CAT will inevitably generate.OH- in the process of scavenging H_2O_2. Tri-manganese (III) salen-based cryptands, an analog of CAT, can minimize the production of.OH- while removing H_2O_2 (141). Glutathione peroxidase (GPx) is the main enzyme in the body to scavenge ROS, removing H_2O_2 and other peroxides. Furthermore, GPx is the key to inhibiting neuronal ferroptosis (142). A selenocysteine-containing peptide, Tat SelPep, can increase GPx expression by binding to nuclear DNA and effectively improve stroke outcomes (143). Thioredoxin (Trx), also one of the body's antioxidant enzymes, has been found to improve outcomes after stroke. Melatonin (MT), a neurohormone in the human body, can achieve anti-oxidation

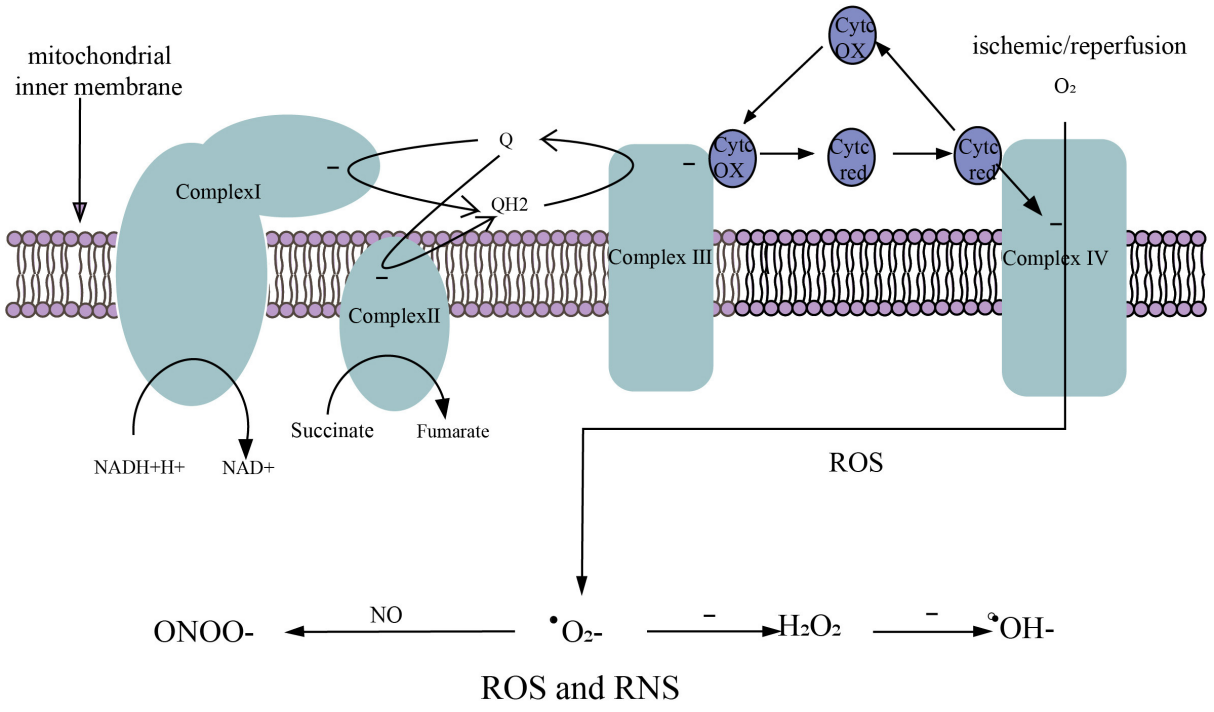

FIGURE 3
Generation of ROS and RNS. (1) NADH oxidation respiratory chain. NADH-H+ transfers electrons to complex I and is oxidized to NAD^+. (2) Succinate oxidation respiratory chain. Succinate transfers electrons to complex II and is oxidized to fumarate. (3) The electrons in complex I/II are captured by ubiquinone (Q) to form QH2. The electrons in QH2 are transferred to complex III, then QH2 that loses electrons becomes Q to participate in the electron transfer again. Electrons in complex III are transferred to complex IV *via* cytochrome c (Cytc). (4) O_2 obtains electrons from complex IV to generate ROS. (5) After ischemic/reperfusion injury, O_2 gets a single electron to generate superoxide anion ($\cdot O_2^-$), $\cdot O_2^-$ accepts a single electron to generate hydrogen peroxide (H_2O_2), and H_2O_2 accepts a single electron to be reduced to hydroxyl radical ($\cdot OH^-$), $\cdot O_2^-$ can rapidly oxidize NO to produce nitrite ($ONOO^-$).

by regulating Trx (144). However, the performance of most antioxidant drugs in animal experiments is not satisfactory, which may be related to the inability of antioxidants to target mitochondria, the birthplace of ROS and RNS (145). Therefore, this kind of antioxidant drug targeting mitochondria needs to be further considered in the future.

Scavenging oxidative free radicals and increasing the reserves of antioxidant enzymes have been one of the strategies for the treatment of IS. However, only edaravone is currently approved for the clinical treatment of IS. Additional mechanisms related to oxidative stress help resolve this dilemma. For example, ferroptosis is a new form of cell death caused by an increase in iron ion-dependent lipid peroxides (146). Although the specific mechanism of ferroptosis in IS has not been elucidated, inhibition of lipid peroxidation and regulation of iron metabolism is promising for treating IS.

Complement system

The complement system is composed of complement intrinsic components, complement regulatory proteins, and complement receptors, and its activation pathways are mainly three-fold: classical pathway (CP), bypass pathway (AP), and lectin pathway (LP). The complement system is part of innate immunity and is involved in the subsequent phases of humoral immunity. Since B lymphocytes are barely detectable in brain tissue within a week after stroke (11, 12), CP that rely on immune complexes (ICs) for activation may be limited in the acute phase of IS. However, there is a natural immunoglobulin M (IgM) in the brain, which can recognize a new epitope on damaged neurons and still activate CP to exert pathological damage after stroke (147, 148). The endpoint of the complement cascade is the formation of a membrane attack complex (MAC), resulting in cell rupture and death. In recent years, neuroprotective agents targeting C3 and C5 or complement fragments have entered our field of vision. As a fusion construct, B4cry inhibits IgM binding to the new epitope, C3 cleavage, and the activation of microglia, which can reduce complement deposition in ischemic lesions and improve neurological function after stroke (149, 150). C5a-C5AaR axis capable of promoting neutrophil migration (151) is widely involved in COVID-19-related coagulopathy, viral hepatitis, cancer, and myocarditis (152–155), and this axis may serve as a target for the treatment of IS. Mannan-binding lectin

(MBL) plays an important role in the activation of LP. Using polyman2 (the synthesized mannosylated molecule selected for its binding to MBL) or anti-MBL antibody, can inhibit the activation of mannan-binding lectin-associated serine protease-2 (MASP-2) to block the subsequent complement cascade, and exert a neuroprotective effect on TMCAO or permanent middle cerebral artery occlusion (PMCAO) rats (156). Complement regulatory proteins are one of the long-term targets that complement drug developers are focusing on. C1 inhibitor (C1-INH) capable of inhibiting C1s or MASP-2 can inhibit complement cascade from the upstream of C3. Animal experiments have shown that compared with TMCAO mice, C1-INH-deficient TMCAO mice showed larger ischemic foci and worse neurobehavioral performance (157).

There are many components in the complement system, but there is little research on the complement system in IS. Since the development of neuroprotective agents against IS has mostly failed, the complement system seems to be a good path.

Thrombus and pericytes

Thrombus is the fundamental factor causing cerebral vascular obstruction and cerebral ischemia and hypoxia. For thrombus formation, the importance of the involvement of ultra-large (UL) von Willebrand factor (ULVWF) and collagen–von Willebrand factor–glycoprotein Ib axis has been highlighted in many studies (175, 176). ULVWF multimers are regulated by a metalloproteinase Adamts13. Some studies have shown that low activity of Adamts13 is associated with an increased risk of IS and TIA (177). The interaction of platelets and neutrophils is critical in thrombosis. Platelets promote the formation of neutrophil extracellular traps (NETs) (178), which can be cleared by patients' deoxyribonuclease-1 (DNase-1) in stroke treatment (179). Antithrombotic therapy has always been the main method for IS treatment. However, some limitations of thrombolysis, such as the therapeutic window and low efficacy of reperfusion, are difficult to solve. In recent years, the combination of thrombolysis and neuroprotection seems to be an excellent strategy for treating IS. For example, uric acid helps to scavenge ROS. In a pre-clinical study by Romanos et al. (138), uric acid and recombinant tissue plasminogen activator (rtPA) showed a synergistic effect in the model of thromboembolic cerebral ischemia in rats. A clinical trial (NCT00860366) on the combination of rtPA and uric acid in the treatment of IS has been completed. Unfortunately, we have not found relevant research results. In addition, in a double-blind, placebo-controlled, phase 2b trial, the combination of rtPA and uric acid may prevent early ischemic deterioration after acute stroke in patients with thrombolysis (180). This strategy of thrombolysis combined with neuroprotection can be a good point in the treatment of IS in the future.

In addition to being interrupted by a thrombus, cerebral blood flow is also regulated by capillary pericytes. Ischemic pericytes constrict and compress capillaries after stroke, reducing cerebral blood flow. More importantly, pericyte death attributed to sustained ischemia and hypoxia can lead to irreversible constriction of capillaries and permanent interruption of blood flow (10). The time of pericyte resistance to ischemia and hypoxia may affect the time window of intravenous thrombolysis or mechanical thrombectomy. Preventing the shrinkage and death of pericytes and improving the tolerance of pericytes to ischemia and hypoxia may be of great significance to prolong this time window.

How to deliver drugs to the CNS?

We have described many promising drugs earlier, but all of these drugs have to face a common problem: It is difficult to deliver them to the CNS. The BBB is the main barrier preventing drugs from entering the CNS. It has been reported that <2% of small molecule drugs with CNS affects approved by the Food and Drug Administration (FDA) can pass through the intact BBB (181, 182). The existence of the BBB is necessary for maintaining the homeostasis of the cerebral microenvironment and ensuring the normal functions of the CNS. Nevertheless, the BBB also impedes the intracerebral delivery of therapeutic agents (183). Although studies have shown that the occurrence of IS can destroy and increase the permeability of the BBB, the BBB remains the main obstacle for drugs to overcome (184).

At present, scientists and industry have developed a variety of technologies to deliver drugs to the CNS. For instance, the emergence of nanoparticles provides a new strategy for drugs to enter the CNS. Nanoparticles have been proven to deliver a great variety of drugs across the BBB, and this mechanism of crossing BBB now appears to be receptor-mediated endocytosis of the brain capillary endothelial cells, followed by transcytosis (185, 186). By combining with different drugs, nanoparticles perform three major approaches for ischemic stroke therapy: recanalization, neuroprotection, and combination therapy (184). More importantly, nanoparticles are capable of increasing drug bioavailability, enhancing therapeutic efficacy, and reducing unwanted toxicity (184). In recent years, exosomes have been mentioned as a strategy for the treatment of IS. Exosomes are endosome-derived membrane-bound vesicles with diameters of 30–150 nm, and they are released by most cell types (187–189). Among the cargoes carried by exosomes, miRNAs are valued by researchers because they may be the core of the therapeutic effects of exosomes (189). Therefore, how to select miRNA contained in exosomes may become a strategy for treating IS. Additionally, in the treatment of IS, engineered exosomes that contain selected miRNA have been proven to be more effective compared with naïve exosomes (189). Although the ability of exosomes to directly cross the BBB is uncertain,

several studies have shown that some exosomes could cross the BBB in healthy and inflamed brains (189–191).

In addition to the aforementioned nanoparticles and exosomes, neurotropic virus mediation emerged as a strategy for the treatment of IS. Neurotropic viruses, with an affinity for nerve, can cross the BBB through multiple pathways, such as direct transcytosis, virus-infected immune cells, and retrograde transport from peripheral nerves to the CNS (192). Additionally, this property makes neurotropic viruses a CNS-targeting strategy. Carrier-mediated transcytosis (CMT) and receptor-mediated transcytosis (RMT) are long-term concerns of researchers. However, when delivering drugs to the CNS through RMT or CMT, the target receptor or carrier protein should be highly expressed in the endothelial cells of the cerebral vasculature, especially those in the microvascular (192). Therefore, this carrier protein that can match the receptors abundantly expressed in cerebral microvascular needs to be elaborately designed.

Crossing the BBB seems to be an inescapable obstacle for delivering drugs to the CNS, even though there are now many ways to bypass the BBB, such as highly invasive intracerebral injection, intranasal, retro-orbital, or intrathecal administration. However, these methods of bypassing the BBB are difficult to achieve clinically, which may be attributed to their operational complexity, high invasiveness, and low bioavailability. With the development of technology, RMT-based strategies, neurotropic virus-based approaches, nanoparticles, exosomes, etc. have shown great potential to deliver drugs to the CNS.

Difficulties in clinical transformation

At present, most neuroprotective drugs are facing difficulties in clinical transformation. These difficulties are caused by many aspects. Animal models of IS, where middle cerebral arteries are often blocked by nylon fibers, have been questioned because they do not reflect the occurrence of vascular embolism under natural conditions (193). Moreover, there are many differences between animal models and humans, such as age, species, and underlying diseases. The side effects of drugs are also important factors. For example, in the development of complement drugs, complement inhibitors inevitably inhibit the activity of serum complement while inhibiting complement activation, which increases the risk of infection (194). Therefore, it is necessary to develop more targeted drugs that have higher precision. More importantly, incomplete mechanism research will also lead to failure in drug development. For example, although the use of anti-IL-17A drugs has seen efficacy in the treatment of psoriasis, it is clinically invalid in the treatment of amyotrophic lateral sclerosis (ALS), rheumatoid disease, and experimental autoimmune encephalomyelitis (EAE) (195). This contradictory role in different diseases may be attributed to the incompleteness of the mechanism such as the possible duality of IL-17, the different pathogenicities of Th17, and the negative feedback of IL-17 (195, 196). In addition, the ability of the drug to penetrate the BBB, oral bioavailability, half-life, and statistical bias are also factors that determine whether the drug can be successfully translated into the clinic.

Conclusion

Since most clinical patients with ischemic stroke fail in conventional treatments such as intravenous thrombolysis and mechanical thrombectomy due to missed time windows, it is of great significance to find other strategies to protect CNS. In the complex pathological mechanisms of IS, we can obtain many methods and targets for the treatment of IS. It is very promising to develop new drugs for IS from these mechanisms and targets. Additionally, combining these new drugs with brain delivery technologies and more precise targeted therapy may go further in the clinical treatment of IS.

Author contributions

YJ and YT conceived the topic and determined the outline of this review. YJ, YT, and ZL contributed to the manuscript writing. YD and SS collected the literature and finished the figures and tables. YT, ZL, and YL critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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References

1. Chen Z, Jiang B, Ru X, Sun H, Sun D, Liu X, et al. Mortality of stroke and its subtypes in china: results from a nationwide population-based survey. *Neuroepidemiology.* (2017) 48:95–102. doi: 10.1159/000477494

2. Chamorro A, Dirnagl U, Urra X, Planas AM. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *Lancet Neurol.* (2016) 15:869–81. doi: 10.1016/S1474-4422(16)00114-9

3. Powers WJ. Acute ischemic stroke. *N Engl J Med.* (2020) 383:252–60. doi: 10.1056/NEJMcp1917030

4. Amarenco P. Transient ischemic attack. *Reply N Engl J Med.* (2020) 383:1598. doi: 10.1056/NEJMc2022610

5. Logallo N, Novotny V, Assmus J, Kvistad CE, Alteheld L, Ronning OM, et al. Tenecteplase versus alteplase for management of acute ischaemic stroke (NOR-TEST): a phase 3, randomised, open-label, blinded endpoint trial. *Lancet Neurol.* (2017) 16:781–8. doi: 10.1016/S1474-4422(17)30253-3

6. Menon BK, Buck BH, Singh N, Deschaintre Y, Almekhlafi MA, Coutts SB, et al. Intravenous tenecteplase compared with alteplase for acute ischaemic stroke in Canada (AcT): a pragmatic, multicentre, open-label, registry-linked, randomised, controlled, non-inferiority trial. *Lancet.* (2022) 400:161–9.

7. Campbell BCV, Mitchell PJ, Churilov L, Yassi N, Kleinig TJ, Dowling RJ, et al. Tenecteplase versus alteplase before thrombectomy for ischemic stroke. *N Engl J Med.* (2018) 378:1573–82.

8. Patel P, Yavagal D, Khandelwal P. Hyperacute management of ischemic strokes: JACC focus seminar. *J Am Coll Cardiol.* (2020) 75:1844–56. doi: 10.1016/j.jacc.2020.03.006

9. Gerschenfeld G, Muresan IP, Blanc R, Obadia M, Abrivard M, Piotin M, et al. Two paradigms for endovascular thrombectomy after intravenous thrombolysis for acute ischemic stroke. *JAMA Neurol.* (2017) 74:549–56. doi: 10.1001/jamaneurol.2016.5823

10. Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, et al. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature.* (2014) 508:55–60. doi: 10.1038/nature13165

11. Doyle KP, Quach LN, Sole M, Axtell RC, Nguyen TV, Soler-Llavina GJ, et al. B-lymphocyte-mediated delayed cognitive impairment following stroke. *J Neurosci.* (2015) 35:2133–45. doi: 10.1523/JNEUROSCI.4098-14.2015

12. Kleinschnitz C, Schwab N, Kraft P, Hagedorn I, Dreykluft A, Schwarz T, et al. Early detrimental T-cell effects in experimental cerebral ischemia are neither related to adaptive immunity nor thrombus formation. *Blood.* (2010) 115:3835–42. doi: 10.1182/blood-2009-10-249078

13. Colonna M, Butovsky O. Microglia function in the central nervous system during health and neurodegeneration. *Annu Rev Immunol.* (2017) 35:441–68. doi: 10.1146/annurev-immunol-051116-052358

14. Thion MS, Ginhoux F, Garel S. Microglia and early brain development: an intimate journey. *Science.* (2018) 362:185–9. doi: 10.1126/science.aat0474

15. Xiong X, Gu L, Wang Y, Luo Y, Zhang H, Lee J, et al. Glycyrrhizin protects against focal cerebral ischemia *via* inhibition of T cell activity and HMGB1-mediated mechanisms. *J Neuroinflammation.* (2016) 13:241. doi: 10.1186/s12974-016-0705-5

16. Manberg A, Skene N, Sanders F, Trusohamn M, Remnestal J, Szczepinska A, et al. Altered perivascular fibroblast activity precedes ALS disease onset. *Nat Med.* (2021) 27:640–6. doi: 10.1038/s41591-021-01295-9

17. Yilmaz M, Yalcin E, Presumey J, Aw E, Ma M, Whelan CW, et al. Overexpression of schizophrenia susceptibility factor human complement C4A promotes excessive synaptic loss and behavioral changes in mice. *Nat Neurosci.* (2021) 24:214–24. doi: 10.1038/s41593-020-00763-8

18. Wood H. Microglial changes associated with meningeal inflammation in multiple sclerosis. *Nat Rev Neurol.* (2021) 17:262. doi: 10.1038/s41582-021-00494-9

19. Franco R, Fernandez-Suarez D. Alternatively activated microglia and macrophages in the central nervous system. *Prog Neurobiol.* (2015) 131:65–86. doi: 10.1016/j.pneurobio.2015.05.003

20. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. *Physiol Rev.* (2011) 91:461–553. doi: 10.1152/physrev.00011.2010

21. Eldahshan W, Fagan SC, Ergul A. Inflammation within the neurovascular unit: focus on microglia for stroke injury and recovery. *Pharmacol Res.* (2019) 147:104349. doi: 10.1016/j.phrs.2019.104349

22. Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S, et al. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke.* (2012) 43:3063–70. doi: 10.1161/STROKEAHA.112.659656

23. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci.* (2009) 29:13435–44. doi: 10.1523/JNEUROSCI.3257-09.2009

24. Ito S, Kimura K, Haneda M, Ishida Y, Sawada M, Isobe K. Induction of matrix metalloproteinases (MMP3, MMP12 and MMP13) expression in the microglia by amyloid-beta stimulation via the PI3K/Akt pathway. *Exp Gerontol.* (2007) 42:532–7. doi: 10.1016/j.exger.2006.11.012

25. Toyama T, Hoshi T, Noguchi T, Saito Y, Matsuzawa A, Naganuma A, et al. Methylmercury induces neuronal cell death by inducing TNF-alpha expression through the ASK1/p38 signaling pathway in microglia. *Sci Rep.* (2021) 11:9832. doi: 10.1038/s41598-021-89210-7

26. Zhao Y, Wei ZZ, Lee JH, Gu X, Sun J, Dix TA, et al. Pharmacological hypothermia induced neurovascular protection after severe stroke of transient middle cerebral artery occlusion in mice. *Exp Neurol.* (2020) 325:113133. doi: 10.1016/j.expneurol.2019.113133

27. Boche D, Perry VH, Nicoll JA. Review: activation patterns of microglia and their identification in the human brain. *Neuropathol Appl Neurobiol.* (2013) 39:3–18. doi: 10.1111/nan.12011

28. Hu X, Zhang M, Leak RK, Gan Y, Li P, Gao Y, et al. Delivery of neurotherapeutics across the blood brain barrier in stroke. *Curr Pharm Des.* (2012) 18:3704–20. doi: 10.2174/138161212802002715

29. Wang X, Xuan W, Zhu ZY, Li Y, Zhu H, Zhu L, et al. The evolving role of neuro-immune interaction in brain repair after cerebral ischemic stroke. *CNS Neurosci Ther.* (2018) 24:1100–14. doi: 10.1111/cns.13077

30. Nishimoto R, Derouiche S, Eto K, Deveci A, Kashio M, Kimori Y, et al. Thermosensitive TRPV4 channels mediate temperature-dependent microglia movement. *Proc Natl Acad Sci U S A.* (2021) 118:e2012894118. doi: 10.1073/pnas.2012894118

31. Han J, Fan Y, Zhou K, Blomgren K, Harris RA. Uncovering sex differences of rodent microglia. *J Neuroinflammation.* (2021) 18:74. doi: 10.1186/s12974-021-02124-z

32. Ma S, Wang J, Wang Y, Dai X, Xu F, Gao X, et al. Diabetes mellitus impairs white matter repair and long-term functional deficits after cerebral ischemia. *Stroke.* (2018) 49:2453–63. doi: 10.1161/STROKEAHA.118.021452

33. Jackson-Cowan L, Eldahshan W, Dumanli S, Dong G, Jamil S, Abdul Y, et al. Delayed administration of angiotensin receptor (AT2R) agonist C21 improves survival and preserves sensorimotor outcomes in female diabetic rats post-stroke through modulation of microglial activation. *Int J Mol Sci.* (2021) 22:1356. doi: 10.3390/ijms22031356

34. Olah M, Patrick E, Villani AC, Xu J, White CC, Ryan KJ, et al. A transcriptomic atlas of aged human microglia. *Nat Commun.* (2018) 9:539. doi: 10.1038/s41467-018-02926-5

35. Kobayashi K, Imagama S, Ohgomori T, Hirano K, Uchimura K, Sakamoto K, et al. Minocycline selectively inhibits M1 polarization of microglia. *Cell Death Dis.* (2013) 4:e525. doi: 10.1038/cddis.2013.54

36. Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, et al. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. *Neurology.* (2007) 69:1404–10. doi: 10.1212/01.wnl.0000277487.04281.db

37. Darsalia V, Hua S, Larsson M, Mallard C, Nathanson D, Nystrom T, et al. Exendin-4 reduces ischemic brain injury in normal and aged type 2 diabetic mice and promotes microglial M2 polarization. *PLoS ONE.* (2014) 9:e103114. doi: 10.1371/journal.pone.0103114

38. Dong LD, Ma YM, Xu J, Guo YZ, Yang L, Guo FY, et al. Effect of hyperglycemia on microglial polarization after cerebral ischemia-reperfusion injury in rats. *Life Sci.* (2021) 279:119660. doi: 10.1016/j.lfs.2021.119660

39. Cheng M, Yang L, Dong Z, Wang M, Sun Y, Liu H, et al. Folic acid deficiency enhanced microglial immune response *via* the Notch1/nuclear factor kappa B p65 pathway in hippocampus following rat brain I/R injury and BV2 cells. *J Cell Mol Med.* (2019) 23:4795–807. doi: 10.1111/jcmm.14368

40. Group VTS. B vitamins in patients with recent transient ischaemic attack or stroke in the VITAmins TO Prevent Stroke (VITATOPS) trial: a randomised, double-blind, parallel, placebo-controlled trial. *Lancet Neurol.* (2010) 9:855–65. doi: 10.1016/S1474-4422(10)70187-3

41. Hankey GJ, Ford AH, Yi Q, Eikelboom JW, Lees KR, Chen C, et al. Effect of B vitamins and lowering homocysteine on cognitive impairment in patients with previous stroke or transient ischemic attack: a prespecified secondary analysis of a randomized, placebo-controlled trial and meta-analysis. *Stroke.* (2013) 44:2232–9. doi: 10.1161/STROKEAHA.113.001886

42. Han D, Liu H, Gao Y. The role of peripheral monocytes and macrophages in ischemic stroke. *Neurol Sci.* (2020) 41:3589–607. doi: 10.1007/s10072-020-04777-9

43. Li Q, Barres BA. Microglia and macrophages in brain homeostasis and disease. *Nat Rev Immunol.* (2018) 18:225–42. doi: 10.1038/nri.2017.125

44. Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science.* (2007) 317:666–70. doi: 10.1126/science.1142883

45. Huang Y, Wang J, Cai J, Qiu Y, Zheng H, Lai X, et al. Targeted homing of CCR2-overexpressing mesenchymal stromal cells to ischemic brain enhances post-stroke recovery partially through PRDX4-mediated blood-brain barrier preservation. *Theranostics.* (2018) 8:5929–44. doi: 10.7150/thno.28029

46. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science.* (2010) 330:841–5. doi: 10.1126/science.1194637

47. Yamasaki R, Lu H, Butovsky O, Ohno N, Rietsch AM, Cialic R, et al. Differential roles of microglia and monocytes in the inflamed central nervous system. *J Exp Med.* (2014) 211:1533–49. doi: 10.1084/jem.20132477

48. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature.* (2015) 518:547–51. doi: 10.1038/nature13989

49. Werner Y, Mass E, Ashok Kumar P, Ulas T, Handler K, Horne A, et al. Cxcr4 distinguishes HSC-derived monocytes from microglia and reveals monocyte immune responses to experimental stroke. *Nat Neurosci.* (2020) 23:351–62. doi: 10.1038/s41593-020-0585-y

50. Liu Q, Johnson EM, Lam RK, Wang Q, Bo Ye H, Wilson EN, et al. Peripheral TREM1 responses to brain and intestinal immunogens amplify stroke severity. *Nat Immunol.* (2019) 20:1023–34. doi: 10.1038/s41590-019-0421-2

51. Baruch K, Schwartz M. Circulating monocytes in between the gut and the mind. *Cell Stem Cell.* (2016) 18:689–91. doi: 10.1016/j.stem.2016.05.008

52. Mohle L, Mattei D, Heimesaat MM, Bereswill S, Fischer A, Alutis M, et al. Ly6C(hi) monocytes provide a link between antibiotic-induced changes in gut microbiota and adult hippocampal neurogenesis. *Cell Rep.* (2016) 15:1945–56. doi: 10.1016/j.celrep.2016.04.074

53. Kim E, Yang J, Beltran CD, Cho S. Role of spleen-derived monocytes/macrophages in acute ischemic brain injury. *J Cereb Blood Flow Metab.* (2014) 34:1411–9. doi: 10.1038/jcbfm.2014.101

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

55. Garcia-Culebras A, Duran-Laforet V, Pena-Martinez C, Ballesteros I, Pradillo JM, Diaz-Guzman J, et al. Myeloid cells as therapeutic targets in neuroinflammation after stroke: specific roles of neutrophils and neutrophil-platelet interactions. *J Cereb Blood Flow Metab.* (2018) 38:2150–64. doi: 10.1177/0271678X18795789

56. Ames A 3rd, Wright RL, Kowada M, Thurston JM, Majno G. Cerebral ischemia. II. The no-reflow phenomenon. *Am J Pathol.* (1968) 52:437–53.

57. Marin-Esteban V, Turbica I, Dufour G, Semiramoth N, Gleizes A, Gorges R, et al. Afa/Dr diffusely adhering *Escherichia coli* strain C1845 induces neutrophil extracellular traps that kill bacteria and damage human enterocyte-like cells. *Infect Immun.* (2012) 80:1891–9. doi: 10.1128/IAI.00050-12

58. Genchi A, Semerano A, Gullotta GS, Strambo D, Schwarz G, Bergamaschi A, et al. Cerebral thrombi of cardioembolic etiology have an increased content of neutrophil extracellular traps. *J Neurol Sci.* (2021) 423:117355. doi: 10.1016/j.jns.2021.117355

59. Roberts RE, Elumalai GL, Hallett MB. Phagocytosis and motility in human neutrophils is competent but compromised by pharmacological inhibition of ezrin phosphorylation. *Curr Mol Pharmacol.* (2018) 11:305–15. doi: 10.2174/1874467211666180516100613

60. Sas AR, Carbajal KS, Jerome AD, Menon R, Yoon C, Kalinski AL, et al. A new neutrophil subset promotes CNS neuron survival and axon regeneration. *Nat Immunol.* (2020) 21:1496–505. doi: 10.1038/s41590-020-00813-0

61. Bird L. Neurorestorative neutrophils. *Nat Rev Immunol.* (2021) 21:2–3. doi: 10.1038/s41577-020-00485-9

62. Sionov RV, Fridlender ZG, Granot Z. The multifaceted roles neutrophils play in the tumor microenvironment. *Cancer Microenviron.* (2015) 8:125–58. doi: 10.1007/s12307-014-0147-5

63. Mantovani A. The yin-yang of tumor-associated neutrophils. *Cancer Cell.* (2009) 16:173–4. doi: 10.1016/j.ccr.2009.08.014

64. Cuartero MI, Ballesteros I, Moraga A, Nombela F, Vivancos J, Hamilton JA, et al. N2 neutrophils, novel players in brain inflammation after stroke: modulation by the PPARgamma agonist rosiglitazone. *Stroke.* (2013) 44:3498–508. doi: 10.1161/STROKEAHA.113.002470

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

66. Seifert HA, Hall AA, Chapman CB, Collier LA, Willing AE, Pennypacker KR, et al. transient decrease in spleen size following stroke corresponds to splenocyte release into systemic circulation. *J Neuroimmune Pharmacol.* (2012) 7:1017–24. doi: 10.1007/s11481-012-9406-8

67. Ran Y, Liu Z, Huang S, Shen J, Li F, Zhang W, et al. Splenectomy fails to provide long-term protection against ischemic stroke. *Aging Dis.* (2018) 9:467–79. doi: 10.14336/AD.2018.0130

68. Wolf SA, Steiner B, Akpinarli A, Kammertoens T, Nassenstein C, Braun A, et al. CD4-positive T lymphocytes provide a neuroimmunological link in the control of adult hippocampal neurogenesis. *J Immunol.* (2009) 182:3979–84. doi: 10.4049/jimmunol.0801218

69. Durafourt BA, Moore CS, Zammit DA, Johnson TA, Zaguia F, Guiot MC, et al. Comparison of polarization properties of human adult microglia and blood-derived macrophages. *Glia.* (2012) 60:717–27. doi: 10.1002/glia.22298

70. Wang S, Zhang H, Xu Y. Crosstalk between microglia and T cells contributes to brain damage and recovery after ischemic stroke. *Neurol Res.* (2016) 38:495–503. doi: 10.1080/01616412.2016.1188473

71. Wong CH, Jenne CN, Tam PP, Leger C, Venegas A, Ryckborst K, et al. Prolonged activation of invariant natural killer T cells and TH2-skewed immunity in stroke patients. *Front Neurol.* (2017) 8:6. doi: 10.3389/fneur.2017.00006

72. Xiao W, Guo S, Chen L, Luo Y. The role of Interleukin-33 in the modulation of splenic T-cell immune responses after experimental ischemic stroke. *J Neuroimmunol.* (2019) 333:576970. doi: 10.1016/j.jneuroim.2019.576970

73. Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, et al. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat Immunol.* (2009) 10:514–23. doi: 10.1038/ni.1716

74. Kulkarni N, Meitei HT, Sonar SA, Sharma PK, Mujeeb VR, Srivastava S, et al. CCR6 signaling inhibits suppressor function of induced-Treg during gut inflammation. *J Autoimmun.* (2018) 88:121–30. doi: 10.1016/j.jaut.2017.10.013

75. Das M, Tang X, Han JY, Mayilsamy K, Foran E, Biswal MR, et al. CCL20-CCR6 axis modulated traumatic brain injury-induced visual pathologies. *J Neuroinflammation.* (2019) 16:115. doi: 10.1186/s12974-019-1499-z

76. Rodriguez-Perea AL, Gutierrez-Vargas J, Cardona-Gomez GP, Guarin CJ, Rojas M, Hernandez PA. Atorvastatin modulates regulatory T cells and attenuates cerebral damage in a model of transient middle cerebral artery occlusion in rats. *J Neuroimmune Pharmacol.* (2017) 12:152–62. doi: 10.1007/s11481-016-9706-5

77. Liesz A, Suri-Payer E, Veltkamp C, Doerr H, Sommer C, Rivest S, et al. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat Med.* (2009) 15:192–9. doi: 10.1038/nm.1927

78. Li P, Gan Y, Sun BL, Zhang F, Lu B, Gao Y, et al. Adoptive regulatory T-cell therapy protects against cerebral ischemia. *Ann Neurol.* (2013) 74:458–71. doi: 10.1002/ana.23815

79. Wan YY. Multi-tasking of helper T cells. *Immunology.* (2010) 130:166–71. doi: 10.1111/j.1365-2567.2010.03289.x

80. Yilmaz G, Granger DN. Leukocyte recruitment and ischemic brain injury. *Neuromolecular Med.* (2010) 12:193–204. doi: 10.1007/s12017-009-8074-1

81. Yilmaz G, Arumugam TV, Stokes KY, Granger DN. Role of T lymphocytes and interferon-gamma in ischemic stroke. *Circulation.* (2006) 113:2105–12. doi: 10.1161/CIRCULATIONAHA.105.593046

82. Selvaraj UM, Ujas TA, Kong X, Kumar A, Plautz EJ, Zhang S, et al. Delayed diapedesis of CD8 T cells contributes to long-term pathology after ischemic stroke in male mice. *Brain Behav Immun.* (2021) 95:502–13. doi: 10.1016/j.bbi.2021.05.001

83. Fan L, Zhang CJ, Zhu L, Chen J, Zhang Z, Liu P, et al. FasL-PDPK1 pathway promotes the cytotoxicity of CD8(+) T cells during ischemic stroke. *Transl Stroke Res.* (2020) 11:747–61. doi: 10.1007/s12975-019-00749-0

84. Brea D, Poon C, Murphy M, Lubitz G, Iadecola C, Anrather J. Ablation of nasal-associated lymphoid tissue does not affect focal ischemic brain injury in mice. *PLoS ONE.* (2018) 13:e0205470. doi: 10.1371/journal.pone.0205470

85. Prinz I, Silva-Santos B, Pennington DJ. Functional development of gammadelta T cells. *Eur J Immunol.* (2013) 43:1988–94. doi: 10.1002/eji.201343759

86. Benakis C, Brea D, Caballero S, Faraco G, Moore J, Murphy M, et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal gammadelta T cells. *Nat Med.* (2016) 22:516–23. doi: 10.1038/nm.4068

87. Shichita T, Sugiyama Y, Ooboshi H, Sugimori H, Nakagawa R, Takada I, et al. Pivotal role of cerebral interleukin-17-producing gammadeltaT cells in the delayed phase of ischemic brain injury. *Nat Med.* (2009) 15:946–50. doi: 10.1038/nm.1999

88. Gelderblom M, Weymar A, Bernreuther C, Velden J, Arunachalam P, Steinbach K, et al. Neutralization of the IL-17 axis diminishes neutrophil invasion and protects from ischemic stroke. *Blood.* (2012) 120:3793–802. doi: 10.1182/blood-2012-02-412726

89. Nakajima S, Tanaka R, Yamashiro K, Chiba A, Noto D, Inaba T, et al. Mucosal-associated invariant T cells are involved in acute ischemic stroke by regulating neuroinflammation. *J Am Heart Assoc.* (2021) 10:e018803. doi: 10.1161/JAHA.120.018803

90. Kreutmair S, Unger S, Nunez NG, Ingelfinger F, Alberti C, De Feo D, et al. Distinct immunological signatures discriminate severe COVID-19 from non-SARS-CoV-2-driven critical pneumonia. *Immunity.* (2021) 54:1578–93.e5. doi: 10.1016/j.immuni.2021.05.002

91. Ma C, Han M, Heinrich B, Fu Q, Zhang Q, Sandhu M, et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer *via* NKT cells. *Science.* (2018) 360:eaan5931. doi: 10.1126/science.aan5931

92. Yan J, Mitra A, Hu J, Cutrera JJ, Xia X, Doetschman T, et al. Corrigendum to "Interleukin-30 (IL27p28) alleviates experimental sepsis by modulating cytokine profile in NKT cells". *J Hepatol.* (2016) 64:1128–36. doi: 10.1016/j.jhep.2016.04.028

93. Amoo M, O'Halloran PJ, Henry J, Husien MB, Brennan P, Campbell M, et al. Permeability of the blood-brain barrier after traumatic brain injury; radiological considerations. *J Neurotrauma.* (2021) 39:20–34. doi: 10.1089/neu.2020.7545

94. Gwak MG, Chang SY. Gut-brain connection: microbiome, gut barrier, and environmental sensors. *Immune Netw.* (2021) 21:e20. doi: 10.4110/in.2021.21.e20

95. Llovera G, Benakis C, Enzmann G, Cai R, Arzberger T, Ghasemigharagoz A, et al. The choroid plexus is a key cerebral invasion route for T cells after stroke. *Acta Neuropathol.* (2017) 134:851–68. doi: 10.1007/s00401-017-1758-y

96. Cai W, Shi L, Zhao J, Xu F, Dufort C, Ye Q, et al. Neuroprotection against ischemic stroke requires a specific class of early responder T cells in mice. *J Clin Invest.* (2022) 132:e157678. doi: 10.1172/JCI157678

97. Shichita T, Ito M, Morita R, Komai K, Noguchi Y, Ooboshi H, et al. MAFB prevents excess inflammation after ischemic stroke by accelerating clearance of damage signals through MSR1. *Nat Med.* (2017) 23:723–32. doi: 10.1038/nm.4312

98. Godino Mdel C, Romera VG, Sanchez-Tomero JA, Pacheco J, Canals S, Lerma J, et al. Amelioration of ischemic brain damage by peritoneal dialysis. *J Clin Invest.* (2013) 123:4359–63. doi: 10.1172/JCI67284

99. Zaghmi A, Dopico-Lopez A, Perez-Mato M, Iglesias-Rey R, Hervella P, Greschner AA, et al. Sustained blood glutamate scavenging enhances protection in ischemic stroke. *Commun Biol.* (2020) 3:729. doi: 10.1038/s42003-020-01406-1

100. da Silva-Candal A, Perez-Diaz A, Santamaria M, Correa-Paz C, Rodriguez-Yanez M, Arda A, et al. Clinical validation of blood/brain glutamate grabbing in acute ischemic stroke. *Ann Neurol.* (2018) 84:260–73. doi: 10.1002/ana.25286

101. Sonnewald U, Qu H, Aschner M. Pharmacology and toxicology of astrocyte-neuron glutamate transport and cycling. *J Pharmacol Exp Ther.* (2002) 301:1–6. doi: 10.1124/jpet.301.1.1

102. Nagy Z, Nardai S. Cerebral ischemia/repefusion injury: from bench space to bedside. *Brain Res Bull.* (2017) 134:30–7. doi: 10.1016/j.brainresbull.2017.06.011

103. Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, et al. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature.* (2005) 433:73–7. doi: 10.1038/nature03180

104. Mostafeezur RM, Shinoda M, Unno S, Zakir HM, Takatsuji H, Takahashi K, et al. Involvement of astroglial glutamate-glutamine shuttle in modulation of the jaw-opening reflex following infraorbital nerve injury. *Eur J Neurosci.* (2014) 39:2050–9. doi: 10.1111/ejn.12562

105. Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature.* (2014) 515:431–5. doi: 10.1038/nature13909

106. Dringen R, Hirrlinger J. Glutathione pathways in the brain. *Biol Chem.* (2003) 384:505–16. doi: 10.1515/BC.2003.059

107. Albrecht P, Lewerenz J, Dittmer S, Noack R, Maher P, Methner A. Mechanisms of oxidative glutamate toxicity: the glutamate/cystine antiporter system xc- as a neuroprotective drug target. *CNS Neurol Disord Drug Targets.* (2010) 9:373–82. doi: 10.2174/187152710791292567

108. Phillis JW, Ren J, O'Regan MH. Transporter reversal as a mechanism of glutamate release from the ischemic rat cerebral cortex: studies with DL-threo-beta-benzyloxyaspartate. *Brain Res.* (2000) 868:105–12. doi: 10.1016/S0006-8993(00)02303-9

109. Griffith OW. Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radic Biol Med.* (1999) 27:922–35. doi: 10.1016/S0891-5849(99)00176-8

110. Conrad M, Sato H. The oxidative stress-inducible cystine/glutamate antiporter, system x (c) (-) : cystine supplier and beyond. *Amino Acids.* (2012) 42:231–46. doi: 10.1007/s00726-011-0867-5

111. Moss HG, Brown TR, Wiest DB, Jenkins DD. N-Acetylcysteine rapidly replenishes central nervous system glutathione measured *via* magnetic resonance spectroscopy in human neonates with hypoxic-ischemic encephalopathy. *J Cereb Blood Flow Metab.* (2018) 38:950–8. doi: 10.1177/0271678X18765828

112. Sekhon B, Sekhon C, Khan M, Patel SJ, Singh I, Singh AK. N-Acetyl cysteine protects against injury in a rat model of focal cerebral ischemia. *Brain Res.* (2003) 971:1–8. doi: 10.1016/S0006-8993(03)02244-3

113. Liu Y, Liu WC, Sun Y, Shen X, Wang X, Shu H, et al. Normobaric hyperoxia extends neuro- and vaso-protection of N-acetylcysteine in transient focal ischemia. *Mol Neurobiol.* (2017) 54:3418–27. doi: 10.1007/s12035-016-9932-0

114. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res.* (2018) 46:D1074–82. doi: 10.1093/nar/gkx1037

115. Tymianski M. Emerging mechanisms of disrupted cellular signaling in brain ischemia. *Nat Neurosci.* (2011) 14:1369–73. doi: 10.1038/nn.2951

116. Liu Y, Wong TP, Aarts M, Rooyakkers A, Liu L, Lai TW, et al. NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both *in vitro* and *in vivo*. *J Neurosci.* (2007) 27:2846–57. doi: 10.1523/JNEUROSCI.0116-07.2007

117. Petralia RS, Wang YX, Hua F, Yi Z, Zhou A, Ge L, et al. Organization of NMDA receptors at extrasynaptic locations. *Neuroscience.* (2010) 167:68–87. doi: 10.1016/j.neuroscience.2010.01.022

118. Hardingham GE. Pro-survival signalling from the NMDA receptor. *Biochem Soc Trans.* (2006) 34:936–8. doi: 10.1042/BST0340936

119. Zhang SJ, Zou M, Lu L, Lau D, Ditzel DA, Delucinge-Vivier C, et al. Nuclear calcium signaling controls expression of a large gene pool: identification of a gene program for acquired neuroprotection induced by synaptic activity. *PLoS Genet.* (2009) 5:e1000604. doi: 10.1371/journal.pgen.1000604

120. Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat Neurosci.* (2002) 5:405–14. doi: 10.1038/nn835

121. Ivanov A, Pellegrino C, Rama S, Dumalska I, Salyha Y, Ben-Ari Y, et al. Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons. *J Physiol.* (2006) 572:789–98. doi: 10.1113/jphysiol.2006.105510

122. Xia P, Chen HS, Zhang D, Lipton SA. Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. *J Neurosci.* (2010) 30:11246–50. doi: 10.1523/JNEUROSCI.2488-10.2010

123. Joe E, Ringman JM. Cognitive symptoms of Alzheimer's disease: clinical management and prevention. *BMJ.* (2019) 367:l6217. doi: 10.1136/bmj.l6217

124. McShane R, Westby MJ, Roberts E, Minakaran N, Schneider L, Farrimond LE, et al. Memantine for dementia. *Cochrane Database Syst Rev.* (2019) 3:CD003154. doi: 10.1002/14651858.CD003154.pub6

125. Montagne A, Hebert M, Jullienne A, Lesept F, Le Behot A, Louessard M, et al. Memantine improves safety of thrombolysis for stroke. *Stroke.* (2012) 43:2774–81. doi: 10.1161/STROKEAHA.112.669374

126. Culmsee C, Junker V, Kremers W, Thal S, Plesnila N, Krieglstein J. Combination therapy in ischemic stroke: synergistic neuroprotective effects of memantine and clenbuterol. *Stroke.* (2004) 35:1197–202. doi: 10.1161/01.STR.0000125855.17686.6d

127. Trotman M, Vermehren P, Gibson CL, Fern R. The dichotomy of memantine treatment for ischemic stroke: dose-dependent protective

and detrimental effects. *J Cereb Blood Flow Metab.* (2015) 35:230–9. doi: 10.1038/jcbfm.2014.188

128. Tajima N, Karakas E, Grant T, Simorowski N, Diaz-Avalos R, Grigorieff N, et al. Activation of NMDA receptors and the mechanism of inhibition by ifenprodil. *Nature.* (2016) 534:63–8. doi: 10.1038/nature17679

129. Zhu S, Paoletti P. Allosteric modulators of NMDA receptors: multiple sites and mechanisms. *Curr Opin Pharmacol.* (2015) 20:14–23. doi: 10.1016/j.coph.2014.10.009

130. Christopherson KS, Hillier BJ, Lim WA, Bredt DS. PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. *J Biol Chem.* (1999) 274:27467–73. doi: 10.1074/jbc.274.39.27467

131. Sattler R, Xiong Z, Lu WY, Hafner M, MacDonald JF, Tymianski M. Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science.* (1999) 284:1845–8. doi: 10.1126/science.284.5421.1845

132. Soriano FX, Martel MA, Papadia S, Vaslin A, Baxter P, Rickman C, et al. Specific targeting of pro-death NMDA receptor signals with differing reliance on the NR2B PDZ ligand. *J Neurosci.* (2008) 28:10696–710. doi: 10.1523/JNEUROSCI.1207-08.2008

133. Zong P, Feng J, Yue Z, Li Y, Wu G, Sun B, et al. Functional coupling of TRPM2 and extrasynaptic NMDARs exacerbates excitotoxicity in ischemic brain injury. *Neuron*. (2022) 110:1944–58.e8. doi: 10.1016/j.neuron.2022.03.021

134. Nishi T, Maier CM, Hayashi T, Saito A, Chan PH. Superoxide dismutase 1 overexpression reduces MCP-1 and MIP-1 alpha expression after transient focal cerebral ischemia. *J Cereb Blood Flow Metab.* (2005) 25:1312–24. doi: 10.1038/sj.jcbfm.9600124

135. Beckman JS. Oxidative damage and tyrosine nitration from peroxynitrite. *Chem Res Toxicol.* (1996) 9:836–44. doi: 10.1021/tx9501445

136. del Zoppo GJ, Mabuchi T. Cerebral microvessel responses to focal ischemia. *J Cereb Blood Flow Metab.* (2003) 23:879–94. doi: 10.1097/01.WCB.0000078322.96027.78

137. Squadrito GL, Cueto R, Splenser AE, Valavanidis A, Zhang H, Uppu RM, et al. Reaction of uric acid with peroxynitrite and implications for the mechanism of neuroprotection by uric acid. *Arch Biochem Biophys.* (2000) 376:333–7. doi: 10.1006/abbi.2000.1721

138. Romanos E, Planas AM, Amaro S, Chamorro A. Uric acid reduces brain damage and improves the benefits of rt-PA in a rat model of thromboembolic stroke. *J Cereb Blood Flow Metab.* (2007) 27:14–20. doi: 10.1038/sj.jcbfm.9600312

139. Onetti Y, Dantas AP, Perez B, Cugota R, Chamorro A, Planas AM, et al. Middle cerebral artery remodeling following transient brain ischemia is linked to early postischemic hyperemia: a target of uric acid treatment. *Am J Physiol Heart Circ Physiol.* (2015) 308:H862–74. doi: 10.1152/ajpheart.00001.2015

140. Culotta VC, Yang M, O'Halloran TV. Activation of superoxide dismutases: putting the metal to the pedal. *Biochim Biophys Acta.* (2006) 1763:747–58. doi: 10.1016/j.bbamcr.2006.05.003

141. Ning Y, Huo Y, Xue H, Du Y, Yao Y, Sedgwick AC, et al. Tri-Manganese(III) salen-based cryptands: a metal cooperative antioxidant strategy that overcomes ischemic stroke damage *in vivo*. *J Am Chem Soc.* (2020) 142:10219–27. doi: 10.1021/jacs.0c03805

142. Yang K, Zeng L, Yuan X, Wang S, Ge A, Xu H, et al. The mechanism of ferroptosis regulating oxidative stress in ischemic stroke and the regulation mechanism of natural pharmacological active components. *Biomed Pharmacother.* (2022) 154:113611. doi: 10.1016/j.biopha.2022.113611

143. Alim I, Caulfield JT, Chen Y, Swarup V, Geschwind DH, Ivanova E, et al. Selenium drives a transcriptional adaptive program to block ferroptosis and treat stroke. *Cell*. (2019) 177:1262–79.e25. doi: 10.1016/j.cell.2019.03.032

144. Ling L, Alattar A, Tan Z, Shah FA, Ali T, Alshaman R, et al. A potent antioxidant endogenous neurohormone melatonin, rescued MCAO by attenuating oxidative stress-associated neuroinflammation. *Front Pharmacol.* (2020) 11:1220. doi: 10.3389/fphar.2020.01220

145. Hoye AT, Davoren JE, Wipf P, Fink MP, Kagan VE. Targeting mitochondria. *Acc Chem Res.* (2008) 41:87–97. doi: 10.1021/ar700135m

146. Zhou Y, Liao J, Mei Z, Liu X, Ge J. Insight into crosstalk between ferroptosis and necroptosis: novel therapeutics in ischemic stroke. *Oxid Med Cell Longev.* (2021) 2021:9991001. doi: 10.1155/2021/9991001

147. Narang A, Qiao F, Atkinson C, Zhu H, Yang X, Kulik L, et al. Natural IgM antibodies that bind neoepitopes exposed as a result of spinal cord injury, drive secondary injury by activating complement. *J Neuroinflammation.* (2017) 14:120. doi: 10.1186/s12974-017-0894-6

148. Elvington A, Atkinson C, Kulik L, Zhu H, Yu J, Kindy MS, et al. Pathogenic natural antibodies propagate cerebral injury following ischemic stroke in mice. *J Immunol.* (2012) 188:1460–8. doi: 10.4049/jimmunol.1102132

149. Alawieh A, Langley EF, Tomlinson S. Targeted complement inhibition salvages stressed neurons and inhibits neuroinflammation after stroke in mice. *Sci Transl Med*. (2018) 10:eaao6459. doi: 10.1126/scitranslmed.aao6459

150. Alawieh AM, Langley EF, Feng W, Spiotta AM, Tomlinson S. Complement-dependent synaptic uptake and cognitive decline after stroke and reperfusion therapy. *J Neurosci.* (2020) 40:4042–58. doi: 10.1523/JNEUROSCI.2462-19.2020

151. Li Y, Lin F. Decoy nanoparticles bearing native C5a receptors as a new approach to inhibit complement-mediated neutrophil activation. *Acta Biomater.* (2019) 99:330–8. doi: 10.1016/j.actbio.2019.08.033

152. Li J, Liu B. The roles and potential therapeutic implications of C5a in the pathogenesis of COVID-19-associated coagulopathy. *Cytokine Growth Factor Rev.* (2021) 58:75–81. doi: 10.1016/j.cytogfr.2020.12.001

153. Zha H, Han X, Zhu Y, Yang F, Li Y, Li Q, et al. Blocking C5aR signaling promotes the anti-tumor efficacy of PD-1/PD-L1 blockade. *Oncoimmunology.* (2017) 6:e1349587. doi: 10.1080/2162402X.2017.1349587

154. Mavroidis M, Davos CH, Psarras S, Varela A, C Athanasiadis N, Katsimpoulas M, et al. Complement system modulation as a target for treatment of arrhythmogenic cardiomyopathy. *Basic Res Cardiol.* (2015) 110:27. doi: 10.1007/s00395-015-0485-6

155. Xu GL, Chen J, Yang F, Li GQ, Zheng LX, Wu YZ. C5a/C5aR pathway is essential for the pathogenesis of murine viral fulminant hepatitis by way of potentiating Fgl2/fibroleukin expression. *Hepatology.* (2014) 60:114–24. doi: 10.1002/hep.27114

156. Orsini F, Villa P, Parrella S, Zangari R, Zanier ER, Gesuete R, et al. Targeting mannose-binding lectin confers long-lasting protection with a surprisingly wide therapeutic window in cerebral ischemia. *Circulation.* (2012) 126:1484–94. doi: 10.1161/CIRCULATIONAHA.112.103051

157. Mercurio D, Piotti A, Valente A, Oggioni M, Ponstein Y, Van Amersfoort E, et al. Plasma-derived and recombinant C1 esterase inhibitor: binding profiles and neuroprotective properties in brain ischemia/reperfusion injury. *Brain Behav Immun.* (2021) 93:299–311. doi: 10.1016/j.bbi.2021.01.002

158. Liesz A, Zhou W, Mracsko E, Karcher S, Bauer H, Schwarting S, et al. Inhibition of lymphocyte trafficking shields the brain against deleterious neuroinflammation after stroke. *Brain.* (2011) 134:704–20. doi: 10.1093/brain/awr008

159. Lu Y, Zhou M, Li Y, Li Y, Hua Y, Fan Y. Minocycline promotes functional recovery in ischemic stroke by modulating microglia polarization through STAT1/STAT6 pathways. *Biochem Pharmacol.* (2021) 186:114464. doi: 10.1016/j.bcp.2021.114464

160. Abdul Y, Abdelsaid M, Li W, Webb RC, Sullivan JC, Dong G, et al. Inhibition of toll-like receptor-4 (TLR-4) improves neurobehavioral outcomes after acute ischemic stroke in diabetic rats: possible role of vascular endothelial TLR-4. *Mol Neurobiol.* (2019) 56:1607–17. doi: 10.1007/s12035-018-1184-8

161. Wang Y, Ge P, Zhu Y. TLR2 and TLR4 in the brain injury caused by cerebral ischemia and reperfusion. *Mediators Inflamm.* (2013) 2013:124614. doi: 10.1155/2013/124614

162. Zhai Y, Zhu Y, Liu J, Xie K, Yu J, Yu L, et al. Dexmedetomidine post-conditioning alleviates cerebral ischemia-reperfusion injury in rats by inhibiting high mobility group protein B1 group (HMGB1)/toll-like receptor 4 (TLR4)/nuclear factor kappa B (NF-kappaB) Signaling Pathway. *Med Sci Monit.* (2020) 26:e918617. doi: 10.12659/MSM.918617

163. Chen Y, Brennan-Minnella AM, Sheth S, El-Benna J, Swanson RA. Tat-NR2B9c prevents excitotoxic neuronal superoxide production. *J Cereb Blood Flow Metab.* (2015) 35:739–42. doi: 10.1038/jcbfm.2015.16

164. Zhou L, Li F, Xu HB, Luo CX, Wu HY, Zhu MM, et al. Treatment of cerebral ischemia by disrupting ischemia-induced interaction of nNOS with PSD-95. *Nat Med.* (2010) 16:1439–43. doi: 10.1038/nm.2245

165. Lai TW, Zhang S, Wang YT. Excitotoxicity and stroke: identifying novel targets for neuroprotection. *Prog Neurobiol.* (2014) 115:157–88. doi: 10.1016/j.pneurobio.2013.11.006

166. Xiong Z, Chang L, Qu Y, Pu Y, Wang S, Fujita Y, et al. Neuronal brain injury after cerebral ischemic stroke is ameliorated after subsequent administration of (R)-ketamine, but not (S)-ketamine. *Pharmacol Biochem Behav.* (2020) 191:172904. doi: 10.1016/j.pbb.2020.172904

167. Huang B, Chen P, Huang L, Li S, Zhu R, Sheng T, et al. Geniposide attenuates post-ischaemic neurovascular damage *via* GluN2A/AKT/ERK-dependent mechanism. *Cell Physiol Biochem.* (2017) 43:705–16. doi: 10.1159/000480657

168. Liu Y, Fu X, Liu Y, Zhang T, Cui P, Wang S, et al. Neuroprotective effect of pseudoginsenoside-F11 on permanent cerebral ischemia in rats by regulating

calpain activity and NR2A submit-mediated AKT-CREB signaling pathways. *Phytomedicine*. (2022) 96:153847. doi: 10.1016/j.phymed.2021.153847

169. Wang XX, Wang F, Mao GH, Wu JC, Li M, Han R, et al. NADPH is superior to NADH or edaravone in ameliorating metabolic disturbance and brain injury in ischemic stroke. *Acta Pharmacol Sin*. (2022) 43:529–40. doi: 10.1038/s41401-021-00705-5

170. Haberman F, Tang SC, Arumugam TV, Hyun DH, Yu QS, Cutler RG, et al. Soluble neuroprotective antioxidant uric acid analogs ameliorate ischemic brain injury in mice. *Neuromolecular Med*. (2007) 9:315–23. doi: 10.1007/s12017-007-8010-1

171. Zhi SM, Fang GX, Xie XM, Liu LH, Yan J, Liu DB, et al. Melatonin reduces OGD/R-induced neuron injury by regulating redox/inflammation/apoptosis signaling. *Eur Rev Med Pharmacol Sci*. (2020) 24:1524–36.

172. Hou Y, Wang Y, He Q, Li L, Xie H, Zhao Y, et al. Nrf2 inhibits NLRP3 inflammasome activation through regulating Trx1/TXNIP complex in cerebral ischemia reperfusion injury. *Behav Brain Res*. (2018) 336:32–9. doi: 10.1016/j.bbr.2017.06.027

173. Chang G, Chen Y, Zhang H, Zhou W. Trans sodium crocetinate alleviates ischemia/reperfusion-induced myocardial oxidative stress and apoptosis *via* the SIRT3/FOXO3a/SOD2 signaling pathway. *Int Immunopharmacol*. (2019) 71:361–71. doi: 10.1016/j.intimp.2019.03.056

174. Zhao B, Sun LK, Jiang X, Zhang Y, Kang J, Meng H, et al. Genipin protects against cerebral ischemia-reperfusion injury by regulating the UCP2-SIRT3 signaling pathway. *Eur J Pharmacol*. (2019) 845:56–64. doi: 10.1016/j.ejphar.2018.12.028

175. De Ceunynck K, De Meyer SF, Vanhoorelbeke K. Unwinding the von Willebrand factor strings puzzle. *Blood*. (2013) 121:270–7. doi: 10.1182/blood-2012-07-442285

176. De Meyer SF, Denorme F, Langhauser F, Geuss E, Fluri F, Kleinschnitz C. Thromboinflammation in stroke brain damage. *Stroke*. (2016) 47:1165–72. doi: 10.1161/STROKEAHA.115.011238

177. Sonneveld MA, de Maat MP, Portegies ML, Kavousi M, Hofman A, Turecek PL, et al. Low ADAMTS13 activity is associated with an increased risk of ischemic stroke. *Blood*. (2015) 126:2739–46. doi: 10.1182/blood-2015-05-643338

178. Kim SJ, Jenne CN. Role of platelets in neutrophil extracellular trap (NET) production and tissue injury. *Semin Immunol*. (2016) 28:546–54. doi: 10.1016/j.smim.2016.10.013

179. Laridan E, Denorme F, Desender L, Francois O, Andersson T, Deckmyn H, et al. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann Neurol*. (2017) 82:223–32. doi: 10.1002/ana.24993

180. Amaro S, Laredo C, Renu A, Llull L, Rudilosso S, Obach V, et al. Uric acid therapy prevents early ischemic stroke progression: a tertiary analysis of the URICO-ICTUS trial (efficacy study of combined treatment with uric acid and r-tPA in acute ischemic stroke). *Stroke*. (2016) 47:2874–6. doi: 10.1161/STROKEAHA.116.014672

181. Pardridge WM. Blood-brain barrier drug targeting: the future of brain drug development. *Mol Interv*. (2003) 3:90–105.51. doi: 10.1124/mi.3.2.90

182. D'Souza A, Dave KM, Stetler RA, S Manickam D. Targeting the blood-brain barrier for the delivery of stroke therapies. *Adv Drug Deliv Rev*. (2021) 171:332–51. doi: 10.1016/j.addr.2021.01.015

183. Patel T, Zhou J, Piepmeier JM, Saltzman WM. Polymeric nanoparticles for drug delivery to the central nervous system. *Adv Drug Deliv Rev*. (2012) 64:701–5. doi: 10.1016/j.addr.2011.12.006

184. He W, Zhang Z, Sha X. Nanoparticles-mediated emerging approaches for effective treatment of ischemic stroke. *Biomaterials*. (2021) 277:121111. doi: 10.1016/j.biomaterials.2021.121111

185. Gabathuler R. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. *Neurobiol Dis*. (2010) 37:48–57. doi: 10.1016/j.nbd.2009.07.028

186. Wohlfart S, Gelperina S, Kreuter J. Transport of drugs across the blood-brain barrier by nanoparticles. *J Control Release*. (2012) 161:264–73. doi: 10.1016/j.jconrel.2011.08.017

187. Rak J. Extracellular vesicles - biomarkers and effectors of the cellular interactome in cancer. *Front Pharmacol*. (2013) 4:21. doi: 10.3389/fphar.2013.00021

188. Lener T, Gimona M, Aigner L, Borger V, Buzas E, Camussi G, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. *J Extracell Vesicles*. (2015) 4:30087. doi: 10.3402/jev.v4.30087

189. Zhang ZG, Buller B, Chopp M. Exosomes - beyond stem cells for restorative therapy in stroke and neurological injury. *Nat Rev Neurol*. (2019) 15:193–203. doi: 10.1038/s41582-018-0126-4

190. Hwang DW, Choi H, Jang SC, Yoo MY, Park JY, Choi NE, et al. Noninvasive imaging of radiolabeled exosome-mimetic nanovesicle using (99m)Tc-HMPAO. *Sci Rep*. (2015) 5:15636. doi: 10.1038/srep15636

191. Webb RL, Kaiser EE, Scoville SL, Thompson TA, Fatima S, Pandya C, et al. Human neural stem cell extracellular vesicles improve tissue and functional recovery in the murine thromboembolic stroke model. *Transl Stroke Res*. (2018) 9:530–9. doi: 10.1007/s12975-017-0599-2

192. Terstappen GC, Meyer AH, Bell RD, Zhang W. Strategies for delivering therapeutics across the blood-brain barrier. *Nat Rev Drug Discov*. (2021) 20:362–83. doi: 10.1038/s41573-021-00139-y

193. Hossmann KA. The two pathophysiologies of focal brain ischemia: implications for translational stroke research. *J Cereb Blood Flow Metab*. (2012) 32:1310–6. doi: 10.1038/jcbfm.2011.186

194. Mastellos DC, Ricklin D, Lambris JD. Clinical promise of next-generation complement therapeutics. *Nat Rev Drug Discov*. (2019) 18:707–29. doi: 10.1038/s41573-019-0031-6

195. Chong WP, Mattapallil MJ, Raychaudhuri K, Bing SJ, Wu S, Zhong Y, et al. The cytokine IL-17A limits Th17 pathogenicity *via* a negative feedback loop driven by autocrine induction of IL-24. *Immunity*. (2020) 53:384–97.e5. doi: 10.1016/j.immuni.2020.06.022

196. Lee JY, Hall JA, Kroehling L, Wu L, Najar T, Nguyen HH, et al. Serum amyloid A proteins induce pathogenic Th17 cells and promote inflammatory disease. *Cell*. (2020) 183:2036–9. doi: 10.1016/j.cell.2020.12.008

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No sex difference was found in the safety and efficacy of intravenous alteplase before endovascular therapy

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Background and purpose: Prior studies on sex disparities were *post-hoc* analyses, had limited treatment modalities, and had controversial findings. Our study aimed to examine whether sex difference modifies the effect of intravenous alteplase before endovascular therapy.

Methods: We conducted a multicenter prospective cohort study of 850 eligible patients with acute ischemic stroke who underwent endovascular therapy. A propensity score was utilized as a covariate to achieve approximate randomization of alteplase pretreatment. The baseline characteristics of women and men were compared. Logistic regression with interaction terms, adjusted for potential confounders, was used to investigate the effect of sex on the prognosis of bridging therapy.

Results: In comparison to men, women were older [78.00 (70.00–84.00) vs. 67 (61.00–74.00), $P < 0.001$], had more atrial fibrillation (61.4 vs. 35.2%, $P < 0.001$), had a lower ASPECTS [10.00 (8.00–10.00) vs. 10 (9.00–10.00), $P = 0.0047$], and had a higher NIHSS score [17.00 (14.00–20.00) vs. 16 (13.00–19.00), $P = 0.005$]. Women tended to receive less bridging therapy (26.3 vs. 33%, $P = 0.043$) and more retrieval attempts [2.00 (1.00–2.00) vs. 1 (1.00–2.00), $P = 0.026$]. There was no sex difference in functional independence at 90 days after bridging therapy (OR 0.968, 95% CI 0.575–1.63), whereas men benefited more after EVT alone (OR 0.654, 95% CI 0.456–0.937). There were no sex-treatment interactions observed regardless of the location of the occlusion. There were no significant sex differences in all safety outcomes.

Conclusion: Our study could not confirm that sex modifies the treatment effect of intravenous alteplase before endovascular therapy. At the same time, we advocate for women to seek timely medical treatment.

KEYWORDS

acute ischemic stroke, endovascular therapy (EVT), functional status, intravenous alteplase, sex characteristics

Introduction

The safety and efficacy of intravenous alteplase before endovascular therapy (EVT) for patients with acute ischemic stroke have been debated (1–3). Previous studies demonstrated that bridging therapy (intravenous alteplase before EVT) was beneficial for patients undergoing endovascular therapy for a large vessel occlusion in the anterior circulation (4, 5). However, intravenous alteplase before EVT may delay the time to initiate EVT and increase the risk of hemorrhagic complications. The DEVT randomized clinical trial (RCT) (6) and the DIRECT-MT clinical trial (7) showed that endovascular thrombectomy alone was non-inferior to bridging therapy, while the SKIP randomized clinical trial (8) and MR CLEAN-NO IV (9) did not. A meta-analysis of three randomized controlled trials found no differences in functional independence of IV thrombolysis-eligible patients with an acute large vascular occlusion undergoing direct EVT compared to bridging therapy (10). Meanwhile, a series of studies attempted to identify differences in outcomes after bridging therapy among different subgroups of patients based on their baseline characteristics, including the location of the occlusion, the volume of the ischemic score, and the National Institutes of Health Stroke Scale (NIHSS) score on admission (11–14). However, studies on the prognostic impact of patient background factors on patients after bridging treatment remain scarce.

Sex is an unchangeable risk factor for stroke. Sex differences in the incidence and development of stroke have been confirmed by research. Women have a higher incidence of stroke because of a longer life expectancy and an older age at the onset of stroke (15). Various pregnancy complications and oophorectomy increase the risk of stroke in women (16). The more severe the degree of stroke, the higher the incidence of atrial fibrillation, and more pre-stroke functional limitations lead to a higher rate of mortality in women (17). Sex differences in clinical outcomes in patients with acute ischemic stroke after endovascular treatment (EVT) have also been discussed in a great number of studies. Some of them suggested that women suffered from poor functional outcomes and were more likely to die, whereas others came up with the opposite conclusion or considered sex to be non-influential on clinical outcomes after EVT (18–22). The explanation for studies with contradictory results might be selection, evident in different baseline characteristics (23, 24). In addition, different confounding variables were included, resulting in inadequate corrections.

Whether sex difference affects the safety and efficacy of intravenous alteplase before EVT has not been discussed. In this prospective cohort study, we studied the effect of sex differences on the prognosis of EVT alone and bridging therapy separately. In addition, the interaction between sex and these treatment modalities was analyzed. Based on the location of the occlusion in the anterior and posterior circulation, a subgroup analysis was performed. We hypothesize that sex might not affect the prognosis of adjunctive alteplase therapy.

Methods

Standard protocol approvals, registrations, and patient consent

We conducted a prospective cohort study of patients with AIS who underwent EVT at 3 comprehensive stroke centers in China between January 2017 and September 2019. The study was registered and approved by the local institutional review board. Written informed consent was obtained from all patients.

Sample size analysis

A power analysis was performed. Prior frequencies of sex and clinical characteristics were estimated from 95 patients enrolled in Shanghai Tenth People's Hospital between 2017 and 2018. The threshold for a significant level was set at 0.05. Sample size and corresponding power were estimated using the chi-square test function in a pwr R-package. The study had more than 75% power for the primary outcome (Supplementary Figure S1).

Patient selection

The patients' inclusion criteria were (1) adult patients (age $\geq$ 18 years old) diagnosed with AIS, (2) time to hospital from clinical onset within 24 h, (3) NIHSS score on admission $\geq$6, (4) occlusion in intracranial arteries including the anterior and posterior circulation according to digital subtraction angiography (DSA), (5) Alberta stroke program early CT score (ASPECTS) or posterior circulation Alberta stroke program early CT score (pc-ASPECTS) on admission $\geq$6, and (6) clinical outcome follow-up reports available at 3 months. Patients were excluded from the study if they were pregnant or had a myocardial infarction (within 1 month before the study), severe liver or kidney disease, malignant tumors, and blood diseases.

Variables of interest and outcomes

We recorded the demographic and clinical characteristics of patients at the time of admission. Baseline characteristics included age; sex; blood pressure on admission; smoking and drinking habits; history of taking statins, anticoagulants, and antiplatelet drugs; CHA2DS2-VASc score, modified Rankin scale (mRS) score, NIHSS score, and ASPECTS or pc-ASPECTS on admission; the location of the occlusion; and the TOAST type

of stroke. We included coronary heart disease, hypertension, diabetes, atrial fibrillation, and a history of ischemic stroke as comorbidities. Key factors during the treatment process, such as pretreatment with IVT, the number of retrieval attempts, and the EVT modality, were also considered. The time from onset to perform CT (computed tomography) examination, onset to groin puncture, groin puncture to recanalization, onset to recanalization, and performing CT examinations to recanalization were recorded. Furthermore, laboratory data, including platelet count, volume on admission, and neutrophil-lymphocyte ratio (NLR) on admission, were collected.

The primary outcome was functional independence defined as mRS $\leq$2 at 3 months after EVT. The secondary outcomes included successful reperfusion, defined as final modified thrombolysis in cerebral infarction (mTICI) of 2b to 3, and early neurological improvement, defined as a $\geq$4 point

TABLE 1 Baseline characteristics of male and female patients.

Baseline characteristics	Male $n = 500$	Female $n = 350$	p
Bridging Therapy (%)	165 (33.0)	92 (26.3)	0.043
Age, years, [median (IQR)]	67.00 [61.00, 74.00]	78.00 [70.00, 84.00]	<0.001
Hypertension (%)	337 (67.4)	241 (68.9)	0.709
Diabetes (%)	138 (27.6)	75 (21.4)	0.05
Atrial fibrillation (%)	176 (35.2)	215 (61.4)	<0.001
History of stroke or TIA (%)	97 (19.4)	63 (18.0)	0.671
History of coronary artery disease (%)	78 (15.6)	52 (14.9)	0.842
Current smoking (%)	99 (19.8)	2 (0.6)	<0.001
Current drinking (%)	48 (9.6)	1 (0.3)	<0.001
Previous use of antiplatelet (%)	39 (7.8)	44 (12.6)	0.029
Previous use of anticoagulant (%)	22 (4.4)	27 (7.7)	0.06
Previous use of statins (%)	18 (3.6)	21 (6.0)	0.141
CHA2DS2-VASc score on admission [median (IQR)]	2.00 [1.00, 3.00]	4.00 [2.00, 5.00]	<0.001
mRS on admission [median (IQR)]	5.00 [5.00, 5.00]	5.00 [5.00, 5.00]	0.259
ASPECT or pc-ASPECT on admission [median (IQR)]	10.00 [9.00, 10.00]	10.00 [8.00, 10.00]	0.047
NIHSS score on admission [median (IQR)]	16.00 [13.00, 19.00]	17.00 [14.00, 20.00]	0.005
Systolic blood pressure on admission mmHg [median (IQR)]	143.00 [131.00, 159.00]	144.00 [131.00, 159.00]	0.619
Diastolic blood pressure on admission mmHg [median (IQR)]	85.00 [76.00, 90.00]	82.00 [73.00, 90.00]	0.077
Platelet number on admission [median (IQR)]	176.00 [149.00, 219.25]	185.50 [150.25, 223.00]	0.485
Platelet volume on admission [median (IQR)]	10.70 [10.00, 11.40]	10.70 [10.10, 11.50]	0.289
Neutrophil to lymphocyte ratio (NLR) on admission [median (IQR)]	7.80 [4.12, 13.60]	7.60 [4.03, 13.40]	0.529
Anterior circulation occlusion (%)	0.83 (0.38)	0.92 (0.27)	<0.001
Toast classification (%)			<0.001
Large-artery atherosclerosis	252 (50.4)	106 (30.3)	
Cardioembolism	190 (38.0)	223 (63.7)	
Stroke of other determined etiology	20 (4.0)	1 (0.3)	
Stroke of undetermined etiology	38 (7.6)	20 (5.7)	
Time from onset to perform CT, min, (median [IQR])	110.00 [72.00, 184.00]	111.00 [70.00, 180.00]	0.969
Time from onset to groin puncture, min, (median [IQR])	234.50 [180.00, 300.00]	235.00 [163.25, 300.00]	0.457
Time from groin puncture to recanalization, min, (median [IQR])	58.50 [40.00, 78.25]	58.50 [40.00, 80.00]	0.512
Time from performing CT to recanalization, min, (median [IQR])	90.00 [58.50, 131.50]	91.00 [60.00, 127.75]	0.985
Time from onset to recanalization, min, (median [IQR])	302.00 [230.00, 373.25]	301.50 [220.00, 372.00]	0.65
Endovascular treatment modality (%)			<0.001
Arterial thrombolysis	8 (1.6)	3 (0.9)	
Direct mechanical thrombectomy	349 (69.9)	298 (85.1)	
Angioplasty and stenting	106 (21.2)	33 (9.4)	
Angioplasty without thrombectomy	36 (7.2)	16 (4.6)	
Retrieval attempts (median [IQR])	1.00 [1.00, 2.00]	2.00 [1.00, 2.00]	0.026

decrease in NIHSS score 24 h after EVT compared with the NIHSS score on admission. Safety outcomes were intracranial hemorrhage and symptomatic intracerebral hemorrhage (sICH) during the hospitalization and death within 90 days after the treatment. Symptomatic intracerebral hemorrhage was defined as new intracranial hemorrhage detected by brain imaging and associated with ≥4 points in total NIHSS at the time of diagnosis compared to immediately before worsening or ≥2 points in one NIHSS category or the need for major medical/surgical intervention or the absence of an alternative explanation for deterioration (25).

Statistical analysis

In terms of statistical description, categorical variables were presented as numbers and percentages, whereas continuous variables were expressed as either mean and SD or median and interquartile range.

The missing values of baseline variables were attributed to multiple imputations. Baseline data were presented according to sex (Table 1). The probability of each patient undergoing bridging therapy (the propensity score), which was utilized to achieve approximate randomization of alteplase pretreatment,

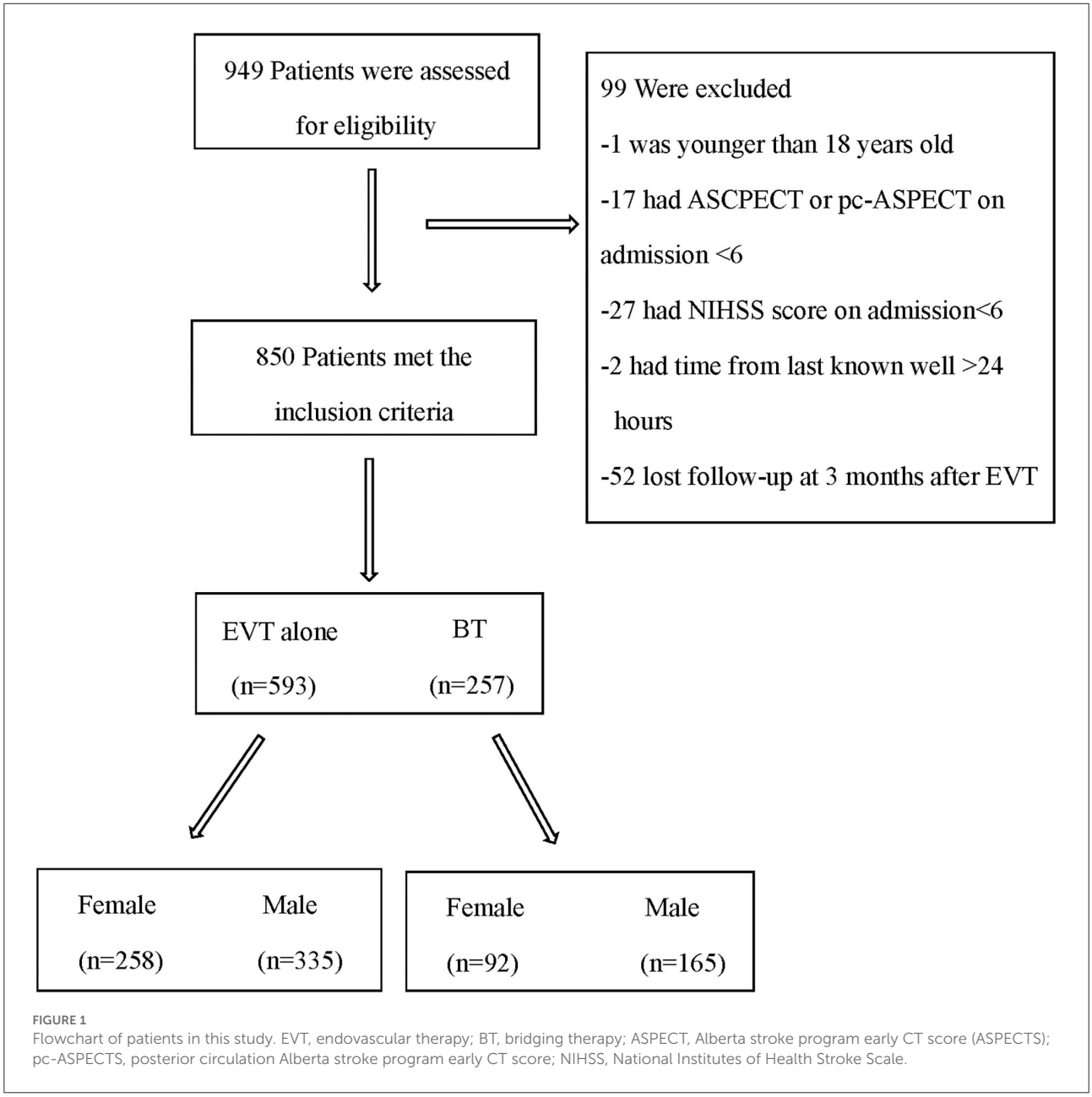


FIGURE 1
Flowchart of patients in this study. EVT, endovascular therapy; BT, bridging therapy; ASPECT, Alberta stroke program early CT score (ASPECTS); pc-ASPECTS, posterior circulation Alberta stroke program early CT score; NIHSS, National Institutes of Health Stroke Scale.

was obtained from a logistic regression model. Selected covariates were age; sex; blood pressure on admission; smoking and drinking habits; history of taking statins, anticoagulants, and antiplatelet drugs; the CHA2DS2-VASc score; modified Rankin scale (mRS) score; the NIHSS score; ASPECTS or pc-ASPECTS on admission; the history of coronary heart disease, hypertension, diabetes, atrial fibrillation, and ischemic stroke; the location of the occlusion; the TOAST type of stroke; time from onset to perform CT (computed tomography) examination; platelet count; platelet volume; and NLR on admission (Supplementary Table S1). To assess the association of sex and primary and secondary outcomes, logistic regression models, which were adjusted for age, diabetes, history of atrial fibrillation and taking antiplatelet drugs, smoking and drinking habits, CHA2DS2-VASc score, NIHSS score, and ASPECTS or pc-ASPECTS on admission, the location of the occlusion, the TOAST type of stroke, the endovascular treatment modality, and the retrieval attempts during EVT, were used. The calculated propensity scores were included in the adjusted models to achieve approximate randomization of alteplase pretreatment. We also investigated the interaction of sex and treatments by adding multiplicative interaction terms into adjusted logistic regression models. For safety outcomes, the chi-square test or the Fisher exact test was used as appropriate. For the report, ORs with 95% CIs were used, and a p-value of <0.05 was considered statistically significant. For subgroup analysis, we divided the patients into two groups based on the location of the occlusion. All analyses were performed using the R statistics program (version 4.1.1, R Core Team 2021, Vienna, Austria).

Results

Baseline characteristics

During the study period, 949 patients from three centers were screened for eligibility, and 850 patients were selected (Figure 1). Baseline characteristics are presented in Table 1. According to our statistics, women were less likely to receive bridging therapy (26.3 vs. 33%, $p = 0.043$). Women were at a higher risk of stroke according to the CHA2DS2-VASc score on admission [4.00 (2.00–5.00) vs. 2 (1.00–3.00), $P < 0.001$] as they were older [78.00 (70.00–84.00) vs. 67 (61.00–74.00), $P < 0.001$] and had more atrial fibrillation (61.4 vs. 35.2%, $P < 0.001$). In addition, women had a lower ASPECTS on admission [10.00 (8.00–10.00) vs. 10 (9.00–10.00), $P = 0.0047$], had a higher NIHSS score [17.00 (14.00–20.00) vs. 16 (13.00–19.00), $P = 0.005$] on admission, and received more retrieval attempts [2.00 (1.00–2.00) vs. 1 (1.00–2.00), $P = 0.026$] than men.

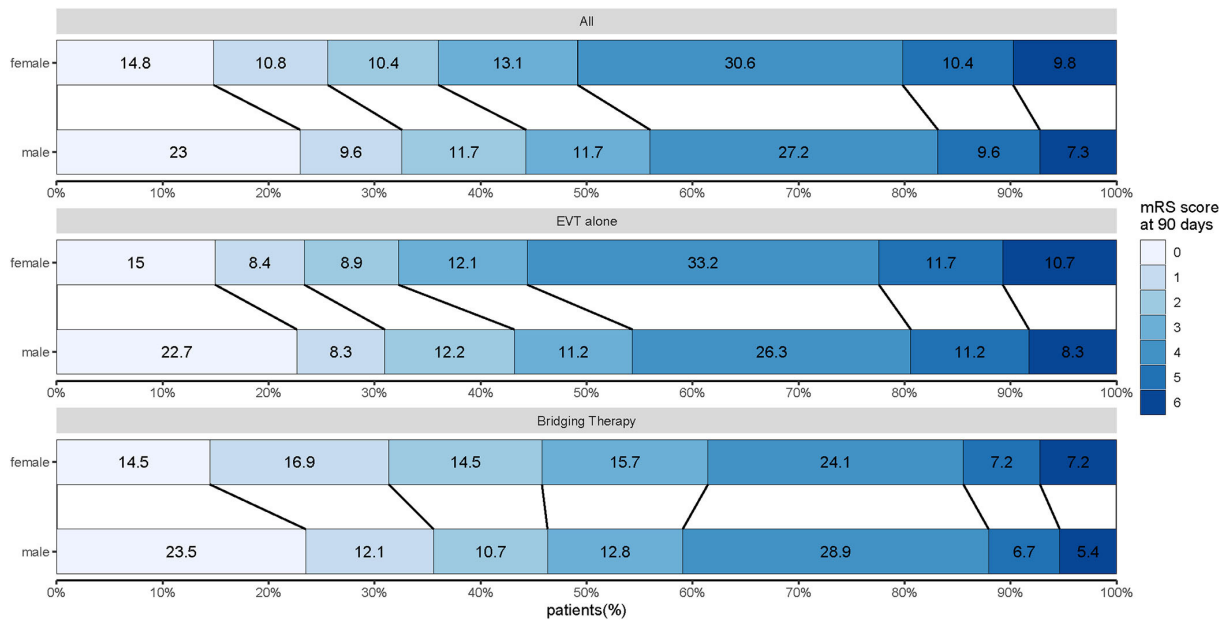


FIGURE 2
Distribution of modified Rankin scale scores at 90 days for women and men treated with EVT alone and bridging therapy. Scores range from 0 to 6, with 0 indicating no symptoms, 1 indicating no clinically significant disability, 2 indicating slight disability (patient can function without assistance but cannot carry out all previous activities), 3 indicating moderate disability (patient requires some help but can walk unassisted), 4 indicating moderately severe disability (patient cannot attend to bodily needs without assistance and cannot walk unassisted), 5 indicating severe disability (patient requires constant nursing care and attention), and 6 indicating death.

Effect of sex difference on the primary outcomes

In patients treated with bridging therapy, the functional status of 90 days was not impacted by sex (OR 0.968, 95% CI 0.575–1.63). However, in patients treated with EVT alone, there was a significant association between sex and functional independence at 90 days (OR 0.654, 95% CI 0.456–0.937). While there was insufficient statistical evidence to support an interaction between sex and these two treatments ($P_{interaction} = 0.226$) (Figure 2; Table 2), we found differences in the effect of sex on EVT alone and bridging therapy for the primary outcomes.

Effect of sex difference on the secondary outcomes

In patients treated with EVT alone or bridging therapy, the success rate of reperfusion was similar in both men and women (EVT alone: OR 0.723, 95% CI 0.453–1.153; bridging therapy: OR 1.087, 95% CI 0.512–2.307). There was no significant interaction between sex and treatments ($P_{interaction} = 0.357$). Sex also had no effect on early neurological improvement (EVT alone: OR 1.119, 95% CI 0.801–1.565; bridging therapy: OR 1.249, 95% CI 0.714–2.188), and there was no interaction about treatment based on sex ($P_{interaction} = 0.733$) (Table 2).

Effect of sex difference on the safety outcomes

Women had a similar incidence of intracranial hemorrhage during hospitalization after bridging therapy compared with men (52.7 vs. 40.7%, $p = 0.066$). This result remained constant when women were treated with EVT alone (45.3 vs. 42.4%, $p = 0.478$). The difference in terms of SICH was not significant for women and men (EVT alone: 8.9 vs. 7.9%, $p = 0.652$; bridging therapy: 4.4 vs. 6.8%, $p = 0.442$). Similar deaths were observed within 90 days after treatment (EVT alone: 21.3 vs. 20.6%, $p = 0.833$; bridging therapy: 15.4 vs. 11.7%, $p = 0.409$) (Table 3).

Subgroup analysis

Patients were divided into two groups based on the location of the occlusion. For patients in the anterior circulation occlusion subgroup, the effect of sex on the treatments for the primary outcome was found to have differences (EVT alone: 0.6 OR, 95% CI 0.405–0.888; bridging therapy: OR 0.93, 95% CI 0.542–1.596), while no significant interaction between sex and these two treatments was discovered ($P_{interaction} = 0.199$) (Figure 3A). Neither sex differences nor interactions about treatment based on sex for all secondary outcomes were shown in the subgroup with anterior circulation occlusion. For patients in the posterior circulation occlusion subgroup, men were no longer observed to reach a better functional status compared with women when treated with EVT alone (OR 0.812, 95% CI 0.274–2.402). In addition, no significant interactions between sex and treatments were observed (Figure 3B). Differences in all safety outcomes were not significant between women and men regardless of the treatments and the occlusion location (Table 3).

Discussion

According to our analysis, sex did not affect the prognosis of intravenous alteplase before EVT, but it was associated with

TABLE 2 Sex differences in primary and secondary outcomes after being adjusted.

Outcomes	Treatment	Female	Male	Adjusted effect OR (95%CI)	*P*-value	P (interaction)
Primary						
mRS 0–2 90 days after EVT, *n* (%)						0.226
	EVT alone	68 (26.4)	120 (36.4)	0.654 (0.456–0.937)	0.021	
	Bridging therapy	48 (31.8)	69 (42.6)	0.968 (0.575–1.63)	0.902	
Secondary						
Final mTICI 2b to 3, *n* (%)						0.357
	EVT alone	216 (83.7)	289 (87.6)	0.723 (0.453–1.153)	0.173	
	Bridging therapy	79 (86.8)	139 (85.6)	1.087 (0.512–2.307)	0.828	
4 points decrease in NHISS score 24 h after EVT, *n* (%)						0.733
	EVT alone	158 (61.2)	195 (59.1)	1.119 (0.801–1.565)	0.51	
	Bridging therapy	65 (71.4)	108 (66.7)	1.249 (0.714–2.188)	0.436	

mTICI, modified thrombolysis in cerebral infarction.

TABLE 3 Safety events in women and men.

ALL patients	EVT alone			Bridging therapy		
Safety outcomes	**Female**	**Male**	***P*-value**	**Female**	**Male**	***P*-value**
Intracranial hemorrhage, *n* (%)	117 (45.3)	140 (42.4)	0.478	48 (52.7)	66 (40.7)	0.066
Symptomatic intracerebral hemorrhage, *n* (%)	23 (8.9)	26 (7.9)	0.652	4 (4.4)	11 (6.8)	0.442
Deaths within 90 days after EVT, *n* (%)	55 (21.3)	68 (20.6)	0.833	14 (15.4)	19 (11.7)	0.409
Anterior circulation						
Intracranial hemorrhage, *n* (%)	107 (46.3)	113 (43.6)	0.55	47 (53.4)	60 (43.5)	0.146
Symptomatic intracerebral hemorrhage, *n* (%)	20 (8.7)	20 (7.7)	0.706	4 (4.5)	9 (6.5)	0.536
Deaths within 90 days after EVT, *n* (%)	46 (19.9)	50 (19.3)	0.866	13 (14.8)	13 (9.4)	0.222
Posterior circulation						
Intracranial hemorrhage, *n* (%)	9 (39.1)	21 (34.4)	0.688	1 (33.3)	5 (21.7)	0.657
Symptomatic intracerebral hemorrhage, *n* (%)	3 (13)	5 (8.2)	0.503	0 (0)	1 (4.3)	0.998
Deaths within 90 days after EVT, *n* (%)	9 (39.1)	17 (27.9)	0.322	1 (33.3)	6 (26.1)	0.791

functional independence in patients treated with EVT alone. No significant interaction between sex and treatments was found for all predefined results. The location of the occlusion did not affect the prognosis of women and men treated with bridging therapy.

Similar to Madsen et al. (18) our group found that women were less likely to be functionally independent at 90 days after being treated with EVT alone. However, this phenomenon disappeared when patients were treated with intravenous alteplase before EVT, indicating a shorter time between the onset of the disease to the onset of receiving diagnosis and treatment, and no interaction about treatment based on sex was found. Based on our baseline characteristics, men were more likely to receive bridging therapy, indicating that the time between the onset of the disease and the onset to receiving diagnosis and treatment is shorter in men. As we all know, the effect of treatment in AIS is time-dependent. Various studies demonstrated that reducing workflow time to shorten onset to treatment times was beneficial to patients (26–28). Hence, we concluded that time might have a greater impact on the effect of EVT alone compared to sex. However, there were no sex differences in the five time points at which patients were examined and underwent EVT after admission (Table 1). This might be due to the lack of other time points in our study, such as the time from the onset of disease to arrival at the hospital, which might affect the patient outcomes.

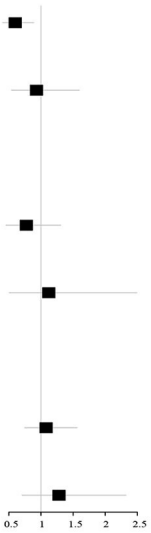
Several studies investigated the interaction between sex and different treatments in patients with AIS. According to Chalos et al. (20) the effect of EVT on the ordinal mRS was similar in women [adjusted common odds ratio (acOR), 2.13; 95% CI, 1.47–3.07] and men (acOR, 2.16; 95% CI, 1.59–2.96), with a *p*-value of 0.926 for interaction, indicating that sex does not influence the clinical outcome after endovascular treatment. In addition, several *post-hoc* analyses based on pooled data from RCTs suggest that sex does not alter the treatment effect of tPA on clinical outcomes (29–32). Interestingly, for patients with late-window stroke, sex was not associated with functional outcomes, while sex was found to influence the association between age and safety outcomes, with men experiencing worse outcomes with advancing age (33). The indirect effects of sex on efficacy and safety outcomes should be taken into consideration. Consistent with these studies, no significant interaction between sex and bridging therapy was found in our study.

de Ridder et al. (21) found the interaction between sex and intra-arterial treatment (IAT) in the MR CLEAN trial and demonstrated that men were more likely to benefit from IAT compared with women. This might be due to the broad inclusion criteria of MR CLEAN, which resulted in a population with a poor prognosis at baseline, especially in female patients. In addition, thirty patients treated with IAT underwent a simultaneous revascularization procedure, which may add complexity to the interpretation of results (34). An assessment from the Cochrane review with subgroup analysis in forest plots using data from randomized controlled trials only showed that significant interactions about sex based on treatment were slightly more common than expected by chance and had limited biological plausibility or clinical significance (35). Most of the interactions about sex based ontreatment were performed in RCTs, which were often subjected to extensive subgroup analyses without correction for multiple testing, increasing the probability of false positives. In addition, as subgroup analyses were not prespecified or insufficiently described in the protocols of RCTs, spurious findings might be reported (36). In contrast to those interaction studies, our results were based on a prospective real-world clinical practice, and confounders were selected after clinical judgment and statistical testing.

Fewer studies focused on occlusion in the posterior circulation. An analysis in patients with acute basilar artery occlusion observed that there were no significant sex differences

A anterior circulation

Outcome	Samples	OR	P−value	P−value (interaction)
mRS score of 0−2 at 90 days				
Interaction of treatment and sex				0.199
EVT alone	490			
Female		0.6(0.405−0.888)	0.011	
Bridging Therapy	226			
Female		0.93(0.542−1.596)	0.791	
Final mTICI 2b to 3				
Interaction of treatment and sex				0.439
EVT alone	490			
Female		0.772(0.456−1.307)	0.336	
Bridging Therapy	226			
Female		1.121(0.505−2.491)	0.779	
4 points decrease in NHISS score 24 hours after EVT				
Interaction of treatment and sex				0.632
EVT alone	490			
Female		1.079(0.746−1.561)	0.685	
Bridging Therapy	226			
Female		1.28(0.705−2.324)	0.418	



B posterior circulation

Outcome	Samples	OR	P−value	P−value (interaction)
mRS score of 0−2 at 90 days				
Interaction of treatment and sex				0.991
EVT alone	84			
Female		0.812(0.274−2.402)	0.706	
Bridging Therapy	26			
Female		0(0−100)	0.994	
Final mTICI 2b to 3				
Interaction of treatment and sex				0.967
EVT alone	84			
Female		0.397(0.127−1.242)	0.112	
Bridging Therapy	26			
Female		0.421(0.03−5.851)	0.519	
4 points decrease in NHISS score 24 hours after EVT				
Interaction of treatment and sex				0.991
EVT alone	84			
Female		0.552(0.204−1.499)	0.244	
Bridging Therapy	26			
Female		0(0−100)	0.994	

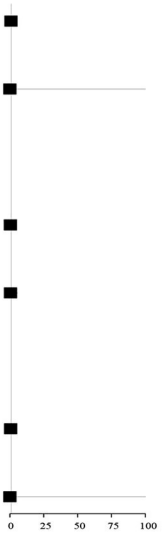


FIGURE 3
Forest plots of the association between sex and treatments in primary and secondary outcomes. **(A)** Forest plots of patients with anterior circulation ischemic stroke; **(B)** Forest plots of patients with posterior circulation ischemic stroke.

for outcomes and recanalization, regardless of treatment modalities, including antithrombotic treatment alone, IVT or combined IVT-IAT, or IAT (37). In our study, similar results were demonstrated for patients treated with bridging therapy. However, this might be related to the small number of patients with posterior circulation ischemic stroke.

Limitations

In this multicenter prospective, real-world study, we used propensity score for covariate adjustment to achieve approximate randomization of alteplase pretreatment. We also included patients with posterior circulation ischemic stroke and made an investigation among them. However, as we only used data from three comprehensive stroke centers in China, the conclusion lacks robustness and is difficult to extrapolate. A great number of patients were lost of follow-up at one center, which might lead to selection bias. In addition, the sample size of patients with posterior circulation ischemic stroke was small, and the confidence intervals were large when analyzing all predefined outcomes.

Conclusion

In this multicenter prospective cohort study, we could not confirm that sex modifies the treatment effect of intravenous alteplase before endovascular therapy. Sex should not be taken into consideration when selecting patients for bridging therapy. Simultaneously, we advocate for women to seek timely medical treatment.

Data availability statement

All data included in this study are available upon request by contacting the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Shanghai Tenth People's Hospital of Tongji University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MF was the main contributor to design, statistical analysis, and writing the first manuscript. CX was responsible for data collection. YS and LM revised the manuscript and checked the collected data. Plotting and editing analysis tables and graphs were allocated to XZ and JD. XL constructed the study and was in charge of overall direction and planning. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.989166/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Power analysis for sample size calculation.

SUPPLEMENTARY TABLE S1
Baseline characteristics of patients who underwent EVT alone and bridging therapy.

References

1. Fischer U, Kaesmacher J, Mendes Pereira V, Chapot R, Siddiqui AH, Froehler MT, et al. Direct mechanical thrombectomy versus combined intravenous and mechanical thrombectomy in large-artery anterior circulation stroke: a topical review. *Stroke.* (2017) 48:2912–8. doi: 10.1161/STROKEAHA.117.017208

2. Kaesmacher J, Mordasini P, Arnold M, Lopez-Cancio E, Cerda N, Boeckh-Behrens T, et al. Direct mechanical thrombectomy in tPA-ineligible and -eligible patients versus the bridging approach: a meta-analysis. *J Neurointerv Surg.* (2019) 11:20–7. doi: 10.1136/neurintsurg-2018-013834

3. Bellwald S, Weber R, Dobrocky T, Nordmeyer H, Jung S, Hadisurya J, et al. Direct mechanical intervention versus bridging therapy in stroke patients eligible for intravenous thrombolysis: a pooled analysis of 2 registries. *Stroke.* (2017) 48:3282–8. doi: 10.1161/STROKEAHA.117.018459

4. Desilles JP, Loyau S, Syvannarath V, Gonzalez-Valcarcel J, Cantier M, Louedec L, et al. Alteplase reduces downstream microvascular thrombosis and improves the benefit of large artery recanalization in stroke. *Stroke.* (2015) 46:3241–8. doi: 10.1161/STROKEAHA.115.010721

5. Seners P, Turc G, Maier B, Mas JL, Oppenheim C, Baron JC. Incidence and predictors of early recanalization after intravenous thrombolysis: a systematic review and meta-analysis. *Stroke.* (2016) 47:2409–12. doi: 10.1161/STROKEAHA.116.014181

6. Zi W, Qiu Z, Li F, Sang H, Wu D, Luo W, et al. Effect of endovascular treatment alone vs intravenous alteplase plus endovascular treatment on functional independence in patients with acute ischemic stroke: the DEVT randomized clinical trial. *JAMA.* (2021) 325:234–43. doi: 10.1001/jama.2020.23523

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

8. Suzuki K, Matsumaru Y, Takeuchi M, Morimoto M, Kanazawa R, Takayama Y, et al. Effect of mechanical thrombectomy without vs with intravenous thrombolysis on functional outcome among patients with acute ischemic stroke: the SKIP randomized clinical trial. *JAMA.* (2021) 325:244–53. doi: 10.1001/jama.2020.23522

9. Treurniet KM, LeCouffe NE, Kappelhof M, Emmer BJ, van Es A, Boiten J, et al. MR CLEAN-NO IV: intravenous treatment followed by endovascular treatment versus direct endovascular treatment for acute ischemic stroke caused by a proximal intracranial occlusion-study protocol for a randomized clinical trial. *Trials.* (2021) 22:141. doi: 10.1186/s13063-021-05063-5

10. Katsanos AH, Turc G, Psychogios M, Kaesmacher J, Palaiodimou L, Stefanou MI, et al. Utility of intravenous alteplase prior to endovascular stroke treatment: a systematic review and meta-analysis of RCTs. *Neurology.* (2021) 97:e777–84. doi: 10.1212/WNL.0000000000012390

11. Zhou Y, Xing P, Li Z, Zhang X, Zhang L, Zhang Y, et al. Effect of occlusion site on the safety and efficacy of intravenous alteplase before endovascular thrombectomy: a prespecified subgroup analysis of DIRECT-MT. *Stroke.* (2022) 53:7–16. doi: 10.1161/STROKEAHA.121.035267

12. Behme D, Kabbasch C, Kowoll A, Dorn F, Liebig T, Weber W, et al. Intravenous thrombolysis facilitates successful recanalization with stent-retriever mechanical thrombectomy in middle cerebral artery occlusions. *J Stroke Cerebrovasc Dis.* (2016) 25:954–9. doi: 10.1016/j.jstrokecerebrovasdis.2016.01.007

13. Nappini S, Arba F, Pracucci G, Saia V, Caimano D, Limbucci N, et al. Bridging versus direct endovascular therapy in basilar artery occlusion. *J Neurol Neurosurg Psychiatry.* (2021) 92:956–62. doi: 10.1136/jnnp-2020-325328

14. Sarraj A, Grotta J, Albers GW, Hassan AE, Blackburn S, Day A, et al. Clinical and neuroimaging outcomes of direct thrombectomy vs bridging therapy in large vessel occlusion: analysis of the SELECT cohort study. *Neurology.* (2021) 96:e2839–53. doi: 10.1212/WNL.0000000000012063

15. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurol.* (2009) 8:355–69. doi: 10.1016/S1474-4422(09)70025-0

16. Poorthuis MH, Algra AM, Algra A, Kappelle LJ, Klijn CJ. Female- and male-specific risk factors for stroke: a systematic review and meta-analysis. *JAMA Neurol.* (2017) 74:75–81. doi: 10.1001/jamaneurol.2016.3482

17. Phan HT, Blizzard CL, Reeves MJ, Thrift AG, Cadilhac D, Sturm J, et al. Sex differences in long-term mortality after STroke in the INSTRUCT (INternational STRoke oUtComes sTudy): a meta-analysis of individual participant data. *Circ Cardiovasc Qual Outcomes.* (2017) 10:e003436. doi: 10.1161/str.47.suppl_1.wmp53

18. Madsen TE, DeCroce-Movson E, Hemendinger M, McTaggart RA, Yaghi S, Cutting S, et al. Sex differences in 90-day outcomes after mechanical thrombectomy for acute ischemic stroke. *J Neurointerv Surg.* (2019) 11:221–5. doi: 10.1136/neurintsurg-2018-014050

19. Sheth SA, Lee S, Warach SJ, Gralla J, Jahan R, Goyal M, et al. Sex differences in outcome after endovascular stroke therapy for acute ischemic stroke. *Stroke.* (2019) 50:2420–7. doi: 10.1161/STROKEAHA.118.023867

20. Chalos V, de Ridder IR, Lingsma HF, Brown S, van Oostenbrugge RJ, Goyal M, et al. Does sex modify the effect of endovascular treatment for ischemic stroke? *Stroke.* (2019) 50:2413–9. doi: 10.1161/STROKEAHA.118.023743

21. de Ridder IR, Fransen PS, Beumer D, Berkhemer OA, van den Berg LA, Wermer MJ, et al. Is intra-arterial treatment for acute ischemic stroke less effective in women than in men? *Interv Neurol.* (2016) 5:174–8. doi: 10.1159/000447331

22. Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet.* (2016) 387:1723–31. doi: 10.1016/S0140-6736(16)00163-X

23. Lisabeth LD, Reeves MJ, Baek J, Skolarus LE, Brown DL, Zahuranec DB, et al. Factors influencing sex differences in poststroke functional outcome. *Stroke.* (2015) 46:860–3. doi: 10.1161/STROKEAHA.114.007985

24. Reeves MJ, Bushnell CD, Howard G, Gargano JW, Duncan PW, Lynch G, et al. Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. *Lancet Neurol.* (2008) 7:915–26. doi: 10.1016/S1474-4422(08)70193-5

25. von Kummer R, Broderick JP, Campbell BC, Demchuk A, Goyal M, Hill MD, et al. The heidelberg bleeding classification: classification of bleeding events after ischemic stroke and reperfusion therapy. *Stroke.* (2015) 46:2981–6. doi: 10.1161/STROKEAHA.115.010049

26. Mulder M, Jansen IGH, Goldhoorn RB, Venema E, Chalos V, Compagne KCJ, et al. Time to endovascular treatment and outcome in acute ischemic stroke: MR CLEAN registry results. *Circulation.* (2018) 138:232–40. doi: 10.1161/CIRCULATIONAHA.117.032600

27. Requena M, Olive-Gadea M, Muchada M, Hernandez D, Rubiera M, Boned S, et al. Direct to angiography suite without stopping for computed tomography imaging for patients with acute stroke: a randomized clinical trial. *JAMA Neurol.* (2021) 78:1099–107. doi: 10.1001/jamaneurol.2021.2385

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

29. Wardlaw JM, Lindley RI, Dennis M, Cohen G, Murray G, Innes K, et al. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet.* (2012) 379:2352–63. doi: 10.1016/S0140-6736(12)60768-5

30. Lindley RI, Wardlaw JM, Whiteley WN, Cohen G, Blackwell L, Murray GD, et al. Alteplase for acute ischemic stroke: outcomes by clinically important subgroups in the third international stroke trial. *Stroke.* (2015) 46:746–56. doi: 10.1161/STROKEAHA.114.006573

31. Hametner C, MacIsaac RL, Kellert L, Abdul-Rahim AH, Ringleb PA, Lees KR, et al. Sex and stroke in thrombolyzed patients and controls. *Stroke.* (2017) 48:367–74. doi: 10.1161/STROKEAHA.116.014323

32. Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet.* (2014) 384:1929–35. doi: 10.1016/S0140-6736(14)60584-5

33. Bala F, Casetta I, Nannoni S, Herlihy D, Goyal M, Fainardi E, et al. Sex-related differences in outcomes after endovascular treatment of patients with late-window stroke. *Stroke.* (2022) 53:311–8. doi: 10.1161/STROKEAHA.121.037127

34. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med.* (2015) 372:11–20. doi: 10.1056/NEJMoa1411587

35. Wallach JD, Sullivan PG, Trepanowski JF, Steyerberg EW, Ioannidis JP. Sex based subgroup differences in randomized controlled trials: empirical evidence from Cochrane meta-analyses. *BMJ.* (2016) 355:i5826. doi: 10.1136/bmj.i5826

36. Kasenda B, Schandelmaier S, Sun X, von Elm E, You J, Blumle A, et al. Subgroup analyses in randomised controlled trials: cohort study on trial protocols and journal publications. *BMJ.* (2014) 349:g4539. doi: 10.1136/bmj.g4539

37. Arnold M, Fischer U, Compter A, Gralla J, Findling O, Mattle HP, et al. Acute basilar artery occlusion in the basilar artery international cooperation study: does gender matter? *Stroke.* (2010) 41:2693–6. doi: 10.1161/STROKEAHA.110.594036

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Genetic insights into the risk of snoring on stroke and ischemic stroke: A single-variable and multivariable Mendelian randomization

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Background: Multiple risk factors of stroke have been identified in previous studies; however, the causal role of snoring in the onset of stroke is less investigated. To clarify the causal association of snoring on stroke and its subtypes, this study is performed.

Methods: The single nucleotide polymorphisms in relation to snoring were retrieved from the UK biobank cohort with 408,317 participants. The data for stroke and its subtypes of European ancestry (67,162 cases and 453,702 controls) were obtained from the MEGASTROKE consortium. In single-variable Mendelian randomization (SVMR) and multivariable MR (MVMR) analyses, inverse variance weighting was used as the primary estimate, complemented with sensitivity analyses more robust to pleiotropy.

Results: Genetically predicted snoring increased the risk of stroke (odds ratio [OR] = 2.69, 95% confidence interval [CI] = 1.19–6.08, $P = 0.016$) and ischemic stroke (IS) (OR = 2.82, 95% CI = 1.23–6.44, $P = 0.013$), but not large artery stroke (LAS) (OR = 3.02, 95% CI = 0.31–29.44, $P = 0.339$), cardioembolic stroke (CES) (OR = 1.51, 95% CI = 0.58–3.92, $P = 0.395$). We provide novel genetic evidence that snoring increases the risk of stroke and IS, but not LAS, CES, and SVS.

Conclusion: Our findings provide novel genetic evidence that snoring increases the risk of stroke and IS, but not LAS, CES, and SVS.

KEYWORDS

snoring, stroke, ischemic stroke, large artery stroke, cardioembolic stroke, small vessel stroke, Mendelian randomization, causal association

Introduction

As reported by the Global Burden of Disease Stroke Collaborators, stroke is the second-leading cause of death (1). In 2019, the number of stroke incidents worldwide was 12.2 million, and the related deaths were 6.55 million. Although acute clinical interventions for stroke have advanced substantially since 2015 (2), the disease burden remains significant. Currently, stroke prevention is considered an effective strategy, and 85% of all strokes may be preventable (3). Particularly, the modifiable risk factors, such as smoking, cigarette consumption, total cholesterol, and cigarette consumption, attract growing interest in stroke prevention, as stroke has decreased in incidence by approximately 42% in developed countries within the last 30 years (4). Therefore, identifying and intervening modifiable risk factors may facilitate decreasing the incidence of stroke.

Snoring is the vibration of the upper airway structures causing noise as the air passes in and out during sleep. Habitual snoring is prevalent, and it is estimated that the prevalence is 35–45% in males and 15–28% in females (5). More seriously, the overall incidence of snoring increases with age. Although most patients with obstructive sleep apnea are accompanied by snoring, 20–25% of them with central sleep apnea do not have the symptom of snoring and belong to habitual non-apneic benign snorers (6). Compared with sleep apnea, the potential effect of snoring on stroke has been less studied. In addition, the findings of the association between snoring and stroke remain inconsistent in previous observational studies. For example, snoring in postmenopausal women was associated with stroke (7); however, in a community-based sample over 17 years of follow-up, no significant association was observed between them (8). The discrepancy may be attributed to the study design, limited sample size, and especially the confounders such as snorers accompanying diseases from the cross-sectional or retrospective design in clinics. These biases may impede the yielding of unbiased causal estimates.

To address the inconsistent results in observational design, Mendelian randomization (MR) that can overcome the endogeneity and confounders and then yield causal estimates is selected in this study. MR uses the genetic variants, namely single-nucleotide polymorphisms (SNPs), as instrumental variables (IVs) to examine the causal association between the exposures (i.e., snoring) and outcomes (i.e., stroke) (9). SNPs are assorted randomly in the forming of a zygote during gestation (10). Therefore, MR is similar to the random assortment of interventions in a randomized clinical trial (RCT) and can avoid reverse causation and overcome confounding factors that are typical of non-randomized observational studies (10, 11). At present, no study has been performed to reveal the causal association of snoring on stroke. To clarify the role of snoring on stroke, we performed a single-variable MR (SVMR) and multivariable MR (MVMR) analysis to address the discrepancy and then yield their causal links.

Methods

Data sources of snoring, stroke, and its subtypes

The SNPs associated with snoring were obtained from the European ancestry in the UK Biobank (408,000 non-snorers and 152,000 snorers) (12). Snoring was assessed with a question: "Does your partner or a close relative or friend complain about your snoring?". The corresponding response options were "Yes", "No", "Don't know", or "Prefer not to answer". The answers "Don't know" or "Prefer not to answer" were removed from the dataset due to the vagueness.

We extracted summary-level datasets of stroke and its subtypes from one meta-analysis by the MEGASTROKE consortium in the European ancestry (13). The dataset of outcomes included stroke (67,162 cases), IS (60,341 cases), LAS (6,688 cases), CES (9,006 cases), and SVS (11,710 cases). The diagnosis of the stroke was based on the World Health Organization (WHO) definition, which was rapidly developing signs of focal (or global) cerebral dysfunction, lasting more than 24 hours or resulting in death with no apparent cause other than that of blood vessel origin. Following the Trial of Org 10,172 in Acute Stroke Treatment (TOAST) criteria (14), IS was subdivided into LAS, CES, and SVS. The detailed information about the study populations, study-specific stroke ascertainment, and subtyping could be accessed through previous publications (13).

Genetic instrument selection

To retrieve the conditionally independent IVs of snoring, the statistical significance threshold was set at a genome-wide significance level of $P < 5 \times 10^{-8}$ with linkage disequilibrium (LD) $r^2 < 0.01$ at a 10,000 kb window size based on 1,000 Genomes European reference panel. We also used the MR-Steiger filtering method to confirm that the SNPs explained more variance in exposure (i.e., snoring) than in outcome (i.e., stroke) (15). When the MR-Steiger test indicated an inverse causality of stroke on snoring, the insignificant SNPs were removed. In our MR-Steiger filtering analysis, all the extracted SNPs passed the test. Besides, the palindromic SNPs were removed.

F-statistics represents the strength of genetic instruments and were calculated using the following formula F-statistics = (Beta/Se) (16, 17). Generally, F-statistic less than 10 was accepted as an indicator of weak IVs, which should be removed. In this step, no SNP was pruned. Additionally, to reduce the heterogeneity and avoid pleiotropy, radial-MR and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) methods were performed to detect the significant horizontal pleiotropic outliers (18). In these analyses, no significant outliers were detected and then removed, indicating the absence of pleiotropy.

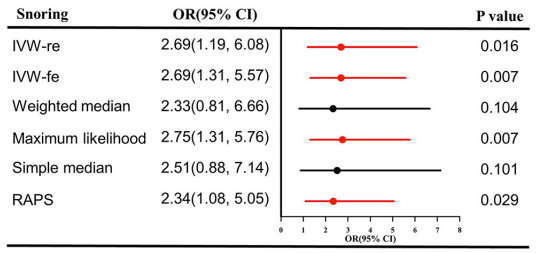

FIGURE 1
Causal effect estimates of snoring on stroke in SVMR. OR, odds ratio; CI, confidence interval; IVW, inverse variance weighted method; RAPS, robust adjusted profile score; MR, Mendelian randomization; SVMR, single-variable Mendelian randomization.

To further verify the absence of possible pleiotropy, we performed a search using an online tool, Phenoscanner (version 2) (http://www.phenoscanner.medschl.cam.ac.uk/), (19) to detect the pleiotropic effects of the selected IVs. We removed 26 SNPs due to their significant links with other diseases and traits ($P < 5 \times 10^{-8}$), and detailed information was displayed in Supplementary Table S2. Finally, the remaining 17 SNPs (Supplementary Table S1) were selected as the IVs and used to estimate the causal relationship between snoring, stroke, and its subtypes.

Main statistical analyses

Fixed and random effects inverse variance weighting (IVW) approaches were deemed as the main analyses to test the causal effect of snoring on stroke. When the horizontal pleiotropy is not detected (no violation of the independence assumption) or was balanced, the IVW method can combine the Wald ratios of each SNP to produce an overall unbiased causal estimate of snoring on stroke (20).

A two-sided *P*-value <0.05 was regarded as statistically significant. All statistical analyses were performed using "TwoSampleMR" (20), "MRPRESSO" (18), "mr.raps" (21), and "forestplot" packages in R software (version 3.6.5, Foundation for Statistical Computing, Vienna, Austria).

Sensitivity analyses

To verify the conformity of the MR results and detect the possible pleiotropy and heterogeneity, we performed four analyses using MR-Egger, MR-PRESSO, Maximum likelihood, and MR robust adjusted profile score (MR-RAPS) methods. In the MR-Egger regression analysis, an intercept term was introduced into the regression model to detect the directional pleiotropy. Even if all the instruments were invalid, MR-Egger could yield valid causal effect estimates (22). MR-PRESSO was used to detect horizontal pleiotropy, correct the significant outliers, and further produce a more robust estimate (18). Maximum likelihood method could obtain a causal effect by the direct maximization of the likelihood, and assume a linear relationship between the exposure (i.e., snoring) and outcome (i.e., stroke). After modeling by MR-RAPS, the robust results could be produced under the assumption of the normal distribution of pleiotropic effects. Even when the weak IVs and systematic and idiosyncratic pleiotropy existed, the findings from MR-RAPS were robust.

The Cochran's Q test was applied to test the heterogeneity across all instrumental SNPs. In addition, the "leave-one-out" sensitivity analysis was used to evaluate whether the snoring-stroke causal links were driven by influential SNPs, otherwise indicating the robustness of the casual estimation.

The statistical power to detect the difference was calculated using an online tool (https://shiny.cnsgenomics.com/mRnd/). When the threshold of type I error rate was 0.05, the statistical power of snoring on stroke was 100%. In addition, we calculated the bias and overlap in the website "https://sb452.shinyapps.io/overlap/". When the threshold of type I error rate was 0.05 and the overlap proportions were 100%, the value of the bias was 0.056. This finding indicated that our results were stable the statistical power was ample and the bias from sample overlap seemed to be minimal in this study.

Multivariable MR of snoring on stroke and its subtypes

To investigate the direct causal effect of snoring on stroke and its subtypes, we performed MVMR analysis (23). MVMR can detect causal effects of multiple risk factors on stroke jointly, and further obtain the independent association of each risk exposure with the outcome (20, 24). In previous studies, snoring could be influenced by other heritable lifestyle factors such as smoking and alcohol drinks (25–27). Therefore, the potential confounders in the MVMR analyses in our study included smoking, alcoholic consumption, low-density lipoprotein (LDL), total cholesterol (TC), and body mass index (BMI). The SNPs in MVMR analyses were the overlapping SNPs between snoring and the confounders.

Results

Causal effect estimates of snoring on stroke in SVMR

As shown in Figure 1, genetically predicted snoring causally lead to a 2.69-fold increase in stroke risk [95% confidence interval (CI)] = 1.19–6.08, $P = 0.016$ for the IVW-re estimator; 95% CI = 1.31–5.57, $P = 0.007$ for the IVW-fe estimator.

The scatter plot in Figure 2A showed that with the increase of IVs' effect on snoring, the SNPs' effect on stroke increased. In sensitivity analyses, the causal association between snoring and stroke still existed (Maximum likelihood method: OR = 2.75, 95% CI = 1.31–5.76, $P = 0.007$; MR-RAPS: OR = 2.34 95% CI = 1.08–5.05, $P = 0.029$; Figure 1).

In sensitivity analysis, there was no signs of pleiotropy (MR-Egger: intercept term = −0.019; $P = 0.251$, Table 1; MR-PRESSO global test: $P = 0.330$). The heterogeneity was not observed according to the results of Cochran's Q statistics ($P > 0.330$) (Table 1) and the funnel plot (Supplementary Figure S1), indicating that no significant outliers were detected. Furthermore, the results from the leave-one-out method revealed that the positive association remained robust after leaving any single SNP out in turn (Supplementary Figure S2). This indicated that no influential SNPs were found. The forest plot visualizing the effect estimate of each SNP on stroke was displayed in Supplementary Figure S3.

Causal effect estimates of snoring on IS in SVMR

Likewise, snoring increased the risk of IS [(OR = 2.82, 95% CI = 1.23–6.44, $P = 0.013$ for the IVW-re estimator; OR = 2.82, 95% CI = 1.28–6.18, $P = 0.009$ for the IVW-fe estimator); Figure 3]. The results were similar in maximum likelihood method (OR = 2.93, 95% CI = 1.31–6.54, $P = 0.008$); simple median (OR = 3.15, 95% CI = 1.04–9.52, $P = 0.041$), and MR-RAPS (OR = 2.58, 95% CI = 1.12–5.93, $P = 0.024$). As shown in Figure 2B, the risk of IS increased as the IVs' effect on snoring increased.

No pleiotropy was identified using MR-Egger [(intercept term = −0.013; $P = 0.442$), Table 1] and MR-PRESSO methods (all $P = 0.473$) in sensitivity analyses. The results of Cochran's Q test revealed the absence of heterogeneity [($P > 0.05$), Table 1]. The heterogeneity results visualized in the funnel plot were presented in Supplementary Figure S4. Additionally, no influential IVs were identified in the leave-one-out analysis when excluding any one of SNP in turn (Supplementary Figure S5). The estimates from each IV were presented in Supplementary Figure S6.

Causal effect estimates of snoring on LAS, CES, and SVS in SVMR

As shown in Supplementary Figures S7–S9, snoring was not causally associated with LAS, CES, and SVS (all $P > 0.05$). The effect of snoring on LAS, CES, and SVS visualizing in the scatter plot revealed that the snoring did not increase their risk (Supplementary Figures S1–S12). There were no signs of heterogeneity according to the results of Cochran's Q tests [($P > 0.05$); Table 1, Supplementary Figures S13–S15]. The results in the leave-one-out analysis for LAS, CES, and SVS remained consistent when excluding any one SNP at a time (Supplementary Figures S16–S18). The casual estimates from each IV on LAS, CES, and SVS were presented in Supplementary Figures S19–S21.

Causal effect estimates of snoring on stroke and its subtypes in MVMR

As shown in Figure 4A, the casual effects of snoring on stroke remained unchanged after adjusting for smoking (OR = 2.11, 95% CI = 1.08–4.11, $P = 0.027$); alcoholic drinks (OR = 2.25, 95% CI = 1.19–4.27, $P = 0.012$), LDL (OR = 2.61, 95% CI = 1.06–6.41, $P = 0.035$), TC (OR = 1.96, 95% CI = 1.01–3.78, $P = 0.043$), and BMI (OR = 2.22, 95% CI = 1.08–4.58, $P = 0.029$), respectively. The positive association still existed for IS in MVMR (smoking: OR = 2.11, 95% CI = 1.07–4.15, $P = 0.029$; alcoholic drinks: OR = 2.39, 95% CI = 1.30–4.38, $P = 0.004$; LDL: OR = 2.59, 95% CI = 1.01–6.71, $P = 0.049$; TC: OR = 2.37, 95% CI = 1.08–5.19, $P = 0.031$; BMI: OR = 2.11, 95% CI = 1.05–4.24, $P = 0.034$), (Figure 4B).

No significant casual association was detected for LAS, CES, and SVS after adjusting the confounders [(all $P > 0.05$), Supplementary Figures S22–S24]. SVMR analysis indicated no causal effects of snoring on these subtypes. Therefore, our analysis provided evidence that snoring could not increase their risk.

Discussion

Snoring is a health problem, and studies in adults and children suggest that the frequency of snoring can predicts symptoms and poorer behavioral and cognitive outcomes (28, 29). Using GWAS summary data, our study identifies the causal association between snoring, stroke, and IS. In addition, no causal association is observed between snoring, LAS, CES, and SVS.

Some previous large population-based cohort studies and meta-analyses may support our conclusion. For instance, during a median follow-up of 9.6 years in a China Kadoorie Biobank (CKB) study of 489,583 participants, habitual snoring increased the risk of IS (hazard ratio = 1.12) among participants aged <50 years (30), while such associations did not exist among individuals in adults aged over 50 years. However, obvious limitations should be mentioned in their study. Firstly, the snoring status of participants was available only at baseline for most CKB participants. However, the condition of these people remained unclear during other period. Moreover, some participants who were unaware of their snoring status were

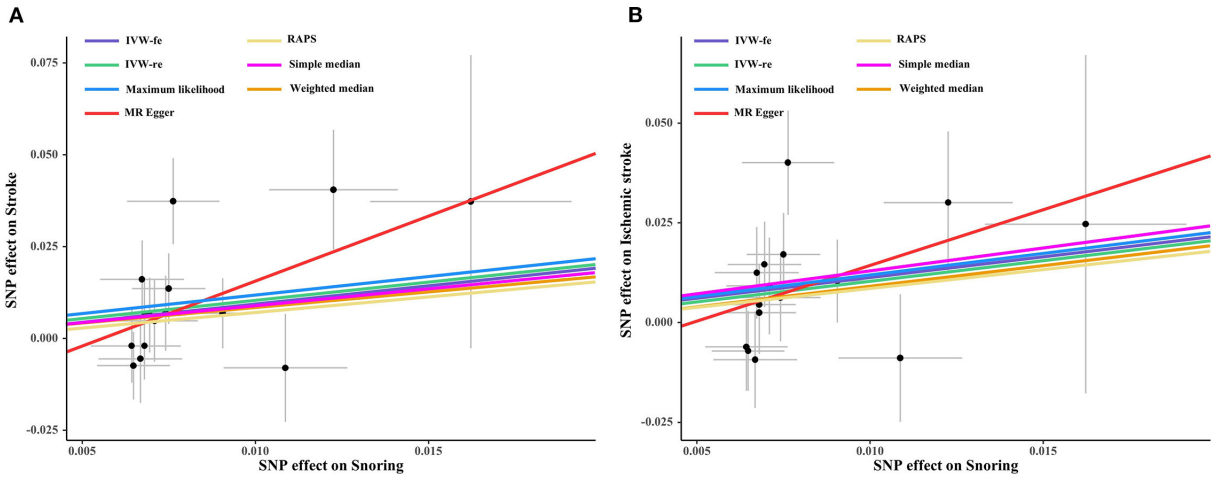

FIGURE 2
Scatter plot of the effect size of each SNP on snoring, stroke **(A)** and IS **(B)** in SVMR. SNP, single nucleotide polymorphism; IS, ischemic stroke; IVW, inverse variance weighted method; MR, Mendelian randomization.

TABLE 1 MR estimates from each method of the causal effect of snoring on stroke and its subtypes.

Traits	MR methods	OR	95% CI	*P*	Cochran's Q statistic	Heterogeneity *P*-value	MR-Egger intercept	Intercept *P*-value
Stroke	MR-Egger	34.48	0.50–2,373.70	0.125	15.861	0.256	−0.019	0.251
	IVW-re	2.69	1.19–6.08	0.016	17.623	0.224	-	-
	Maximum likelihood method	2.75	1.31–5.76	0.007	17.441	0.233	-	
IS	MR-Egger	16.326	0.15–1,366.77	0.238	14.781	0.321	−0.013	0.442
	IVW-re	2.820	1.23–6.44	0.013	15.493	0.345	-	-
	Maximum likelihood method	2.936	1.31–6.54	0.008	15.363	0.353	-	-
LAS	MR-Egger	0.163	5.10e-07–52,318.92	0.783	18.672	0.133	0.022	0.653
	IVW-re	3.027	0.31–29.44	0.339	18.975	0.165	-	-
	Maximum likelihood method	3.145	0.42–23.01	0.259	18.948	0.166	-	-
CES	MR-Egger	5.993	0.01–21,971.55	0.675	5.462	0.963	−0.010	0.743
	IVW-re	1.512	0.58–3.92	0.395	5.574	0.976	-	-
	Maximum likelihood method	1.528	0.33–6.98	0.584	5.571	0.976	-	-
SVS	MR-Egger	4.449	0.01–74,117.93	0.768	9.302	0.749	0.037	0.898
	IVW-re	2.360	0.53–10.45	0.258	9.319	0.810	-	-
	Maximum likelihood method	2.439	0.38–15.35	0.342	9.308	0.810	-	-

MR, Mendelian randomization; *OR*, odds ratio; *CI*, confidence interval; *IS*, ischemic stroke; *LAS*, large artery stroke; *CES*, cardioembolic stroke; *SVS*, small vessel stroke; *IVW*, inverse variance weighted.

assigned as non-snorers. Therefore, these findings about the association between stroke risk and snoring could not be verified whether habitual snoring was associated with stroke risk and required further research. Recently, a result of a meta-analysis including 3,598 stroke patients and 145,901 participants without stroke, suggested that snoring was associated with a significantly increased risk of stroke (relative risk 1.46; 95% CI, 1.29–1.63; $P< 0.001$) (31). A similar meta-analysis conducted by Min Li et al. reported that snoring had a modest but statistically significant positive association with the risk of stroke (32). However, the studies included in the meta-analysis varied in study design, population, adjustment for confounders, and different diagnostic methods for exposure and outcome, which reduced the reliability of their results. Performing RCT about the association between snoring and stroke seems difficult in practice. Our MR analysis provides novel evidence to overcome these confounders and discloses the casual association of snoring on stroke.

The insignificant results between snoring and stroke are also reported elsewhere. In the Busselton Health Study, no significant association was identified between snoring and stroke during a follow-up of 17 years (8). However, this study had

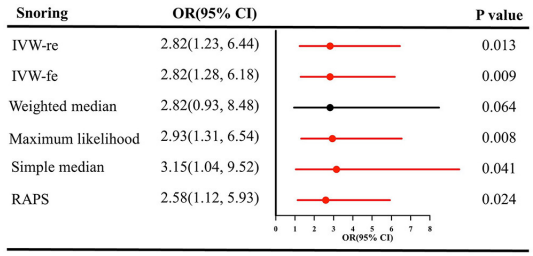

FIGURE 3
Causal estimates of snoring on IS in SVMR. OR, odds ratio; CI, confidence interval; IVW, inverse variance weighted method; IS, ischemic stroke; RAPS, robust adjusted profile score; MR, Mendelian randomization; SVMR, single-variable Mendelian randomization.

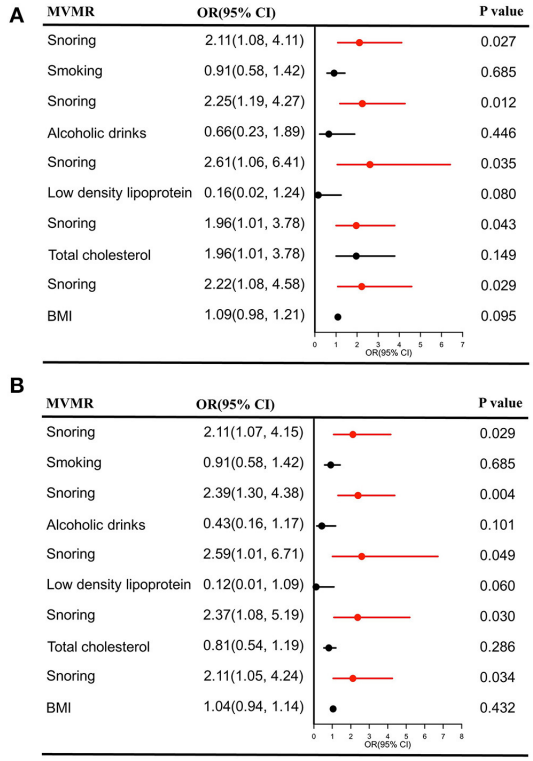

FIGURE 4
Causal estimates of snoring on stroke **(A)** and IS **(B)** in MVMR. OR, odds ratio; CI, confidence interval; IS, Ischemic stroke; MVMR, multivariable Mendelian randomization; BMI, body mass index.

limited sample size (360 participants), reducing the reliability of the conclusion. Besides, a population-based cohort in the Jackson Heart Study with 4,495 participants (787 snorers and 3,708 non-snorers) found that self-reported habitual snoring was not associated with incident stroke (33). Yet, subjects in the study were African Americans, who had the greatest level of difficulty in recalling snoring, especially males (34). Furthermore, some individuals among non-whites remained uncertain whether they had snoring status, or reported snoring inaccurately. These would lead to the low prevalence of snoring and further make an opposite conclusion. The causal association of snoring on stroke is identified in our study. This causal report addresses the discrepancy in previous observational studies and may support the clinical decision about identifying snoring for preventing stroke.

The exact mechanisms linking snoring to stroke remain unclear. One possible explanation may be the hypoxia and the vibration of the upper airway structures during sleep according to the definition of snoring (35, 36). Obstruction or stenosis of the upper airway could lead to hypoxia and further result in the chronic activation of the sympathetic system, oxidative stress, and inflammation, all of which were involved in the pathological process of hypertension and atherosclerosis (37). In western countries, some studies suggested that patients with habitual snoring had a higher risk of hypertension than their non-habitual snoring counterparts (38, 39). Moreover, preload and afterload of the heart increased during snoring due to large swing in pleural pressure, and snoring-related energy could be transmitted to the caroid artery and further induce the process of atherosclerosis or the disruption of atherosclerotic plaques (40, 41). In addition, the high energy generated by vibration during snoring could be transmitted to the proximal tissues including the carotid artery, which might cause cascade effects of numerous cells in the arterial wall and lead to alteration in vascular structure and function (41, 42).

In the analysis of stroke subtypes, association between snoring and IS was observed, while the causal association does not exist between snoring and LAS, CES, and SVS. These results indicate that biological heterogeneity of the genetic effect of snoring on different IS subtypes may exist, and different subtypes may have distinct pathological mechanisms.

There are several strengths in our MR study. Firstly, we clarify the causal association between snoring and stroke using the MR method, which overcomes the underlying impact of confounding factors and yields causal inferences. Moreover, this study is the first study to directly identify the causal effects of snoring on stroke and its subtypes, which can assist doctors in the clinical decision. The association between other sleep disorders, such as insomnia and sleep duration, have been clarified in MR analyses. In our study, we found the casual association between the snoring and stroke and IS. However, there are some shortcomings in our study. The main limitation is that the datasets originate from the European population, which limits the generalization of the conclusion. Furthermore, only self-reported information on snoring was available. In addition, the case of stroke subtypes was relatively small. Therefore, we should evaluate the conclusion with caution. In future studies, replication in other ancestries, more rigorous clinical study design, and large studies with more samples should be performed to verify the conclusions.

Conclusions

In conclusion, our study supports the causal association between snoring, stroke, and IS. In clinical settings, snoring should be noted by doctors, and interventions targeting snoring should be considered.

Data availability statement

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

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

Author contributions

QH and LR analyzed and interpreted the data and wrote the manuscript. HL, WW, and QH analyzed the data. CT, LM, and CY designed the study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1023748/full#supplementary-material

References

1. Collaborators GBDS. Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the global burden of disease study 2019. *Lancet Neurol.* (2021) 20:795–820. doi: 10.1016/S1474-4422(21)00252-0

2. Campbell BCV, Khatri P. Stroke. *Lancet.* (2020) 396:129–42. doi: 10.1016/S0140-6736(20)31179-X

3. O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, et al. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the interstroke study): a case-control study. *Lancet.* (2010) 376:112–23. doi: 10.1016/S0140-6736(10)60834-3

4. Hankey GJ. Nutrition and the risk of stroke. *Lancet Neurol.* (2012) 11:66–81. doi: 10.1016/S1474-4422(11)70265-4

5. Main C, Liu Z, Welch K, Weiner G, Jones SQ, Stein K. Surgical procedures and non-surgical devices for the management of non-apnoeic snoring: a systematic review of clinical effects and associated treatment costs. *Health Technol Assess.* (2009) 13:1–208. doi: 10.3310/hta13030

6. Bauters FA, Hertegonne KB, De Buyzere ML, Joos GF, Chirinos JA, Rietzschel ER. Phenotype and risk burden of sleep apnea: a population-based cohort study. *Hypertension.* (2019) 74:1052–62. doi: 10.1161/HYPERTENSIONAHA.119.13452

7. Sands M, Loucks EB, Lu B, Carskadon MA, Sharkey K, Stefanick M, et al. Self-reported snoring and risk of cardiovascular disease among postmenopausal women (from the women's health initiative). *Am J Cardiol.* (2013) 111:540–6. doi: 10.1016/j.amjcard.2012.10.039

8. Marshall NS, Wong KK, Cullen SR, Knuiman MW, Grunstein RR. Snoring is not associated with all-cause mortality, incident cardiovascular disease, or stroke in the busselton health study. *Sleep.* (2012) 35:1235–40. doi: 10.5665/sleep.2076

9. Burgess S, Foley CN, Allara E, Staley JR, Howson JMM. A robust and efficient method for Mendelian randomization with hundreds of genetic variants. *Nat Commun.* (2020) 11:376. doi: 10.1038/s41467-019-14156-4

10. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol.* (2003) 32:1–22. doi: 10.1093/ije/dyg070

11. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol.* (2013) 37:658–65. doi: 10.1002/gepi.21758

12. Campos AI, Garcia-Marin LM, Byrne EM, Martin NG, Cuellar-Partida G, Renteria ME. Insights into the aetiology of snoring from observational

and genetic investigations in the UK biobank. *Nat Commun.* (2020) 11:817. doi: 10.1038/s41467-020-14625-1

13. Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet.* (2018) 50:524–37. doi: 10.1038/s41588-018-0058-3

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

15. Hemani G, Tilling K, Davey Smith G. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet.* (2017) 13:e1007081. doi: 10.1371/journal.pgen.1007081

16. Burgess S, Small DS, Thompson SG, A. review of instrumental variable estimators for Mendelian randomization. *Stat Methods Med Res.* (2017) 26:2333–55. doi: 10.1177/0962280215597579

17. Xiong Y, Zhang FX, Zhang YC, Wu CJ, Qin F, Yuan JH. Genetically predicted insomnia causally increases the risk of erectile dysfunction. *Asian J Androl.* (2022). doi: 10.4103/aja202261

18. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7

19. Kamat MA, Blackshaw JA, Young R, Surendran P, Burgess S, Danesh J, et al. PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations. *Bioinformatics.* (2019) 35:4851–3. doi: 10.1093/bioinformatics/btz469

20. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-base platform supports systematic causal inference across the human phenome. *Elife.* (2018) 7:1–29. doi: 10.7554/eLife.34408

21. Zhao Q, Wang J, Hemani G, Bowden J. Small DSJTAoS. Statistical inference in two-sample summary-data Mendelian randomization using robust adjusted profile score. *Ann Stat.* (2020) 48:1742–69. doi: 10.1214/19-AOS1866

22. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol.* (2015) 44:512–25. doi: 10.1093/ije/dyv080

23. Burgess S, Thompson SG. Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. *Am J Epidemiol.* (2015) 181:251–60. doi: 10.1093/aje/kwu283

24. Sanderson E, Davey Smith G, Windmeijer F, Bowden J. An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings. *Int J Epidemiol.* (2019) 48:713–27. doi: 10.1093/ije/dyy262

25. Katila M, Saarenpaa-Heikkila O, Saha MT, Vuorela N, Paavonen EJ. Parental reports showed that snoring in infants at three and eight months associated with snoring parents and smoking mothers. *Acta Paediatr.* (2019) 108:1686–94. doi: 10.1111/apa.14758

26. Wei YX, Shen ZW Yu CQ, Du HD Lyu J, Guo Y, et al. Epidemiological characteristics and correlated factors of habitual snoring among Chinese aged 30–79 year-old. *Zhonghua Liu Xing Bing Xue Za Zhi.* (2019) 40:917–23. doi: 10.3760/cma.j.issn.0254-6450.2019.08.009

27. Morales-Munoz I, Koskinen S, Partonen T. The effects of seasonal affective disorder and alcohol abuse on sleep and snoring functions in a population-based study in Finland. *J Sleep Res.* (2018) 27:e12611. doi: 10.1111/jsr.12611

28. Smith DL, Gozal D, Hunter SJ, Kheirandish-Gozal L. Frequency of snoring, rather than apnea-hypopnea index, predicts both cognitive and behavioral problems in young children. *Sleep Med.* (2017) 34:170–8. doi: 10.1016/j.sleep.2017.02.028

29. Svensson M, Franklin KA, Theorell-Haglow J, Lindberg E. Daytime sleepiness relates to snoring independent of the apnea-hypopnea index in women from the general population. *Chest.* (2008) 134:919–24. doi: 10.1378/chest.08-0847

30. Wei Y, Lv J, Guo Y, Bian Z, Fan J, Du H, et al. Age-specific associations between habitual snoring and cardiovascular diseases in China: a 10-year cohort study. *Chest.* (2021) 160:1053–63. doi: 10.1016/j.chest.2021.04.070

31. Bai J, He B, Wang N, Chen Y, Liu J, Wang H, et al. Snoring is associated with increased risk of stroke: a cumulative meta-analysis. *Front Neurol.* (2021) 12:574649. doi: 10.3389/fneur.2021.574649

32. Li M, Li K, Zhang XW, Hou WS, Tang ZY. Habitual snoring and risk of stroke: a meta-analysis of prospective studies. *Int J Cardiol.* (2015) 185:46–9. doi: 10.1016/j.ijcard.2015.03.112

33. Rosen DM, Kundel V, Rueschman M, Kaplan R, Guo N, Wilson JG, et al. Self-reported snoring and incident cardiovascular disease events: results from the Jackson Heart Study. *Sleep Breath.* (2019) 23:777–84. doi: 10.1007/s11325-018-01776-1

34. Kump K, Whalen C, Tishler PV, Browner I, Ferrette V, Strohl KP, et al. Assessment of the validity and utility of a sleep-symptom questionnaire. *Am J Respir Crit Care Med.* (1994) 150:735–41. doi: 10.1164/ajrccm.150.3.8087345

35. Wiener RC, Zhang R, Shankar A. Elevated serum C-reactive protein and markers of sleep disordered breathing. *Int J Vasc Med.* (2012) 2012:914593. doi: 10.1155/2012/914593

36. Fernandez Alvarez R, Rubinos Cuadrado G, Alonso Arias R, Cascon Hernandez JA, Palomo Antequera B, Iscar Urrutia M, et al. Snoring as a determinant factor of oxidative stress in the airway of patients with obstructive sleep apnea. *Lung.* (2016) 194:469–73. doi: 10.1007/s00408-016-9869-0

37. Grassi G, Mark A, Esler M. The sympathetic nervous system alterations in human hypertension. *Circ Res.* (2015) 116:976–90. doi: 10.1161/CIRCRESAHA.116.303604

38. Lindberg E, Janson C, Gislason T, Svardsudd K, Hetta J, Boman G. Snoring and hypertension: a 10 year follow-up. *Eur Respir J.* (1998) 11:884–9. doi: 10.1183/09031936.98.11040884

39. Hu FB, Willett WC, Colditz GA, Ascherio A, Speizer FE, Rosner B, et al. Prospective study of snoring and risk of hypertension in women. *Am J Epidemiol.* (1999) 150:806–16. doi: 10.1093/oxfordjournals.aje.a010085

40. Stradling JR, Crosby JH. Relation between systemic hypertension and sleep hypoxaemia or snoring: analysis in 748 men drawn from general practice. *BMJ.* (1990) 300:75–8. doi: 10.1136/bmj.300.6717.75

41. Cho JG, Witting PK, Verma M, Wu BJ, Shanu A, Kairaitis K, et al. Tissue vibration induces carotid artery endothelial dysfunction: a mechanism linking snoring and carotid atherosclerosis? *Sleep.* (2011) 34:751–7. doi: 10.5665/SLEEP.1042

42. Curry BD, Bain JL, Yan JG, Zhang LL, Yamaguchi M, Matloub HS, et al. Vibration injury damages arterial endothelial cells. *Muscle Nerve.* (2002) 25:527–34. doi: 10.1002/mus.10058

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The influential factors and non-pharmacological interventions of cognitive impairment in children with ischemic stroke

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Background: The prevalence of pediatric ischemic stroke rose by 35% between 1990 and 2013. Affected patients can experience the gradual onset of cognitive impairment in the form of impaired language, memory, intelligence, attention, and processing speed, which affect 20–50% of these patients. Only few evidence-based treatments are available due to significant heterogeneity in age, pathological characteristics, and the combined epilepsy status of the affected children.

Methods: We searched the literature published by Web of Science, Scopus, and PubMed, which researched non-pharmacological rehabilitation interventions for cognitive impairment following pediatric ischemic stroke. The search period is from the establishment of the database to January 2022.

Results: The incidence of such impairment is influenced by patient age, pathological characteristics, combined epilepsy status, and environmental factors. Non-pharmacological treatments for cognitive impairment that have been explored to date mainly include exercise training, psychological intervention, neuromodulation strategies, computer-assisted cognitive training, brain-computer interfaces (BCI), virtual reality, music therapy, and acupuncture. In childhood stroke, the only interventions that can be retrieved are psychological intervention and neuromodulation strategies.

Conclusion: However, evidence regarding the efficacy of these interventions is relatively weak. In future studies, the active application of a variety of interventions to improve pediatric cognitive function will be necessary, and neuroimaging and electrophysiological measurement techniques will be of great value in this context. Larger multi-center prospective longitudinal studies are also required to offer more accurate evidence-based guidance for the treatment of patients with pediatric stroke.

KEYWORDS

pediatric, ischemic stroke, cognitive impairment, intervention, non-pharmacological

1. Introduction

Childhood arterial ischemic stroke is defined as a cerebrovascular event that occurs between 1 month and 18 years of age. Arterial ischemic stroke is characterized by an acute-onset neurological deficit due to an infarct in an arterial territory consistent with the clinical syndrome (1). The annual incidence of stroke in children is 1.3–13/100,000 (2), and it has risen by 35% between 1990 and 2013 (3). The prognosis for post-stroke recovery in affected children is no better than that of patients with adult stroke (3). The primary difference between these two patient populations is that in adults, a stroke can result in a loss of functional independence, whereas in children, it can also interfere with their growth and development due to prolonged neurological dysfunction (4–7). Children, young adults, and their parents exhibit high levels of unmet needs across a range of health domains in the months and years after pediatric stroke (8). Due to delays in presentation, only about 2% of children are eligible for treatment with thrombolysis and thrombectomy (9). Therefore, rehabilitation therapies for pediatric stroke are important, which improve outcomes after hyperacute treatment (10).

Approximately 20–50% of patients with pediatric ischemic stroke exhibit signs of cognitive impairment (3), affecting both executive function and behavioral traits, including intelligence, memory, attention, and processing speed. The intelligence quotient (IQ) values of patients with pediatric ischemic stroke are generally reported to be on the lower end of the normal range while being significantly lower on average than those in healthy age-matched populations (11–13). Notably, patients exhibit significantly more damage to operational IQ values relative to speech IQ values (12). In one study of the neuropsychological characteristics of 49 children after ischemic stroke, the average performance of these children in attention and executive function tasks was significantly lower than that of the healthy control children, with 67% of the children exhibiting impairment when completing attention tasks and 30% of the children exhibiting impaired executive function (13). Processing speed (12, 14, 15) and working memory (4, 14) are also significantly impaired in patients with ischemic stroke.

Compared with the adult stroke population, there is conflicting evidence about whether children's prognosis is more favorable and whether children recover better after stroke than adults (10, 16, 17). The plasticity and the selective vulnerability are widely held assumptions (18). Such as, children with ischemic stroke have different cognitive prognoses depending on their age groups (19, 20). Two mechanisms of recovery after nerve injury have been proposed, namely, behavioral recovery and compensation. For the rehabilitation of children with stroke, the influence of natural development should also be considered. The immature brain, however, is a dynamic environment with significant changes to the cellular composition, neural circuitry, and blood flow occurring throughout childhood (21).

Developing a better understanding of the risk of cognitive impairment and other adverse outcomes in children following ischemic stroke occurrence is critically important to parents, clinicians, and patients (22). Relatively few studies have been conducted on cognitive impairment in children after ischemic stroke. Therefore, the present review explores the factors associated with the prognosis of these patients and discusses treatment strategies aimed at alleviating post-ischemic stroke cognitive impairment to provide a foundation for future interventional and patient management strategies.

2. Methods

We searched the literature published by Web of Science, Scopus, and PubMed, which researched non-pharmacological rehabilitation interventions for cognitive impairment following pediatric ischemic stroke. Keywords are as follows: pediatric, ischemic stroke, cognitive, and intervention. The search period is from the establishment of the database to January 2022.

3. Factors affecting cognitive impairment

3.1. Demographic characteristics: Chronological age

Some reports suggested that stroke onset before the age of 1 year is associated with poorer cognitive outcomes (4, 19), while other reports have found poorer outcomes tend to occur in patients below the age of 1 year and over the age of 6 years (20), with children between these two ages having better outcomes on average (19). Still, other studies suggested that children between the ages of 5 and 10 years have the best prognosis after stroke, with children outside of this age range exhibiting a poorer prognosis (11). These studies all seem to agree that stroke outcomes are poor in children under the age of 1 year and over the age of 10 years. One study reported significantly different neuropsychological outcomes when comparing strokes occurring during the perinatal period to those occurring in children (29 days−18 years old) (23). Overall, these results suggest that age is a key factor in the cognitive outcome of children after a stroke.

3.2. Stroke features

3.2.1. Lesion characteristics

Larger infarct area (20), larger lesion volume (4, 12), and simultaneous cortical and subcortical involvement (4) are all associated with poorer cognitive outcomes. One study found that Language and verbal IQ scores were significantly lower

($p < 0.01$) among patients with lesions in the left hemisphere as opposed to the right in 184 children retrospectively (24). In contrast, other studies have detected no differences in cognitive outcomes as a function of lesion laterality (14, 23). Kornfeld et al. suggested that children experience a significant reduction in their resting-state functional connection of the bilateral parietal lobes following stroke incidence while also exhibiting positively correlated reductions in processing speed and perceptual reasoning relative to healthy controls (25). Overall, these data clearly emphasize the relationship between pathological lesion characteristics and prognosis.

3.2.2. Comorbidity (epilepsy)

Many children with arterial ischemic stroke present with acute symptomatic seizures, and survivors frequently develop remote symptomatic seizures and epilepsy. Remote symptomatic seizures were defined as any seizure occurring ≥30 days after stroke ictus. Definite epilepsy was defined as ≥2 unprovoked seizures occurring ≥24 h apart (26). According to the literature, the manifestation of epilepsy after pediatric stroke varies between 13 and 67%, depending on the study population (27). The risk factors included early seizures, young age, cortical lesions, and multiple infarctions at the time of stroke (28). Approximately 20% of children experience epilepsy after ischemic stroke incidence (29). Relative to children without epilepsy, those that experience seizures generally exhibit more substantial cognitive impairment (30) and a decrease in their overall quality of life (31).

3.3. Environmental factors

The quality of the home environment contributes to outcomes in patients with ischemic stroke, suggesting that efforts to support parental and family functioning offer opportunities to optimize children's mental health and social outcomes (32). The impact of environmental factors (socioeconomic status and quality of life) on cognitive abilities (expressive and receptive language, adaptive abilities, and social abilities) increased over time after childhood stroke and even exceeded the impact of impairment factors (33). The relationship between socioeconomic status and pediatric health has been well-documented over many years (34). One study found that socioeconomic status was a better predictor of cognitive outcome in childhood arterial ischemic stroke than clinical factors (35). For example, the financial situation of the family may affect the quantity and quality of treatment, and parental education may be linked to children's cognitive reserve. Therefore, future pediatric studies on the prediction of cognitive function should effectively control participants' socioeconomic status. Most importantly, we need to pay more attention to the treatment of children with low socioeconomic status, such as providing more funding and resources.

The above reports clearly emphasize that age, pathological lesion characteristics, and epilepsy co-occurrence can all affect cognitive outcomes in children following ischemic stroke. Differences in reported findings among studies may be attributable to differences in experimental design (cross-sectional vs. longitudinal studies, variations in patient age, and/or differences in disease course) or the specific characteristics of brain development or plasticity in particular patient populations (15).

4. Treatment of cognitive impairment

Few studies to date have reported on the rehabilitation of cognitive impairment in children following brain injury, and the underlying evidence is thus limited, with research specifically focusing on post-stroke outcomes in this population being even less common. Non-pharmacological treatments for cognitive impairment that have been explored to date mainly include exercise training, psychological intervention, neuromodulation strategies, computer-assisted cognitive training, brain-computer interfaces (BCI), virtual reality (VR), music therapy, and acupuncture. The goal of rehabilitative strategies in children following brain injury is to allow children to return to their homes and schools as quickly as possible.

4.1. Physical exercise

Physical exercise has been explored as a promising neuroprotective and anti-ischemic intervention for patients with ischemic stroke (adults) and animals (36–38), with some evidence suggesting that it can regulate excitatory signal transduction to preserve neurological function (39). Exercise can also boost cerebrovascular efficacy, potentially reducing infarct size and increasing the number of viable cells surrounding the infarcted lesion (36). Such preventative physical activity can also preserve synaptic plasticity in the context of ischemia, and specific therapeutic approaches have been explored as a means of promoting plasticity and improving overall cognitive function (39). Ischemic preconditioning is an interventional approach that has been shown to be effective in individuals suffering from transient non-fatal ischemic periods, conferring adaptive intracellular changes to neuronal electrophysiological properties that can improve the ability of tissues to tolerate future ischemic events (40).

Long-term exercise training after ischemia has been found to enhance the induction of learning-dependent long-term potentiation (LTP) in the CA3 area of the hippocampus (41). Short-term moderate-intensity treadmill exercise was

also shown to improve hippocampus-dependent episodic fear memory and other cognitive functions in two rat models of ischemic brain injury (42). Further evidence suggests that exercise can enhance short-term plasticity by improving paired-pulse facilitation (PPF), which promotes the coding of situational and spatiotemporal information by enhancing hippocampal nerve regeneration and facilitating neuronal circuit reorganization (43).

In adults, some studies have found that physical exercise had a positive effect on the global cognitive functioning of patients with stroke (44–47). Wang et al. showed that the combination of physical exercise and cognitive training was more efficacious than cognitive training alone as a means of improving cognitive impairment after stroke in adults (48). Moriya et al. established that moderate-intensity aerobic exercise enhances prefrontal cortex activity and improves working memory performance in patients with post-stroke as assessed by near-infrared spectroscopy (49), while Cotman et al. observed that aerobic exercise benefits cognition, likely through the upregulation of growth factors including BDNF, IGF-1, and VEGF, thus promoting neurogenesis and angiogenesis, particularly in the hippocampus (50).

In children, from a population perspective, moderate-to-vigorous physical activity, especially vigorous physical activity (51), is associated with improved cognitive function in normal prepubertal children (51, 52), as well as in children with ADHD (53) and cerebral palsy (54). Both long-term intervention (>6 months) (51, 52) and short-term intervention (7 days) (55) increased the hippocampal gray matter volume significantly. There are also significant changes in the EEG theta and alpha band power spectra immediately after intervention (51). From the perspective of an interventional approach, different types of physical activity are thought to differentially activate children's brains either through physiological mechanisms or by activating similar brain regions during physical and cognitive tasks; specific or standardized programs are, however, lacking. There are also studies suggesting that not every child benefits from interventions in the same way and that individual differences vary widely (56). As physical fitness comprises both muscle and neuromuscular components, some researchers believe that physical fitness represents a better outcome predictor than physical activity (57).

Exercise training triggers several complex processes that can interact to protect and preserve neuronal function following ischemic injury (38), ameliorating cognitive recovery by improving synaptic plasticity and promoting new neuronal circuit reorganization. Physical exercise, thus, holds great promise as an interventional approach for treating cognitive impairment following ischemic stroke in children, although further research is necessary to understand the extent to which these preclinical findings are applicable to children with ischemic stroke.

4.2. Psychological interventions

Psychological interventions are critical means of treating cognitive impairment following ischemic stroke in children, offering guidance regarding available resources and rehabilitative strategies that can help children return to school. Such interventions are broadly divided into strategy training and cognitive retraining approaches, with some studies suggesting that strategy training is the more efficacious of the two (58).

Strategy training is the most popular psychological intervention used at present. While children present with specific cognitive deficits following ischemic stroke, their cognitive advantages can be leveraged to overcome these deficits in particular environments (59). Evidence on the utility of strategy training for the treatment of cognitive impairment is designated as NHMRC grade D, consistent with very low-grade evidence (58), although it currently remains the only recommended treatment supported by direct medical evidence. Successful implementation is dependent upon a comprehensive neuropsychological assessment of the child's cognitive deficits and advantages, as well as an understanding of their individual environmental needs. Effective communication among health professionals, families, and schools is also critical to ensure that children are placed in a supportive environment that provides them with the best possible developmental opportunities (59). One meta-analysis of patients with sickle-cell disease (SCD)-related infarct found that those undergoing psychoeducational interventions including cognitive behavioral therapy, particularly in family settings, showed positive outcomes (60). Three studies have reported on the training of working memory and memory strategy as a means of improving cognitive function in children after stroke (61–63). It has been found that tutoring combined with memory training was more effective than individual tutoring alone and was linked to more positive outcomes (61, 62).

Cognitive retraining has been a focus of increasing research interest in recent years. This strategy primarily relies on an assessment of the degree of cognitive impairment followed by training according to their specific cognitive abilities. A randomized controlled study of children with central nervous system injuries found that a cognitive remediation program (CRP) improved both attention and academic performance (64). Recla et al. showed that a 1-month intensive memory-focused training program (IM-FTP) improved children's ability to learn semantically related and irrelevant words, while also improving their immediate prose memory (65). Functional magnetic resonance imaging (fMRI) analyses of these children revealed that the IM-FTP treatment was associated with functional changes in the left lower frontal cortex. The left lower frontal gyrus is closely associated with the left posterior middle temporal gyrus, which plays a vital role in syntactic analysis (66) and vocabulary selection (67), which is why

this is an area that is stimulated during intensive memory training (65).

Through the Swedish Memory and Attention Re-Training and parental coaching program, van't Hooft et al. similarly determined that cognitive retraining of children was able to enhance attention, memory, social interaction, and parental stress outcomes (68). A meta-analysis found that most studies utilizing remote technology-based training programs reported treatment-related improvements in cognition and behavior. For example, remote computerized cognitive training can improve visual-spatial working memory (69). However, substantial heterogeneity exists among the studies published to date (70).

Due to the heterogeneity of neurological behaviors in children after central nervous system injuries, there has been no universal adaption of specific therapeutic programs. Instead, individualized interventional plans are formulated in accordance with the needs of each child. It is thus essential that schools, families, and rehabilitation teams regularly assess and discuss these plans to ensure that children are provided with appropriate environmental and educational programs capable of fostering their cognitive recovery.

4.3. Neuromodulation

Neuromodulation therapy has recently emerged as a promising therapeutic modality capable of remediating cognitive function in the context of cerebral injuries including Parkinson's disease (71) and traumatic brain injury (72). As cerebral oscillation patterns are altered following ischemia, electrical or magnetic stimulation may be able to improve overall neural network function by restoring abnormal electrical activity and plasticity (73). Several non-invasive neuromodulatory approaches have been explored as tools for improving cognitive function in children with ischemic stroke, including transcranial direct current stimulation (tDCS) (74) and repetitive transcranial magnetic stimulation (rTMS) (75). The rTMS approach utilizes a coil to generate a magnetic field capable of penetrating the scalp and inducing changes in excitability through a mechanism similar to LTP-LTD, thereby augmenting neuronal plasticity (76). One meta-analysis found that low-frequency ($\leq$1 Hz) rTMS in the unaffected hemisphere of patients (adults) suffering from post-stroke aphasia could effectively improve overall language function (77). Malone et al. posited that patients with childhood ischemic stroke may benefit from rTMS when appropriate operational parameters are employed (78), while Gillick et al. explored optimal tDCS parameters for use in the treatment of children following ischemic stroke, including current intensity, electrode size, location, and stimulation duration (79). There are also invasive neuromodulatory approaches, such as deep brain stimulation (DBS). DBS necessitates the implantation of a pair of electrodes in the brain parenchyma, with the electrodes connected to a pulse generator implanted in the chest. Much like rTMS, DBS can target specific brain regions, and parameters such as voltage intensity and frequency can be customized according to the patient's condition. Importantly, the transmission of specific electrical activity patterns *via* DBS can influence oscillatory activity (80, 81). Increased levels of brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and synaptic markers such as synaptophysin were detected within 2.5 h of DBS treatment in rats (82). DBS can also improve overall network function by enhancing synaptic plasticity and normalizing disordered oscillatory activity.

While patients with cognitive impairment after brain injury benefit from neuromodulation therapy, the mechanisms underlying these benefits are poorly understood. The development of novel non-invasive neuromodulatory technologies will offer a convenient, cost-effective, safe, and painless means of facilitating cognitive rehabilitation in children following ischemic stroke. As such, future research should focus on optimizing neuromodulatory treatment strategies by the identification of appropriate biomarkers and therapeutic parameters associated with positive patient outcomes.

4.4. Other interventions

Multimodal stimulatory approaches, including auditory, visual, olfactory, and exercise-based stimulation, can enhance neuroplasticity and promote cognitive recovery after stroke. This has been preliminarily confirmed in rat models of traumatic brain injury (83). While the current results are promising, more research is needed to make conclusive statements and successfully apply these methods to daily clinical life. Multidisciplinary collaborations help improve current neurotechnologies and provide guidance for future implementations.

4.4.1. Computer-assisted cognitive training

In recent years, computer-assisted cognitive rehabilitation has been regarded as a good alternative or supplement to traditional cognitive rehabilitation. Computer-assisted cognitive training is beneficial to improve the cognitive ability of patients and restoring the overall functional status of patients. It is widely used in cognitive impairment after stroke in adults (84, 85). However, research regarding its use for cognitive impairment in children has largely focused on psychiatric conditions such as ADHD (86) or autism spectrum disorder (87).

4.4.2. Brain-computer interfaces (BCI)

Brain-computer interfaces-based cognitive training is another emerging area in the neurorehabilitation field; this involves the reception of nerve cell signals, identifying

and classifying their activity, and translating them into computer-recognized instructions. In adults, BCI treatment of post-stroke cognitive impairment (PSCI) reportedly results in improvements in executive function (88, 89), attention (90), memory (90–92), language (91), and visuospatial abilities (91, 93). In children, Munoz et al. applied the EEG-BCI system to improve attention ability in patients with ADHD (94). Friedrich et al. introduced a BCI application combining neurofeedback and biofeedback to treat children with autism spectrum disease (95). Kim et al. found that BCI can improve logical thinking, problem-solving, and attention to external stimuli in children with spastic cerebral palsy (96). However, there are no reports on the application of this approach for treating cognitive impairment in children, excepting stroke which included cerebral palsy.

4.4.3. Virtual reality

In the past decade, VR has been widely concerned, and its technological progress has surpassed clinical research. A particular property of VR is that it creates the illusion that a person is interacting with a synthetic world. In children's cognitive rehabilitation therapy, VR is widely used, such as improving happiness, relaxation, and anxiety (97), promoting upper limb recovery after ischemic stroke (98), autism spectrum disorder (99), and intellectual disabilities (100). The application of cognitive impairment in children after stroke has not been reported.

4.4.4. Music therapy

Brain imaging studies have shown that the neural activity associated with listening to music extends far beyond the auditory cortex, involving a wide-spread bilateral network of frontal, temporal, parietal, and subcortical regions related to attention, semantic and musical syntactic processing, memory, and motor function (101, 102). In adults, regular music listening during the subacute phase of stroke promotes recovery of verbal memory and focused attention (103), and fine-grained structural reorganization (as indicated by increased gray matter volume, GMV) in the network of frontolimbic brain regions (104). In children with neurological disorders, music therapy has been found to stabilize vital signs during and after treatment, reflected by reduced heart and respiratory rates and increased oxygen saturation (105). We hypothesize that music therapy during the early stages of recovery from stroke could serve as a valuable supplement to patient care by providing an individualized, easily implemented, and inexpensive means of promoting cognitive recovery.

4.4.5. Acupuncture

Acupuncture has been shown to be a safe potential alternative intervention for the treatment of post-stroke patients with cognitive impairment (106). Its mechanism may mainly improve cognitive function after stroke by promoting synaptic plasticity (107). However, no corresponding studies have been conducted on children to date.

5. Limitations and future prospects

There is significant heterogeneity in the available studies of pediatric ischemic stroke patients due to differences in experimental design, evaluation methodology, tested interventions, and stroke subgroups. The cognitive function of children is not comparable across age groups, and as such many stroke-related cognitive deficiencies may only manifest over the course of patient growth and development. As such, larger longitudinal studies are essential to fully understand the relative value of different interventional strategies in this vulnerable patient population. To ensure access to effective personalized treatment, it is also critical that biomarkers of cognitive impairment be identified, particularly if such biomarkers can be evaluated using MRI or EEG data modeling approaches. The mechanisms whereby current treatments may benefit patient cognitive function are also not currently understood, and more basic and clinical research is thus essential to facilitate evidence-based treatment.

6. Conclusion

Stroke-related cognitive impairment in children has been a focus of increasing research interest in recent years. Impairment is influenced by patient age, pathological characteristics, combined epilepsy status, and environmental factors. Non-pharmacological treatments for cognitive impairment that have been explored to date primarily include exercise training, psychological intervention, neuromodulation strategies, computer-assisted cognitive training, BCI, VR, music therapy, and acupuncture. Most of these interventions are easily implemented and inexpensive strategies that can promote cognitive recovery. In childhood stroke, the only interventions explored in detail to date are psychological interventions and neuromodulatory strategies. However, evidence regarding the efficacy of these interventions is relatively weak. In future studies, the active application of a range of interventions is warranted to improve pediatric cognitive function, and neuroimaging and electrophysiological measurement techniques should be used to identify biomarkers capable of predicting cognitive impairment, facilitating early diagnosis, guiding treatment, and thereby improving patient prognosis. Larger multi-center prospective longitudinal studies are also required to provide more accurate evidence-based guidance for the treatment of patients with childhood stroke.

Author contributions

GX contributed to conception and design and drafted manuscript. FH, WZ, and JQ contributed to acquisition and interpretation and drafted manuscript. PZ and QZ critically revised manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1072388/full#supplementary-material

References

1. Amlie-Lefond C, Sébire G, Fullerton HJ. Recent developments in childhood arterial ischaemic stroke. *Lancet Neurol.* (2008) 7:425–35. doi: 10.1016/S1474-4422(08)70086-3

2. Greenham M, Anderson V, Cooper A, Hearps S, Ditchfield M, Coleman L, et al. Early predictors of psychosocial functioning 5 years after paediatric stroke. *Dev Med Child Neurol.* (2017) 59:1034–41. doi: 10.1111/dmcn.13519

3. Mackay MT, Steinlin M. Recent developments and new frontiers in childhood arterial ischemic stroke. *Int J Stroke.* (2019) 14:32–43. doi: 10.1177/1747493018790064

4. Westmacott R, Askalan R, MacGregor D, Anderson P, Deveber G. Cognitive outcome following unilateral arterial ischaemic stroke in childhood: effects of age at stroke and lesion location. *Dev Med Child Neurol.* (2010) 52:386–93. doi: 10.1111/j.1469-8749.2009.03403.x

5. Gordon AL, Anderson V, Ditchfield M, Coleman L, Mackay MT, Greenham M, et al. Factors associated with six-month outcome of pediatric stroke. *Int J Stroke.* (2015) 10:1068–73. doi: 10.1111/ijs.12489

6. Cooper AN, Anderson V, Hearps S, Greenham M, Gordon AL. Trajectories of motor recovery in the first year after pediatric arterial ischemic stroke. *Pediatrics.* (2017) 140:e20163870. doi: 10.1542/peds.2016-3870

7. Ferriero DM, Fullerton HJ, Bernard TJ, Billinghurst L, Daniels SR, DeBaun MR, et al. Management of stroke in neonates and children: a scientific statement from the american heart association/american stroke association. *Stroke.* (2019) 50:e51–96. doi: 10.1161/STR.0000000000000183

8. Gordon AL, Nguyen L, Panton A, Mallick AA, Ganesan V, Wraige E, et al. Self-reported needs after pediatric stroke. *Eur J Paediatr Neurol.* (2018) 22:791–6. doi: 10.1016/j.ejpn.2018.06.003

9. Bernard TJ, Rivkin MJ, Scholz K, deVeber G, Kirton A, Gill JC, et al. Emergence of the primary pediatric stroke center: impact of the thrombolysis in pediatric stroke trial. *Stroke.* (2014) 45:2018–23. doi: 10.1161/STROKEAHA.114.004919

10. Malone LA, Felling RJ. Pediatric stroke: unique implications of the immature brain on injury and recovery. *Pediatr Neurol.* (2020) 102:3–9. doi: 10.1016/j.pediatrneurol.2019.06.016

11. Pavlovic J, Kaufmann F, Boltshauser E, Capone Mori A, Gubser Mercati D, Haenggeli CA, et al. Neuropsychological problems after paediatric stroke: two year follow-up of Swiss children. *Neuropediatrics.* (2006) 37:13–9. doi: 10.1055/s-2006-923932

12. Everts R, Pavlovic J, Kaufmann F, Uhlenberg B, Seidel U, Nedeltchev K, et al. Cognitive functioning, behavior, and quality of life after stroke in childhood. *Child Neuropsychol.* (2008) 14:323–38. doi: 10.1080/09297040701792383

13. O'Keeffe F, Liégeois F, Eve M, Ganesan V, King J, Murphy T. Neuropsychological and neurobehavioral outcome following childhood arterial ischemic stroke: attention deficits, emotional dysregulation, and executive dysfunction. *Child Neuropsychol.* (2014) 20:557–82. doi: 10.1080/09297049.2013.832740

14. Westmacott R, MacGregor D, Askalan R, deVeber G. Late emergence of cognitive deficits after unilateral neonatal stroke. *Stroke.* (2009) 40:2012–9. doi: 10.1161/STROKEAHA.108.533976

15. Studer M, Boltshauser E, Capone Mori A, Datta A, Fluss J, Mercati D, et al. Factors affecting cognitive outcome in early pediatric stroke. *Neurology.* (2014) 82:784–92. doi: 10.1212/WNL.0000000000000162

16. Kim CT, Han J, Kim H. Pediatric stroke recovery: a descriptive analysis. *Arch Phys Med Rehabil.* (2009) 90:657–62. doi: 10.1016/j.apmr.2008.10.016

17. Goeggel Simonetti B, Cavelti A, Arnold M, Bigi S, Regényi M, Mattle HP, et al. Long-term outcome after arterial ischemic stroke in children and young adults. *Neurology.* (2015) 84:1941–7. doi: 10.1212/WNL.0000000000001555

18. Jacomb I, Porter M, Brunsdon R, Mandalis A, Parry L. Cognitive outcomes of pediatric stroke. *Child Neuropsychol.* (2018) 24:287–303. doi: 10.1080/09297049.2016.1265102

19. Allman C, Scott RB. Neuropsychological sequelae following pediatric stroke: a nonlinear model of age at lesion effects. *Child Neuropsychol.* (2013) 19:97–107. doi: 10.1080/09297049.2011.639756

20. Jiang B, Hills NK, Forsyth R, Jordan LC, Slim M, Pavlakis SG, et al. Imaging predictors of neurologic outcome after pediatric arterial ischemic stroke. *Stroke.* (2021) 52:152–61. doi: 10.1161/STROKEAHA.120.030965

21. Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in rodents and humans: identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol.* (2013) 106–107:1–16. doi: 10.1016/j.pneurobio.2013.04.001

22. Edwards H, Dunlop M, Mallick A, O'Callaghan F. Outcomes following childhood arterial ischaemic stroke: a Delphi Consensus on what

parents want from future research. *Eur J Paediatr Neurol*. (2015) 19:181–7. doi: 10.1016/j.ejpn.2014.12.006

23. Peterson RK, Williams TS, McDonald KP, Dlamini N, Westmacott R. Cognitive and academic outcomes following childhood cortical stroke. *J Child Neurol*. (2019) 34:897–906. doi: 10.1177/0883073819866609

24. de Montferrand C, Vassel-Hitier J, Yvon-Chaou E, Câmara-Costa H, Dellatolas G, Chevignard M. Language and cognitive outcomes after childhood stroke: theoretical implications for hemispheric specialization. *Cortex*. (2019) 120:509–23. doi: 10.1016/j.cortex.2019.07.020

25. Kornfeld S, Yuan R, Biswal BB, Grunt S, Kamal S, Delgado Rodríguez JA, et al. Resting-state connectivity and executive functions after pediatric arterial ischemic stroke. *Neuroimage Clin*. (2018) 17:359–67. doi: 10.1016/j.nicl.2017.10.016

26. Billinghurst LL, Beslow LA, Abend NS, Uohara M, Jastrzab L, Licht DJ, et al. Incidence and predictors of epilepsy after pediatric arterial ischemic stroke. *Neurology*. (2017) 88:630–7. doi: 10.1212/WNL.0000000000003603

27. von Stülpnagel C, Kutschker S, Sperl W, Berweck S, Staudt M, Berndt M, et al. Childhood stroke: long-term outcomes and health-related quality of life with a special focus on the development of epilepsy. *Neuropediatrics*. (2021) 52:469–74. doi: 10.1055/s-0041-1731805

28. López-Espejo M, Hernández-Chávez M, Huete I. Clinical and radiological risk factors for poststroke epilepsy in childhood. *Epilepsy Behav*. (2018) 88:113–6. doi: 10.1016/j.yebeh.2018.08.012

29. Malik P, Patel UK, Kaul S, Singla R, Kavi T, Arumaithurai K, et al. Risk factors and outcomes of intravenous tissue plasminogen activator and endovascular thrombectomy utilization amongst pediatrics acute ischemic stroke. *Int J Stroke*. (2021) 16:172–83. doi: 10.1177/1747493020904915

30. De Schryver EL, Kappelle LJ, Jennekens-Schinkel A, Boudewyn Peters AC. Prognosis of ischemic stroke in childhood: a long-term follow-up study. *Dev Med Child Neurol*. (2000) 42:313–8. doi: 10.1017/S0012162200000554

31. Smith SE, Vargas G, Cucchiara AJ, Zelonis SJ, Beslow LA. Hemiparesis and epilepsy are associated with worse reported health status following unilateral stroke in children. *Pediatr Neurol*. (2015) 52:428–34. doi: 10.1016/j.pediatrneurol.2014.11.016

32. Greenham M, Hearps S, Gomes A, Rinehart N, Gonzalez L, Gordon A, et al. Environmental contributions to social and mental health outcomes following pediatric stroke. *Dev Neuropsychol*. (2015) 40:348–62. doi: 10.1080/87565641.2015.1095191

33. Anderson V, Darling S, Mackay M, Monagle P, Greenham M, Cooper A, et al. Cognitive resilience following paediatric stroke: biological and environmental predictors. *Eur J Paediatr Neurol*. (2020) 25:52–8. doi: 10.1016/j.ejpn.2019.11.011

34. Poulain T, Vogel M, Sobek C, Hilbert A, Körner A, Kiess W. Associations between socio-economic status and child health: findings of a large German cohort study. *Int J Environ Res Public Health*. (2019) 16. doi: 10.3390/ijerph16050677

35. Bartha-Doering L, Gleiss A, Knaus S, Schmook MT, Seidl R. Influence of socioeconomic status on cognitive outcome after childhood arterial ischemic stroke. *Dev Med Child Neurol*. (2021) 63:465–71. doi: 10.1111/dmcn.14779

36. Pianta S, Lee JY, Tuazon JP, Castelli V, Mantohac LM, Tajiri N, et al. A short bout of exercise prior to stroke improves functional outcomes by enhancing angiogenesis. *Neuromolecular Med*. (2019) 21:517–28. doi: 10.1007/s12017-019-08533-x

37. Wang H, Niu F, Fan W, Shi J, Zhang J, Li B. Modulating effects of preconditioning exercise in the expression of ET-1 and BNP via HIF-1α in ischemically injured brain. *Metab Brain Dis*. (2019) 34:1299–311. doi: 10.1007/s11011-019-00450-z

38. Raimondo DD, Rizzo G, Musiari G, Tuttolomondo A, Pinto A. Role of regular physical activity in neuroprotection against acute ischemia. *Int J Mol Sci*. (2020) 21:1–31. doi: 10.3390/ijms21239086

39. Escobar I, Xu J, Jackson CW, Perez-Pinzon MA. Altered neural networks in the papez circuit: implications for cognitive dysfunction after cerebral ischemia. *J Alzheimers Dis*. (2019) 67:425–46. doi: 10.3233/JAD-180875

40. Neumann JT, Thompson JW, Raval AP, Cohan CH, Koronowski KB, Perez-Pinzon MA. Increased BDNF protein expression after ischemic or PKC epsilon preconditioning promotes electrophysiologic changes that lead to neuroprotection. *J Cereb Blood Flow Metab*. (2015) 35:121–30. doi: 10.1038/jcbfm.2014.185

41. Yu Q, Li X, Wang J, Li Y. Effect of exercise training on long-term potentiation and NMDA receptor channels in rats with cerebral infarction. *Exp Ther Med*. (2013) 6:1431–6. doi: 10.3892/etm.2013.1319

42. Stradecki-Cohan HM, Youbi M, Cohan CH, Saul I, Garvin AA, Perez E, et al. Physical exercise improves cognitive outcomes in 2 models of transient cerebral ischemia. *Stroke*. (2017) 48:2306–9. doi: 10.1161/STROKEAHA.117.017296

43. Vivar C, Peterson BD, van Praag H. Running rewires the neuronal network of adult-born dentate granule cells. *Neuroimage*. (2016) 131:29–41. doi: 10.1016/j.neuroimage.2015.11.031

44. Shu Y, He Q, Xie Y, Zhang W, Zhai S, Wu T. Cognitive gains of aerobic exercise in patients with ischemic cerebrovascular disorder: a systematic review and meta-analysis. *Front Cell Dev Biol*. (2020) 8:582380. doi: 10.3389/fcell.2020.582380

45. Marzolini S, Oh P, McIlroy W, Brooks D. The effects of an aerobic and resistance exercise training program on cognition following stroke. *Neurorehabil Neural Repair*. (2013) 27:392–402. doi: 10.1177/1545968312465192

46. Chin LM, Keyser RE, Dsurney J, Chan L. Improved cognitive performance following aerobic exercise training in people with traumatic brain injury. *Arch Phys Med Rehabil*. (2015) 96:754–9. doi: 10.1016/j.apmr.2014.11.009

47. Sanders L, Hortobágyi T, la Bastide-van Gemert S, van der Zee EA, van Heuvelen M. Dose-response relationship between exercise and cognitive function in older adults with and without cognitive impairment: a systematic review and meta-analysis. *PLoS ONE*. (2019) 14:e0210036. doi: 10.1371/journal.pone.0210036

48. Bo W, Lei M, Tao S, Jie LT, Qian L, Lin FQ, et al. Effects of combined intervention of physical exercise and cognitive training on cognitive function in stroke survivors with vascular cognitive impairment: a randomized controlled trial. *Clin Rehabil*. (2019) 33:54–63. doi: 10.1177/0269215518791007

49. Moriya M, Aoki C, Sakatani K. Effects of physical exercise on working memory and prefrontal cortex function in post-stroke patients. *Adv Exp Med Biol*. (2016) 923:203–8. doi: 10.1007/978-3-319-38810-6_27

50. Cotman CW, Berchtold NC, Christie LA. Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci*. (2007) 30:464–72. doi: 10.1016/j.tins.2007.06.011

51. Coe DP, Pivarnik JM, Womack CJ, Reeves MJ, Malina RM. Effect of physical education and activity levels on academic achievement in children. *Med Sci Sports Exerc*. (2006) 38:1515–9. doi: 10.1249/01.mss.0000227537.13175.1b

52. Cichy I, Kaczmarczyk M, Wawrzyniak S, Kruszwicka A, Przybyla T, Klichowski M, et al. Participating in physical classes using Eduball stimulates acquisition of mathematical knowledge and skills by primary school students. *Front Psychol*. (2020) 11:2194. doi: 10.3389/fpsyg.2020.02194

53. Choi H, Park S, Kim KK, Lee K, Rhyu HS. Acute effects of aerobic stretching, health and happiness improving movement exercise on cortical activity of children. *J Exerc Rehabil*. (2016) 12:320–7. doi: 10.12965/jer.1632602.301

54. Lakes KD, Sharp K, Grant-Beuttler M, Neville R, Haddad F, Sunico R, et al. A six week therapeutic ballet intervention improved gait and inhibitory control in children with cerebral palsy-a pilot study. *Front Public Health*. (2019) 7:137. doi: 10.3389/fpubh.2019.00137

55. Migueles JH, Cadenas-Sanchez C, Esteban-Cornejo I, Torres-Lopez LV, Aadland E, Chastin SF, et al. Associations of objectively-assessed physical activity and sedentary time with hippocampal gray matter volume in children with overweight/obesity. *J Clin Med*. (2020) 9:1080. doi: 10.3390/jcm9041080

56. de Bruijn A, van der Fels I, Renken RJ, Königs M, Meijer A, Oosterlaan J, et al. Differential effects of long-term aerobic versus cognitively-engaging physical activity on children's visuospatial working memory related brain activation: a cluster RCT. *Brain Cogn*. (2021) 155:105812. doi: 10.1016/j.bandc.2021.105812

57. Sember V, Jurak G, Starc G, Morrison SA. Can primary school mathematics performance be predicted by longitudinal changes in physical fitness and activity indicators. *Front Psychol*. (2022) 13:796838. doi: 10.3389/fpsyg.2022.796838

58. Greenham M, Knight S, Rodda J, Scheinberg A, Anderson V, Fahey MC, et al. Australian clinical consensus guideline for the subacute rehabilitation of childhood stroke. *Int J Stroke*. (2021) 16:311–20. doi: 10.1177/1747493020941279

59. Greenham M, Anderson V, Mackay MT. Improving cognitive outcomes for pediatric stroke. *Curr Opin Neurol*. (2017) 30:127–32. doi: 10.1097/WCO.0000000000000422

60. Asnani MR, Quimby KR, Bennett NR, Francis DK. Interventions for patients and caregivers to improve knowledge of sickle cell disease and recognition of its related complications. *Cochrane Database Syst Rev*. (2016) 10:CD011175. doi: 10.1002/14651858.CD011175.pub2

61. Yerys BE, White DA, Salorio CF, McKinstry R, Moinuddin A, DeBaun M. Memory strategy training in children with cerebral infarcts related to sickle cell disease. *J Pediatr Hematol Oncol*. (2003) 25:495–8. doi: 10.1097/00043426-200306000-00014

62. King AA, White DA, McKinstry RC, Noetzel M, Debaun MR. A pilot randomized education rehabilitation trial is feasible in sickle cell and strokes. *Neurology*. (2007) 68:2008–11. doi: 10.1212/01.wnl.0000264421.24415.16

63. Eve M, O'Keeffe F, Jhuty S, Ganesan V, Brown G, Murphy T. Computerized working-memory training for children following arterial ischemic stroke: a pilot study with long-term follow-up. *Appl Neuropsychol Child*. (2016) 5:273–82. doi: 10.1080/21622965.2015.1055563

64. Butler RW, Copeland DR, Fairclough DL, Mulhern RK, Katz ER, Kazak AE, et al. A multicenter, randomized clinical trial of a cognitive remediation program for childhood survivors of a pediatric malignancy. *J Consult Clin Psychol*. (2008) 76:367–78. doi: 10.1037/0022-006X.76.3.367

65. Recla M, Molteni E, Manfredi V, Arrigoni F, Nordio A, Galbiati S, et al. Feasibility randomized trial for an intensive memory-focused training program for school-aged children with acquired brain injury. *Brain Sci*. (2020) 10:430. doi: 10.3390/brainsci10070430

66. Tyler LK, Marslen-Wilson WD, Randall B, Wright P, Devereux BJ, Zhuang J, et al. Left inferior frontal cortex and syntax: function, structure and behaviour in patients with left hemisphere damage. *Brain*. (2011) 134:415–31. doi: 10.1093/brain/awq369

67. Python G, Glize B, Laganaro M. The involvement of left inferior frontal and middle temporal cortices in word production unveiled by greater facilitation effects following brain damage. *Neuropsychologia*. (2018) 121:122–34. doi: 10.1016/j.neuropsychologia.2018.10.026

68. van't Hooft I, Norberg AL. SMART cognitive training combined with a parental coaching programme for three children treated for medulloblastoma. *NeuroRehabilitation*. (2010) 26:105–13. doi: 10.3233/NRE-2010-0541

69. Corti C, Urgesi C, Poggi G, Strazzer S, Borgatti R, Bardoni A. Home-based cognitive training in pediatric patients with acquired brain injury: preliminary results on efficacy of a randomized clinical trial. *Sci Rep*. (2020) 10:1391. doi: 10.1038/s41598-020-57952-5

70. Corti C, Oldrati V, Oprandi MC, Ferrari E, Poggi G, Borgatti R, et al. Remote technology-based training programs for children with acquired brain injury: a systematic review and a meta-analytic exploration. *Behav Neurol*. (2019) 2019:1346987. doi: 10.1155/2019/1346987

71. Selznick L, Savage RC. Using self-monitoring procedures to increase on-task behavior with three adolescent boys with brain injury. *Behav Interv*. (2010) 15:243–60. doi: 10.1002/1099-078X(200007/09)15:3<243::AID-BIN59>3.0.CO;2-1

72. Pevzner A, Izadi A, Lee DJ, Shahlaie K, Gurkoff GG. Making waves in the brain: what are oscillations, and why modulating them makes sense for brain injury. *Front Syst Neurosci*. (2016) 10:30. doi: 10.3389/fnsys.2016.00030

73. Gratianne R, He JW, Yasuo N, Aaron W, Liu J. Perturbation of brain oscillations after ischemic stroke: a potential biomarker for post-stroke function and therapy. *Int J Mol Sci*. (2015) 16:25605–40. doi: 10.3390/ijms161025605

74. Gillick BT, Feyma T, Menk J, Usset M, Vaith A, Wood TJ, et al. Safety and feasibility of transcranial direct current stimulation in pediatric hemiparesis: randomized controlled preliminary study. *Phys Ther*. (2015) 95:337–49. doi: 10.2522/ptj.20130565

75. Gillick BT, Krach LE, Feyma T, Rich TL, Moberg K, Menk J, et al. Safety of primed repetitive transcranial magnetic stimulation and modified constraint-induced movement therapy in a randomized controlled trial in pediatric hemiparesis. *Arch Phys Med Rehabil*. (2015) 96:S104–13. doi: 10.1016/j.apmr.2014.09.012

76. Klomjai W, Katz R, Lackmy-Vallée A. Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Retour Au Numéro*. (2015) 58:208–13. doi: 10.1016/j.rehab.2015.05.005

77. Ren CL, Zhang GF, Xia N, Jin CH, Zhang XH, Hao JF, et al. Effect of low-frequency rTMS on Aphasia in stroke patients: a meta-analysis of randomized controlled trials. *PLoS ONE*. (2014) 9:e102557–102557. doi: 10.1371/journal.pone.0102557

78. Malone LA, Sun LR. Transcranial magnetic stimulation for the treatment of pediatric neurological disorders. *Curr Treat Options Neurol*. (2019) 21:58. doi: 10.1007/s11940-019-0600-3

79. Gillick BT, Kirton A, Carmel JB, Minhas P, Bikson M. Pediatric stroke and transcranial direct current stimulation: methods for rational individualized dose optimization. *Front Hum Neurosci*. (2014) 8:739. doi: 10.3389/fnhum.2014.00739

80. Lu M, Wei X, Loparo KA. Investigating synchronous oscillation and deep brain stimulation treatment in a model of cortico-basal ganglia network. *IEEE Trans Neural Syst Rehabil Eng*. (2017) 25:1950–8. doi: 10.1109/TNSRE.2017.2707100

81. Tsai ST, Harn HJ, Lin SZ, Tseng GF, Chen SY. Deep brain stimulation for amelioration of cognitive impairment in neurological disorders: neurogenesis and circuit reanimation. *Cell Transplant*. (2019) 28:813–8. doi: 10.1177/0963689718804144

82. Gondard E, Chau HN, Mann A, Tierney TS, Hamani C, Kalia SK, et al. Rapid modulation of protein expression in the rat hippocampus following deep brain stimulation of the fornix. *Brain Stimul*. (2015) 8:1058–64. doi: 10.1016/j.brs.2015.07.044

83. Maegele M, Lippert-Gruener M, Ester-Bode T, Sauerland S, Schäfer U, Molcanyi M, et al. Reversal of neuromotor and cognitive dysfunction in an enriched environment combined with multimodal early onset stimulation after traumatic brain injury in rats. *J Neurotrauma*. (2005) 22:772–82. doi: 10.1089/neu.2005.22.772

84. Nie P, Liu F, Lin S, Guo J, Chen X, Chen S, et al. The effects of computer-assisted cognitive rehabilitation on cognitive impairment after stroke: a systematic review and meta-analysis. *J Clin Nurs*. (2021). doi: 10.1111/jocn.16030

85. Ho HY, Chen MD, Tsai CC, Chen HM. Effects of computerized cognitive training on cognitive function, activity, and participation in individuals with stroke: a randomized controlled trial. *Neuro Rehabil*. (2022) 51:79–89. doi: 10.3233/NRE-210271

86. Mozaffari M, Hassani-Abharian P, Kholghi G, Vaseghi S, Zarrindast MR, Nasehi M. Treatment with RehaCom computerized rehabilitation program improves response control, but not attention in children with attention-deficit/hyperactivity disorder (ADHD). *J Clin Neurosci*. (2022) 98:149–53. doi: 10.1016/j.jocn.2022.02.008

87. Pellecchia M, Marcus SC, Spaulding C, Seidman M, Xie M, Rump K, et al. Randomized trial of a computer-assisted intervention for children with autism in schools. *J Am Acad Child Adolesc Psychiatry*. (2020) 59:373–80. doi: 10.1016/j.jaac.2019.03.029

88. Kober SE, Schweiger D, Reichert JL, Neuper C, Wood G. Upper alpha based neurofeedback training in chronic stroke: brain plasticity processes and cognitive effects. *Appl Psychophysiol Biofeedback*. (2017) 42:69–83. doi: 10.1007/s10484-017-9353-5

89. Lyukmanov RK, Aziatskaya GA, Mokienko OA, Varako NA, Kovyazina MS, Suponeva NA, et al. [Post-stroke rehabilitation training with a brain-computer interface: a clinical and neuropsychological study]. *Zh Nevrol Psikhiatr Im S S Korsakova*. (2018) 118:43–51. doi: 10.17116/jnevro201811808143

90. Lee TS, Goh SJ, Quek SY, Phillips R, Guan C, Cheung YB, et al. A brain-computer interface based cognitive training system for healthy elderly: a randomized control pilot study for usability and preliminary efficacy. *PLoS ONE*. (2013) 8:e79419. doi: 10.1371/journal.pone.0079419

91. Gomez-Pilar J, Corralejo R, Nicolas-Alonso LF, Álvarez D, Hornero R. Assessment of neurofeedback training by means of motor imagery based-BCI for cognitive rehabilitation. *Annu Int Conf IEEE Eng Med Biol Soc*. (2014) 2014:3630–3. doi: 10.1109/EMBC.2014.6944409

92. Yeo SN, Lee TS, Sng WT, Heo MQ, Bautista D, Cheung YB, et al. Effectiveness of a personalized brain-computer interface system for cognitive training in healthy elderly: a randomized controlled trial. *J Alzheimers Dis*. (2018) 66:127–38. doi: 10.3233/JAD-180450

93. Tonin L, Pitteri M, Leeb R, Zhang H, Menegatti E, Piccione F, et al. Behavioral and cortical effects during attention driven brain-computer interface operations in spatial neglect: a feasibility case study. *Front Hum Neurosci*. (2017) 11:336. doi: 10.3389/fnhum.2017.00336

94. Munoz JE, Lopez DS, Lopez JF, Lopez A. Design and creation of a BCI videogame to train sustained attention in children with ADHD. In: *2015 10th Computing Colombian Conference (10CCC)*. Bogotá: IEEE (2015). doi: 10.1109/ColumbianCC.2015.7333431

95. Friedrich EV, Suttie N, Sivanathan A, Lim T, Louchart S, Pineda JA. Brain-computer interface game applications for combined neurofeedback and biofeedback treatment for children on the autism spectrum. *Front Neuroeng*. (2014) 7:21. doi: 10.3389/fneng.2014.00021

96. Kim TW, Lee BH. Clinical usefulness of brain-computer interface-controlled functional electrical stimulation for improving brain activity in children with spastic cerebral palsy: a pilot randomized controlled trial. *J Phys Ther Sci*. (2016) 28:2491–4. doi: 10.1589/jpts.28.2491

97. Caruso TJ, Fonseca A, Barreau A, Khoury M, Menendez M, Wang E, et al. Real-time reorientation and cognitive load adjustment allow for broad application of virtual reality in a pediatric hospital. *J Clin Transl Res*. (2021) 7:750–3.

98. Choi YH, Ku J, Lim H, Kim YH, Paik NJ. Mobile game-based virtual reality rehabilitation program for upper limb dysfunction after ischemic

stroke. *Restor Neurol Neurosci*. (2016) 34:455–63. doi: 10.3233/RNN-150626

99. Maskey M, Rodgers J, Grahame V, Glod M, Honey E, Kinnear J, et al. A randomised controlled feasibility trial of immersive virtual reality treatment with cognitive behaviour therapy for specific phobias in young people with autism spectrum disorder. *J Autism Dev Disord*. (2019) 49:1912–27. doi: 10.1007/s10803-018-3861-x

100. Ahn SN. Combined effects of virtual reality and computer game-based cognitive therapy on the development of visual-motor integration in children with intellectual disabilities: a pilot study. *Occup Ther Int*. (2021) 2021:6696779. doi: 10.1155/2021/6696779

101. Janata P, Tillmann B, Bharucha JJ. Listening to polyphonic music recruits domain-general attention and working memory circuits. *Cogn Affect Behav Neurosci*. (2002) 2:121–40. doi: 10.3758/CABN.2.2.121

102. Popescu M, Otsuka A, Ioannides AA. Dynamics of brain activity in motor and frontal cortical areas during music listening: a magnetoencephalographic study. *Neuroimage*. (2004) 21:1622–38. doi: 10.1016/j.neuroimage.2003.11.002

103. Särkämö T, Tervaniemi M, Laitinen S, Forsblom A, Soinila S, Mikkonen M, et al. Music listening enhances cognitive recovery and mood after middle cerebral artery stroke. *Brain*. (2008) 131:866–76. doi: 10.1093/brain/awn013

104. Särkämö T, Ripollés P, Vepsäläinen H, Autti T, Silvennoinen HM, Salli E, et al. Structural changes induced by daily music listening in the recovering brain after middle cerebral artery stroke: a voxel-based morphometry study. *Front Hum Neurosci*. (2014) 8:245. doi: 10.3389/fnhum.2014.00245

105. Kobus S, Bologna F, Maucher I, Gruenen D, Brandt R, Dercks M, et al. Music Therapy Supports Children with Neurological Diseases during Physical Therapy Interventions. *Int J Environ Res Public Health*. (2022) 19:1492. doi: 10.3390/ijerph19031492

106. Hung CY, Wu XY, Chung VC, Tang EC, Wu JC, Lau AY. Overview of systematic reviews with meta-analyses on acupuncture in post-stroke cognitive impairment and depression management. *Integr Med Res*. (2019) 8:145–59. doi: 10.1016/j.imr.2019.05.001

107. Hu Y, Li R, Hao W, Li J, Chang W, Zhang Z, et al. Study on the mechanism of electroacupuncture in the treatment of cognitive impairment after stroke. *J Basic Chin Med*. (2021) 27:1186–90.

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Factors associated with functional disability in patients with acute stroke excluded from alteplase administration due to minor non-disabling neurological deficits

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Background: Although the PRISMS study did not demonstrate the benefit of intravenous alteplase administration in patients with mild stroke within 3 h, about 30% of patients presenting with mild symptoms showed unfavorable functional outcomes. We investigated the factors predictive of functional disability at 90 days in patients who were excluded from alteplase administration due to the National Institutes of Health Stroke Scale (NIHSS) scores of 0–5 and a score between 0 and 2 for each NIHSS score item.

Methods: All patients were diagnosed with acute ischemic stroke or transient ischemic attack within 4.5 h of admission to a tertiary hospital and did not receive alteplase due to a minor stroke between January 2013 and December 2020. Radiological data and clinical information were collected, including baseline and discharge NIHSS scores and modified Rankin Scale (mRS) scores at 90 days. Early neurological deterioration (END) was defined as an increase of two or more NIHSS scores. We defined moderate motor weakness as a NIHSS limb motor score of more than 3 and defined a favorable outcome as a mRS score at 90 days that was 0 or 1.

Results: During the investigation period, 400 patients did not receive alteplase. END occurred significantly more frequently in patients with large artery disease (LAD) than in those with other TOAST classifications. In the multivariate regression analysis, NIHSS per 1-point increase, presenting as moderate motor weakness, and LAD were independent predictors of poor functional outcome (OR, 1.811 NIHSS per 1-point increase; 95% confidence interval [CI], 1.503–2.182; $P < 0.0001$; OR, 2.173 moderate motor weakness; 95% CI 1.028–4.595; $P = 0.042$; OR, 2.033 LAD; 95% CI 1.099–3.762; $P = 0.024$, respectively).

Conclusion: Moderate motor weakness presentation and LAD may be important factors associated with poor functional outcomes in patients with acute stroke excluded from alteplase administration due to mild symptoms.

KEYWORDS

alteplase, acute ischemic stroke, minor, hyperacute treatment, functional outcome after acute stroke

1. Introduction

Intravenous (IV) thrombolytic treatment with alteplase, initiated within 4.5 h after the onset of symptoms, has been shown to reduce patient disability compared with placebo (1, 2). However, the effects of alteplase in patients with mild symptoms are unclear because many clinical trials involving the use of alteplase have excluded these patients (3–5).

A mild stroke symptom is the most common cause for the exclusion of thrombolytic treatment (6). However, many of these patients have recurrent events or disabilities despite mild symptoms at the initial presentation. Prospective data suggest that 30% of patients with the National Institutes of Health Stroke Scale (NIHSS) scores 0–3 or transient ischemic attack (TIA) have a functional disability 90 days after stroke (7). Therefore, to avoid disability, the number of IV alteplase administrations for patients with mild symptoms has increased (8).

The Phase IIIb, Double-Blind, Multicenter Study to Evaluate the Efficacy and Safety of Alteplase in Patients with Mild Stroke: Rapidly Improving Symptoms and Minor Neurologic Deficits (PRISMS) trial tested the efficacy and safety of IV alteplase in patients with NIHSS scores 0–5 and non-disabling deficits; however, IV alteplase administration was not beneficial within 3 h of onset (9). Based on the finding of this trial, IV alteplase is not recommended for patients with mild stroke in the current AHA/ASA guidelines (strength of recommendation class III [No benefit]; quality of evidence level B-R [Randomized]) (10). Although the PRISMS study did not demonstrate an effect in patients with mild stroke, 6–15% of patients with acute ischemic stroke presenting with minor neurological deficits initially experienced neurological deterioration during the acute period, and ∼30% of patients presenting with mild symptoms showed unfavorable functional outcomes (11, 12). Therefore, there is a need to identify factors associated with these outcomes in patients with these gray zones.

Stroke is associated with critical disabilities in humans, and the administration of alteplase is the only medical treatment for the hyperacute phase. Therefore, re-evaluation of indications is necessary, and the analysis of factors associated with poor functional outcomes among patients excluded from IV alteplase can help to modify the exclusion criteria. The objective of this study was to investigate factors associated with functional disabilities at 90 days in patients excluded from alteplase administration due to the initial NIHSS scores of 0–5 and minor non-disabling neurological deficits.

2. Methods

The local Institutional Review Board reviewed the study. It waived the requirement for ethical approval in compliance with governmental laws and regulations and informed consent since we only accessed de-identified previously collected data.

2.1. Study population

This study is a retrospective observational study conducted in a single center. Among all patients diagnosed with acute ischemic stroke or TIA within 4.5 h of admission to a tertiary hospital from 1 January 2013 to 31 December 2020, we enrolled patients with acute ischemic stroke who did not receive alteplase due to NIHSS scores of 0–5 and whose deficits were not initially disabling. Minor non-disabling acute ischemic stroke was identified as patients with baseline National Institutes of Health Stroke Scale (NIHSS) score ≤5 and a score between 0 and 2 for each NIHSS score item. We excluded patients with pre-stroke disability (modified Rankin Scale score of 2–6) and with contraindication for alteplase in the current clinical guidelines. Figure 1 shows patient flow according to inclusion and exclusion criteria. Patients who did not receive alteplase received 100 mg of oral aspirin and 75 mg of clopidogrel. According to our local protocol, patients with acute ischemic stroke or TIA within 4.5 h underwent baseline neuroimaging, such as precontrast brain computer tomography (CT), brain CT angiography, and brain magnetic resonance image (MRI), including diffusion restriction image and fluid-attenuated inversion recovery (FLAIR). In addition, all patients who were hospitalized and received dual antiplatelet therapy were checked for drug resistance tests by using a new-generation impedance aggregometer (Multiplate^{R} analyzer; Roche Diagnostics, Mannheim, Germany). Clinical management was based on institutional protocols and clinical guidelines for stroke care. The patient underwent follow-up neuroimaging (MRI) within 20–28 h after symptom onset.

2.2. Data collection and methods

Radiological data and clinical outcomes that were collected included: (1) baseline neuroimaging, NIHSS score, and mRS; (2) repeat neuroimaging after 20–28 h; (3) discharge NIHSS score, TOAST classification (consisting of small artery disease [SAD], large artery disease [LAD], cardioembolic [CE], other etiology [others], and undetermined etiology [Undetermined]), and mRS; and (4) mRS after 90 days, scored during outpatient follow-up. To compare minor non-disabling stroke presenting as mild dysarthria, ataxia, and mild motor weakness, we defined moderate motor weakness (including dysarthria and facial weakness) when the limb motor score of the NIHSS was ≥3. Early neurological deterioration (END) was defined as an increase of two or more NIHSS scores compared with the best neurological status within 7 days after stroke (13). We defined favorable functional outcomes at 90 days after stroke as a modified Rankin Scale (mRS) score of 0 or 1 (total range, 0 [symptom-free] to 6 [dead]). Fluid-attenuated inversion recovery hyperintense arteries (FLAIR-HAs) were defined as focal, tubular, or serpentine hyperintensities that correspond to a typical arterial course in the FLAIR sequence (14).

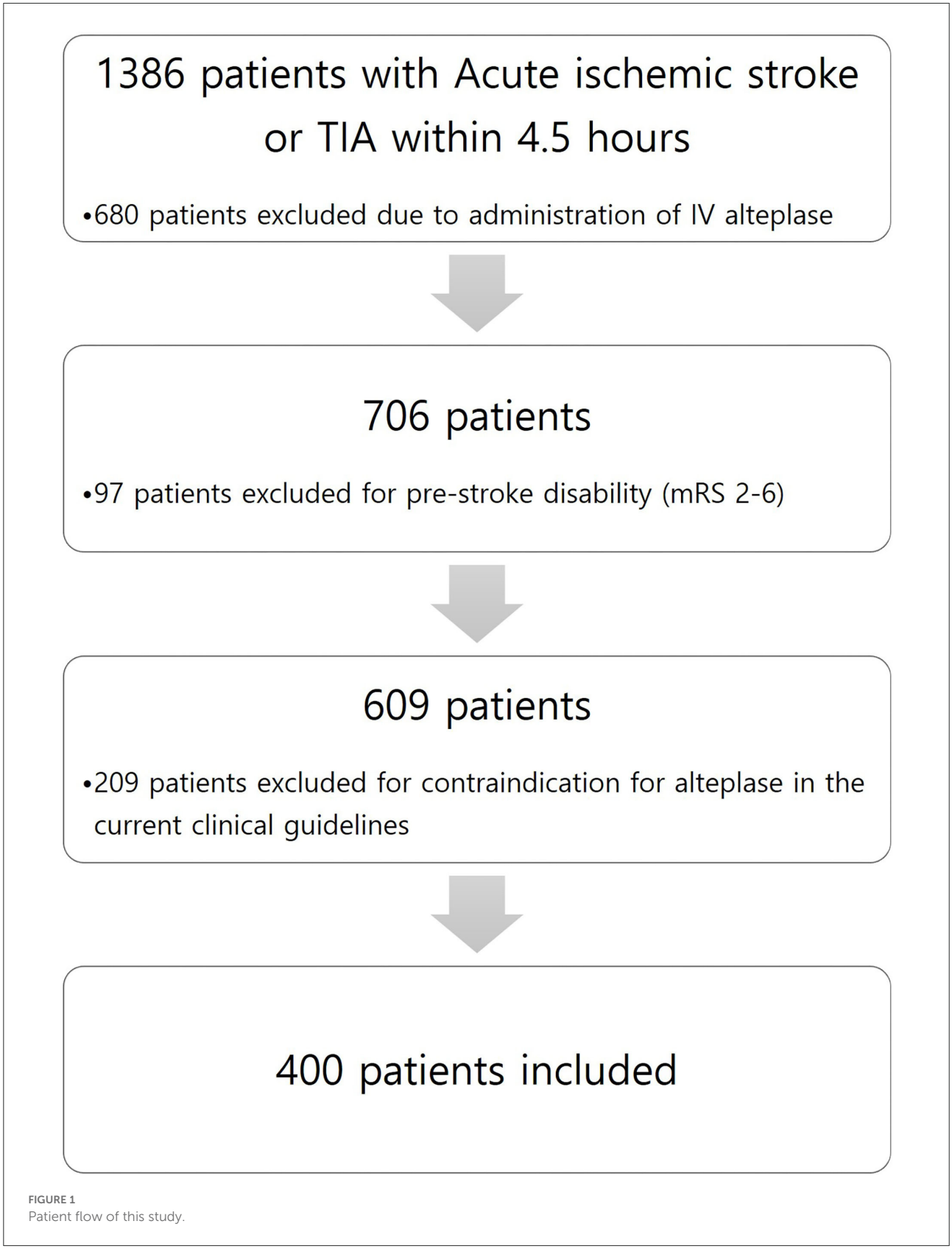

FIGURE 1
Patient flow of this study.

2.3. Statistical analyses

Categorical variables were expressed as numbers and percentages, while continuous variables were expressed as mean ± standard deviation. An analysis was performed to determine whether the etiology of cerebral infarction according to TOAST classification and factors of anterior and posterior circulation infarction affected the clinical outcome and frequency of END. Multiple regression analysis was performed to determine predictive factors for poor functional outcomes in patients who did not receive alteplase 90 days after stroke. Potential predictors ($P \leq 0.2$) in the univariate analysis were included in the full multivariate model. The final multivariate model was adjusted for sex. The study rejected the null hypotheses (no difference between the groups) if *P*-values were <0.05 and considered equivalence if the 95% confidence intervals (CIs) of risk point estimates excluded 1. Statistical Package for Social Sciences 19.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analyses.

3. Results

3.1. Characteristics of the study population

A total of 400 patients who were not administered alteplase [alteplase (–) group] were enrolled from 1 January 2013 to 31 December 2020, with a mean age of 64.8 ± 12.3 years; 246 (61.5%) of these were women. The baseline characteristics of patients are shown in Table 1. The medical risk factors of the study group were as follows: hypertension, 223 patients (55.8%); diabetes mellitus, 113 (28.3%); hyperlipidemia, 66 (16.5%); smoking, 205 (35%); and atrial fibrillation, 62 (15.5%). Notably, 57 (14.3%) and 40 (10.0%) patients had histories of stroke and coronary heart disease, respectively. The most prevalent stroke subtypes were: SAD in 127 patients (31.8%), LAD in 118 (29.5%), and CE in 86 (21.5%). The most prevalent baseline NIHSS was 0 (36.3%) in the alteplase (–) group.

3.2. Analysis for predicting poor functional outcomes at 90 days in the alteplase (–) group

There was no significant relationship in aspects of functional outcome at 3 months in the factors of anterior and posterior circulatory infarction. LAD was significantly more prevalent (42.7%, $P = 0.042$) than other TOAST classifications in 89 patients with poor functional outcomes (2–6 mRS at 90 days) (Table 2).

In the alteplase (–) group, END occurred significantly more frequently in the LAD group than in the non-LAD TOAST

TABLE 1 Baseline characteristics of this study.

	Intravenous alteplase (–) (*n* = 400)
Age, years	64.8 ± 12.3
Male, *n* (%)	246 (61.5)
Risk factors, *n* (%)	
Hypertension	223 (55.8)
Diabetes mellitus	113 (28.3)
Atrial fibrillation	62 (15.5)
Hyperlipidemia	66 (16.5)
Cardiac disease	40 (10.0)
Previous stroke	57 (14.3)
Smoking	149 (37.3)
Baseline laboratory findings	
Hemoglobin, g/dl	13.9 ± 1.7
Glucose, mg/dl	134.3 ± 49.7
Total cholesterol, mg/dl	174.3 ± 39.9
CRP, mg/dl	0.51 ± 1.69
Uric acid, mg/dl	5.5 ± 1.6
Transient ischemic attack, *n* (%)	105 (26.3)
Location of Stroke, *n* (%)	
Anterior circulation	273 (68.3)
Posterior circulation	115 (28.8)
MR negative	12 (3.0)
Stroke subtype, *n* (%)	
LAD	118 (29.5)
Intracranial stenosis	74 (67.7)
Extracranial stenosis	44 (37.3)
SAD	127 (31.8)
CE	86 (21.5)
Others	22 (5.5)
Undetermined	47 (11.8)
Baseline NIHSS, *n* (%)	
0	145 (36.3)
1	122 (30.5)
2	65 (16.3)
3	32 (8.0)
4	16 (4.0)
5	20 (5.0)
mRS score of 0 or 1 at 90 days	311 (77.8)

LAD, large artery disease; SAD, small artery disease; CE, cardioembolism; others, cryptogenic embolism and hematological coagulopathy; NIHSS, NIH Stroke Scale; MR, magnetic resonance.

TABLE 2 Analysis of functional outcome according to TOAST classification in intravenous alteplase (−) group.

Outcomes	mRS 0–1 ($n = 311$)	mRS 2–6 ($n = 89$)	*P*
Stroke subtype, *n* (%)			0.042
LAD	80 (25.7)	38 (42.7)	
SAD	104 (33.4)	23 (25.8)	
CE	71 (22.8)	15 (16.9)	
Others	17 (5.5)	5 (5.6)	
Undetermined	39 (12.5)	8 (9.0)	

LAD, large artery disease; SAD, small artery disease; CE, cardioembolism; others, cryptogenic embolism and hematological coagulopathy; NIHSS, NIH Stroke Scale.

TABLE 3 Correlation between large artery disease and early neurological deterioration in intravenous alteplase (−) group.

Outcomes	LAD ($N = 118$)	Non-LAD ($N = 282$)	*P*
Early neurological deterioration (+)	13 (11.0)	15 (5.3)	0.042
Early neurological deterioration (−)	105 (89.0)	267 (94.7)	

LAD, large artery disease.

classification (11.0 vs. 5.3%; $P = 0.042$, Table 3). However, a factor of intracranial and extracranial atherosclerotic stenosis was not significantly related to END and poor functional outcomes at 90 days. Although the univariate regression analysis demonstrated that age per 1-year increase, NIHSS per 1-point increase, FLAIR-HAs, presenting as moderate motor weakness, and LAD were significant predictors for poor functional outcomes, multivariate regression analysis revealed that NIHSS per 1-point increase, presenting as moderate motor weakness, and LAD were independent predictors for poor functional outcomes (OR, 1.811 NIHSS per 1-point increase; 95% CI 1.503–2.182; $P < 0.0001$; OR, 2.173 presenting as moderate motor weakness; 95% CI 1.028–4.595; $P = 0.042$; OR, 2.033 LAD; 95% CI 1.099–3.762; $P = 0.024$, respectively) (Table 4).

4. Discussion

In this retrospective study, 77.8% of patients with minor, non-disabling acute ischemic stroke that did not receive IV alteplase showed favorable functional outcomes at 90 days. Our study found that NIHSS per 1-point increase, presenting as moderate motor weakness, and LAD were independent predictors of poor functional outcomes at 90 days in patients with acute ischemic stroke who did not receive alteplase due to an NIHSS score of 0–5.

Our study showed that moderate motor weakness (limb motor NIHSS score ≥3) was significantly associated with poor functional outcomes at 90 days compared with minor deficits, including mild dysarthria, ataxia, and mild motor weakness. Choi et al. reported that all 15 items of the NIHSS, except sensory and extinction items, were significantly associated with unfavorable functional outcomes and that using the total NIHSS score effectively predicted functional outcomes of mild stroke (15). However, Fischer et al. reported that a minor stroke defined as a score ≤1 on every NIHSS item or NHISS ≤3 showed favorable outcomes and would be suited to the definition of minor stroke (16). Among his definitions of a minor stroke, the definition of only motor deficits, including dysarthria or ataxia with or without sensory deficit, showed a more significant disability at 3 months. This definition was less suitable for defining a minor stroke. Our findings suggest that patients with minor stroke presenting with moderate motor weakness should be cautiously considered for alteplase administration.

In this study, predictors for poor functional outcomes at 90 days were NIHSS per 1-point increase and LAD in patients who did not receive alteplase due to minor, non-disabling acute ischemic stroke within 4.5 h. There were only two drug resistance (one for aspirin and one for clopidogrel) in 28 END patients; it seems that drug resistance did not affect END. In patients with poor functional outcomes at 90 days despite presenting with minor and non-disabling deficits, the LAD subtype was found to be significantly more prevalent compared with other TOAST classifications. END occurred significantly more frequently in the LAD group than in the non-LAD TOAST group. We believe that the poor functional outcomes in the LAD group can be explained by the more frequent END associated with LAD. In the multivariate analysis, higher baseline NIHSS and LAD were predictors of poor functional outcomes. Sato et al. reported that patients who have large vessel occlusive lesions were 2.80 times more prone to unfavorable functional outcomes at 3 months among acute ischemic stroke patients presenting with NIHSS <3. In addition, Kim et al. showed that END occurred in 14.6% of patients with minor stroke (<3 NIHSS) within 6 h. The only predictor for neurological progression was large vessel occlusion (11, 17). Our results correlate well with those of previous studies. LAD may be an important factor for predicting functional outcomes and should be considered an indicator of alteplase administration in patients with minor stroke within 4.5 h.

Recently, studies on early-warning blood biomarkers and imaging markers in hyperacute cerebral infarction have been actively conducted. The plasma neurofilament light chain is being studied as an index suggesting the possibility of END in patients with acute cerebral infarction (18). Zhou et al. reported that five metabolic markers, such as sphingomyelin (18:0/14:0), 1-methylpyrrolinium, phosphatidylcholine (18:0/18:0), lysophosphatidylcholine (18:0/0:0), and phosphatidylcholine (18:2/18:2), have good diagnostic and predictive ability. The change level of these metabolites is significantly related to ischemic stroke and provides early warning for the diagnosis of

TABLE 4 Multivariate analysis of prediction for poor functional outcome.

Model 1	Estimated ORs for poor functional outcome			
	Crude (95% CI)	*p*	Adjusted (95% CI)	*p*
Age, per 1-year increase	1.034 (1.012–1.057)	0.002	–	–
NIHSS, per 1-point increase	1.755 (1.455–2.117)	<0.0001	1.811 (1.503–2.182)	<0.0001
Atrial fibrillation	1.413 (0.752–2.653)	0.283	–	–
Hypertension	1.041 (0.635–1.705)	0.874	–	–
Diabetes mellitus	1.552 (0.935–2.574)	0.089		
Cardiac disease	1.675 (0.818–3.431)	0.159		
Previous stroke	1.373 (0.725–2.603)	0.331		
Smoking	0.783 (0.473–1.297)	0.342		
Hyperlipidemia	0.476 (0.217–1.043)	0.064	–	–
Female	1.193 (0.726–1.962)	0.486	–	–
FLAIR-HAs	1.910 (1.133–3.218)	0.015	–	–
Moderate motor weakness	2.667 (1.391–5.111)	0.003	2.173 (1.028–4.595)	0.042
LAD	2.115 (1.270–3.522)	0.004	2.033 (1.099–3.762)	0.024

FLAIR-HAs, fluid-attenuated inversion recovery hyperintense arteries; LAD, large artery disease.

atherosclerosis-induced ischemic stroke (19). In acute ischemic stroke patients with severe intracranial arterial stenosis or occlusion, the asymmetrical prominent cortical vein sign might be a useful neuroimaging marker for predicting END (20). In addition, low FLAIR vascular hyperintensity ASPECTS is associated with a higher risk of END in patients who are receiving DAPT (21). Despite these various studies and attempts looking for markers of predicting END, there is no clinically proven predictor for END yet.

Although there is no objective evidence of the benefit of using alteplase in patients who present with an NIHSS score of 5 or less and disabling disability, ∼30% of patients presenting with mild ischemic stroke showed unfavorable functional outcomes at 90 days. There may be a gray zone in patients with minor ischemic stroke contraindicated for IV alteplase administration. In our study, although FLAIR-HAs were not significant in the multivariate analysis, they were a significant predictor of poor functional outcomes in the univariate analysis. The FLAIR-HAs result from retrograde flow through the collateral arterial circulation and are related to the presence of large vessel stenosis or occlusion (22). The results of our study suggested that patients with large vessel occlusive disease should be considered as candidates for IV alteplase treatment even though presenting as minor stroke, and the LAD patient group should be defined into detailed subclassification according to various imaging parameters, such as the ratio of the penumbra to the infarct core in perfusion imaging studies, FLAIR-HAs in MR images, or diffusion–perfusion mismatch ratios. Therefore, a large-scale and well-organized study is needed to define certain imaging-based criteria for alteplase administration in patients presenting with minor and non-disabling ischemic stroke.

This study had several limitations. First, it was conducted retrospectively. Second, the number of enrolled patients was relatively small. Finally, because this study was conducted in a medical center, the results need to be carefully interpreted.

There is no concrete evidence for IV thrombolysis in patients with acute ischemic stroke presenting with mild neurological deficits. However, patients presenting with moderate motor weakness or large-vessel occlusive disease showed significantly higher unfavorable functional outcomes at 90 days. Therefore, aggressive treatment with IV thrombolytic agents may be considered cautiously in acute stroke presenting as moderate motor weakness or LAD, even if the neurological deficits are mild.

Data availability statement

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

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 for

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

Author contributions

YK participated in data analysis and writing of the manuscript. SC participated in data collection and data analysis. TK, H-MP, and DJS participated in data collection. DHS acquired the funds, designed the study, involved in data analysis, writing of the manuscript, and supervised the project. All authors read and approved the final version of the manuscript.

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Conflict of interest

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

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References

1. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med.* (1995) 333:1581–7. doi: 10.1056/NEJM199512143332401

2. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D. et al. Thrombolysis with alteplase 3 to 45 hours after acute ischemic stroke. *N Engl J Med.* (2008) 359:1317–29. doi: 10.1056/NEJMoa0804656

3. Hacke W, Kaste M, Fieschi C, Toni D, Lesaffre E, Von Kummer R, et al. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke. The European Cooperative Acute Stroke Study (ECASS). *JAMA.* (1995) 274:1017–25. doi: 10.1001/jama.274.13.1017

4. Davis SM, Donnan GA, Parsons MW, Levi C, Butcher KS, Peeters A, et al. Effects of alteplase beyond 3 h after stroke in the echoplanar imaging thrombolytic evaluation trial (EPITHET): a placebo-controlled randomised trial. *Lancet Neurol.* (2008) 7:299–309. doi: 10.1016/S1474-4422(08)70044-9

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

6. Messé SR, Khatri P, Reeves MJ, Smith EE, Saver JL, Bhatt DL, et al. Why are acute ischemic stroke patients not receiving IV tPA? Results from a national registry. *Neurology.* (2016) 87:1565–74. doi: 10.1212/WNL.0000000000003198

7. Coutts SB, Modi J, Patel SK, Aram H, Demchuk AM, Goyal M. et al. What causes disability after transient ischemic attack and minor stroke? Results from the CT and MRI in the Triage of TIA and minor cerebrovascular events to identify high risk patients (CATCH) study. *Stroke.* (2012) 43:3018–22. doi: 10.1161/STROKEAHA.112.665141

8. Schwamm LH, Ali SF, Reeves MJ, Smith EE, Saver JL, Messe S, et al. Temporal trends in patient characteristics and treatment with intravenous thrombolysis among acute ischemic stroke patients at Get with the guidelines–stroke hospitals. *Circ Cardiovasc Qual Outcomes.* (2013) 6:543–9. doi: 10.1161/CIRCOUTCOMES.111.000095

9. Khatri P, Kleindorfer DO, Devlin T, Sawyer RN, Starr M, Mejilla J, et al. Effect of alteplase vs. aspirin on functional outcome for patients with acute ischemic stroke and minor nondisabling neurological deficits: the prisms randomized clinical trial. *JAMA.* (2018) 320:156–66. doi: 10.1001/jama.2018.8496

10. Warner JJ, Harrington RA, Sacco RL, Elkind MSV. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke. *Stroke.* (2019) 50:3331–2. doi: 10.1161/STROKEAHA.119.027708

11. Kim JT, Park MS, Chang J, Lee JS, Choi KH, Cho KH. Proximal arterial occlusion in acute ischemic stroke with low NIHSS scores should not be considered as mild stroke. *PLoS ONE.* (2013) 8:e70996. doi: 10.1371/journal.pone.0070996

12. Khatri P, Conaway MR, Johnston KC. Acute Stroke Accurate Prediction Study (ASAP) Investigators. Ninety-day outcome rates of a prospective cohort of consecutive patients with mild ischemic stroke. *Stroke.* (2012) 43:560–2. doi: 10.1161/STROKEAHA.110.593897

13. Kim JM, Bae JH, Park KY, Lee WJ, Byun JS, Ahn SW, et al. Incidence and mechanism of early neurological deterioration after endovascular thrombectomy. *J Neurol.* (2019) 266:609–15. doi: 10.1007/s00415-018-09173-0

14. Zhou Z, Yoshimura S, Delcourt C, Lindley RI, You S, Malavera A, et al. Thrombolysis outcomes in acute ischemic stroke by fluid-attenuated inversion recovery hyperintense arteries. *Stroke.* (2020) 51:2240–3. doi: 10.1161/STROKEAHA.119.028550

15. Choi JC, Kim BJ, Han MK, Lee SJ, Kang K, Park JM, et al. Utility of items of baseline National Institutes of Health stroke scale as predictors of functional outcomes at three months after mild ischemic stroke. *J Stroke Cerebrovasc Dis.* (2017) 26:1306–13. doi: 10.1016/j.jstrokecerebrovasdis.2017.01.027

16. Fischer U, Baumgartner A, Arnold M, Nedeltchev K, Gralla J, De Marchis GM, et al. What is a minor stroke? *Stroke.* (2010) 41:661–6. doi: 10.1161/STROKEAHA.109.572883

17. Sato S, Uehara T, Ohara T, Suzuki R, Toyoda K, Minematsu K, et al. Factors associated with unfavorable outcome in minor ischemic stroke. *Neurology.* (2014) 83:174–81. doi: 10.1212/WNL.0000000000000572

18. Wang Z, Wang S, Li Y, Wang R, Jiang L, Zheng B, et al. Biomarker of early neurological deterioration in minor stroke and proximal large vessel occlusion. *Front Neurol.* (2022) 13:1019530. doi: 10.3389/fneur.2022.1019530

19. Zhou W, Li S, Sun G, Song L, Feng W, Li R, et al. Early Warning of ischemic stroke based on atherosclerosis index combined with serum markers. *J Clin Endocrinol Metabol.* (2022) 107:1956–64. doi: 10.1210/clinem/dgac176

20. Li W, Xiao WM, Luo GP, Liu YL, Qu JF, Fang XW, et al. Asymmetrical cortical vein sign predicts early neurological deterioration in acute ischemic stroke patients with severe intracranial arterial stenosis or occlusion. *BMC Neurol.* (2020) 20:331. doi: 10.1186/s12883-020-01907-w

21. Zhu L, Gong S, Zhu X, Zhang R, Ren K, Zhu Z, et al. FLAIR vascular hyperintensity: an unfavorable marker of early neurological deterioration and short-term prognosis in acute ischemic stroke patients. *Ann Palliat Med.* (2020) 9:3144–51. doi: 10.21037/apm-20-1175

22. Shang WJ, Chen HB, Shu LM, Liao HQ, Huang XY, Xiao S, et al. The association between FLAIR vascular hyperintensity and stroke outcome varies with time from onset. *AJNR Am J Neuroradiol.* (2019) 40:1317–22. doi: 10.3174/ajnr.A6142

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ERCC1 polymorphism and its expression associated with ischemic stroke in Chinese population

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Background: Excision repair cross-complementing group 1 (*ERCC1*) was considered a potential candidate gene for ischemic stroke, and its polymorphisms might be associated with the susceptibility to ischemic stroke.

Methods: A total of 513 patients with ischemic stroke and 550 control subjects were recruited. The expression levels of *ERCC1* messenger RNA (mRNA) in peripheral blood mononuclear cells and its protein in plasma were detected by quantitative real-time PCR (*qPCR*) and enzyme-linked immunosorbent assay (*ELISA*), respectively. *Rs3212986* polymorphism of *ERCC1* was detected by PCR-restriction fragment length polymorphism (*RFLP-PCR*) and was confirmed by sequencing. The association between the *ERCC1 rs3212986* polymorphism or its expression and ischemic stroke was further analyzed.

Results: The *ERCC1* mRNA level in patients with ischemic stroke was lower than that in the control group ($P < 0.05$). However, the *ERCC1* protein level in patients with ischemic stroke was higher than that in the control group ($P < 0.05$). The A allele of *rs3212986* was associated with increased ischemic stroke risk (OR = 1.287, 95% CI = 1.076–1.540, $P = 0.006$). The association between *rs3212986* polymorphism and ischemic stroke susceptibility was found in both recessive (OR = 2.638, 95% CI = 1.744–3.989, $P < 0.001$) and additive models (OR = 1.309, 95% CI = 1.028–1.667, $P = 0.031$), respectively. Similar results were obtained in the recessive model (OR = 2.015, 95% CI = 1.087–3.704, $P = 0.026$) after adjusting for demographic information and other variables. Additionally, the level of *ERCC1* mRNA in the CC/CA genotype was higher than that in the AA genotype ($P < 0.05$).

Conclusion: It was suggested that the *ERCC1 rs3212986* polymorphism was associated with ischemic stroke susceptibility in a Chinese Han population and that an A allele of *rs3212986* was related to increased ischemic stroke risk. The altered *ERCC1* expression level caused by the *rs3212986* polymorphism might participate in the pathophysiological process of ischemic stroke.

KEYWORDS

ischemic stroke, polymorphism, susceptibility, expression, *ERCC1*

1. Introduction

Ischemic stroke is a common age-related cerebrovascular disorder, accounting for 87% of all strokes, with a high disability and mortality rate, causing a huge burden to the economy and society (1, 2). However, the pathophysiological mechanism of ischemic stroke remains unclear (3). Recently, genomic instability due to the unresolved accumulation of DNA variants was considered one of the contributors to age-related diseases (4). Vascular and endothelial function deteriorated with age because those unresolved DNA variants gradually increased, and was hypothesized as a key risk factor in the development and progression of age-related diseases, such as cardiovascular and cerebrovascular diseases. The unresolved accumulations of DNA variants promoted endothelial cell dysfunction, vascular senescence, and plaque rupture and eventually contributed to heart attacks or ischemic strokes (5). Durik et al. conducted a study to investigate whether DNA damage plays an important role in age-related vascular dysfunction in the nucleotide excision repair (NER)-defect mouse model *via* inactivated excision repair cross-complementing group 1 (*ERCC1*), a key component as a highly conserved rate-limiting enzyme in NER, and found that $ERCC1^{d/-}$ mice accelerated the development of vasodilator dysfunction and increased vascular senescence and stiffness. In addition, to discuss if DNA damage-related gene variants could have an impact on human vascular disease and in line with our murine phenotype, they performed genetic studies to evaluate the association of NER-related gene variations with carotid-femoral pulse wave velocity and found a significant association of a SNP (*rs2029298*) of the damage-specific DNA binding protein 2 (*DDB2*) gene required for DNA binding in NER with carotid-femoral pulse wave velocity. These results suggested that genomic instability due to NER-related gene variations played a key role in age-dependent vascular dysfunction as observed in animal models and in humans (6). Recently, the concept that genomic instability due to a DNA repair defect caused by the genetic removal of *ERCC1* was involved in the development of vascular aging and age-related cardiovascular and cerebrovascular diseases was confirmed in the work of Bautista-Niño et al. (7). Furthermore, mice with *ERCC1* deficiency also had elevated serum cholesterol level and change in the expression of genes involved in the metabolism of cholesterol, which were associated with vascular stiffness and have been considered traditional risk factors for ischemic stroke (8, 9). Consequently, based on the above findings, DNA excision repair-related gene variations may alter its mRNA and protein expression, which in turn accelerates vascular stiffness and increases ischemic stroke risk.

DNA excision repair includes base excision repair (BER) and NER. Ghosh et al. found lower expression of X-ray repair cross-complementing 1 (*XRCC1*) proteins required for BER in brain samples from human individuals who died of ischemic stroke compared with individuals who died of non-neurological causes. Furthermore, there was serious brain damage in $XRCC1^{+/-}$ mice than in wild-type mice. These results indicated that impaired BER might be a risk factor for ischemic stroke. Furthermore, impaired *XRCC1* was associated with increased genetic susceptibility to ischemic stroke (10). NER is another important DNA repair pathway and is critical in repairing various DNA lesions. *ERCC1*, a key component as a highly conserved rate-limiting enzyme in NER, forms a heterodimer with excision repair cross-complimentary group 4 (*ERCC4*) and functions as a structure-specific endonuclease in the incision step of the DNA repair system (11). He et al. established an *ERCC1* gene knockdown and overexpression rat middle cerebral artery occlusion (MCAO) model and found both endogenous and exogenous *ERCC1* could protect the brain against ischemic injury. There was the first evidence that *ERCC1* has a protective role in the pathophysiological process of ischemic stroke in the MCAO rat (12). In our previous study, we found that the *ERCC4* expression levels were significantly lower in patients with ischemic stroke than in healthy controls, and the 30028T/C polymorphism *(rs1799801)* of ERCC4 might be associated with ischemic stroke susceptibility. It was suggested that ERCC4, as an important component of the *ERCC1/ERCC4* heterodimer and a rate-limiting enzyme in the NER pathway, might play a key role in the pathophysiological process of ischemic stroke and that its variations were likely to increase ischemic stroke risk (13). Therefore, we hypothesized that ERCC1 polymorphism and its expressions may also be associated with ischemic stroke risk.

Genome-wide association studies have been conducted to confirm the relationship between genetic polymorphisms and susceptibility to ischemic stroke (14, 15). Recently, several common and putatively functional single nucleotide polymorphisms (SNPs) of *ERCC1* have been identified, of which *ERCC1* C118T (*rs11615*) at exon 4 without amino acid change and *ERCC1* C8092A (rs3212986) located at the 3'-untranslated region (3'-UTR) were likely to have some effects on *ERCC1* mRNA expression, which played an important role in cancer susceptibility, clinical phenotype diversity, and therapy (16–18). As everyone knows, the 3'-UTR of a gene is strongly related to regulating transcription and translation. The importance of the polymorphism from 3'-UTR is not only from their direct modification of the related gene functions but also from their genetic linkage with other causative germ-line mutations (19). The previous study showed that the *rs3212986* polymorphism of *ERCC1* has been associated with better objective response to chemotherapy among Asian patients with cancer, while studies on Caucasians have not found a significant association (20). Chen et al. conducted the first study to report a significant association of the *rs3212986* polymorphism in *ERCC1* with the risk of brain tumors and considered that the A/C *rs3212986* polymorphism, which may affect mRNA stability for *ERCC1*, also results in an amino acid substitution of lysine to glutamine in nucleolar protein (*ASE-1*) and T-cell receptor complex subunit CD3 epsilon-associated signal transducer (*CAST*) (21). It was suggested that the *rs3212986* polymorphism of *ERCC1* might play an important role in disease susceptibility. Nevertheless, there was little information about the association of ischemic stroke susceptibility with the *rs3212986* polymorphism located in the 3'-UTR of *ERCC1* and strongly related to altering its mRNA and protein expression. In the current study, we conducted a case-control study to detect *ERCC1* mRNA and protein levels and evaluated associations between the *ERCC1 rs3212986* polymorphism and ischemic stroke susceptibility in the Chinese Han population.

2. Materials and methods

2.1. Study population

The peripheral blood samples were collected from 513 patients with ischemic stroke and 550 healthy controls in the Affiliated Hospital of North Sichuan Medical College. All fresh peripheral

blood samples were collected in an EDTA-coated vacutainer tube. The fresh peripheral blood samples of patients with ischemic stroke were collected within 24 h after the stroke. A total of 84 patients with ischemic stroke and 84 healthy controls were randomly selected to analyze *ERCC1* mRNA and protein levels.

All patients with ischemic stroke were diagnosed and confirmed by computed tomography and/or magnetic resonance according to ICD-9-CM codes 433, 434, and 436 (in accordance with ICD-10 codes I63.0-9). Ischemic stroke subtypes, including large artery atherosclerosis (LAA), cardioembolism (CE), small artery occlusion (SAA), stroke of other determined etiology (SOE), and stroke of undetermined etiology (SUE), were classified based on the criteria of Trial of Org 10172 in acute stroke treatment. Ischemic stroke patients with transient ischemic attack, hemorrhagic stroke, and other strokes caused by tumors, blood disease, traumatic brain injuries, and cerebrovascular malformations were excluded. Healthy controls were free from ischemic stroke, clear ischemic changes, stroke symptoms, malignant tumor, severe hepatic and renal dysfunction, and immunological disease. All individuals enrolled were from the Han Chinese ethnic group. Informed consent was obtained from all participants, and the study protocol and consent form were approved by the Ethics Committee of North Sichuan Medical College.

2.2. Clinical data collection

Clinical data of patients with ischemic stroke and healthy controls were collected, including demographic information (age, sex, height, weight, living area, ethnicity, former/current smoking or drinking, etc.), medical history (hypertension, diabetes, coronary heart disease, prior ischemic stroke, hyperlipidemia, etc.), the main laboratory data of white blood cells (WBC), neutrophil percentage, platelet (PLT), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), very low-density lipoprotein (VLDL), red blood cell (RBC), and hemoglobin (HGB). Body mass index (BMI) was calculated as baseline weight in kilograms divided by squared height in meters (kg/m^2).

2.3. Quantitative real-time polymerase chain reaction

Peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral venous blood of enrolled individuals by Ficoll-Hypaque density gradient centrifugation with lymphocyte separation medium (TBD, China). Total RNA was extracted from PBMCs using RNAiso Plus (Takara, Japan) and subsequently reverse-transcribed using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan) according to the manufacturer's instructions.

qPCR was performed using the SYBR® Premix Ex Taq™ II kit (Takara, Japan) in the LightCycler®96 PCR Machine (Roche, Germany) according to the manufacturer's instructions. β-actin was served as an internal standard. The primer sequences of *ERCC1* were F: 5'-GAGCCTCAAGGGAAAGACTGC-3' and R: 5'-TCGCCCTGCTCTATGCTCTACT-3' (size: 132 bp). The primer sequences of *β-actin* were F: 5'-CCACGAAACTACCTTCAACTCC-3' and R: 5'-GTGATCTCCTTCTGCATCCTGT-3' (size: 132 bp). The PCR amplification was performed with a total volume of 20 μl, containing 0.4 μl each primer (0.4μM), 2μl template cDNA, 10 μl SYBR Premix Ex Taq (X2), and 0.4 μl ROX Reference Dye, adding ddH_2O to 20 μl total reaction volume. The amplification conditions were as follows: 1 cycle of predenaturation at 95°C for 60 s, 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 10 s, and a final extension at 72°C for 10 s. The standard curves of *ERCC1* and *β-actin* were generated by detecting geometric serial dilutions in the range of 10^6-10^1. The efficiencies of amplification were calculated according to the formula $E=[10^{(-1/S)}]^{-1}$, with S being the slope of the standard curve. A melting curve analysis was performed at 95°C for 5 s, 65°C for 30 s, and 97°C for 1 s. Amplification size was 132 bp for both *ERCC1* and β-actin. No template samples were used as blank or negative controls. All reactions were performed three times, and the average value of each sample was used for further data analysis. The *ERCC1* mRNA relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method ($\Delta CT=CT^{ERCC1}-CT^{\beta-actin}$; $\Delta\Delta CT=\Delta CT^{case}-\Delta CT^{control}$).

2.4. Enzyme-linked immunosorbent assay

Peripheral venous blood was centrifuged at 1,500 rpm for 10 min at 4°C to separate plasma. *ERCC1* protein levels in plasma were measured using the ELISA kits (USCN, China) according to the manufacturer's instructions at 450 nm in the microplate reader (Bio-Rad, USA). The sensitivity of the *ERCC1* ELISA kit was 0.056 ng/ml. The cut-off value was set as the mean absorbance in the negative controls. Concentrations were calculated from standard curves. *ERCC1* protein level was calculated using the following equation: $ERCC1 = 1.154^{*}OD^2 + 0.5232^{*}OD + 0.1886$ ($R^2 = 0.9984$). OD was represented by optical density. The results were expressed in nanograms per milliliter. Each sample was repeated three times.

2.5. Genotyping

Genomic DNA was extracted from 200 μl of peripheral venous blood using a commercial DNA isolation kit (BioTeke, China) according to the manufacturer's instructions and then stored at −20°C.

PCR restriction fragment length polymorphism (*PCR-RFLP*) was used to detect the *ERCC1 rs3212986* genotype. The PCR primers were F: 5′-ACCCCACTCTAGATTTACCCAGGAA-3′ and R: 5′-AAGAAGCAGAGTCAGGAAAGC-3′ (size: 442 bp). PCR was performed in a total reaction volume of 10 μl, containing 100 ng genomic DNA, 0.2 μl each primer (0.4 μM), and 4 μl 2×Taq PCR MasterMix (Qiagen, Germany), adding ddH_2O to 10 μl total reaction volume. After an initial denaturation at 94°C for 3 min, the DNA was amplified for 40 cycles at 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s, followed by a final extension at 72°C for 5 min in BIO-RAD PCR amplification instrument (BIO-RAD, USA).

The PCR products were digested with 2U *MboII* (New England Biolabs, USA) at 37°C in a reaction volume of 10 μl for 4 h. The restriction enzyme *MboII* was used to identify the genotype of *rs3212986*, and the 442 bp, 315 bp, and 127 bp restriction fragments were obtained. In the presence of the A allele, the PCR products were divided into two fragments of 315 bp and 127 bp, while products

containing the C allele were not cleavable and remained a 442 bp fragment. The variants (sizes of bands for each genotype) were determined using 3% agarose gel electrophoresis. Allele and genotype frequencies of *ERCC1 rs3212986* were determined by direct counting. For quality control and validation purposes, more than 10% of PCR-amplified samples were confirmed by DNA sequencing analysis, with 100% reported reproducibility (Supplementary Figure S1).

2.6. Statistical analysis

All data were presented as the mean±standard deviation (SD) for continuous variables and as numbers or percentages (%) for categorical variables. Differences between the case and control groups were evaluated by Student's *t*-test or the rank-sum test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. The Hardy–Weinberg equilibrium (HWE) was detected by the chi-square test. Associations between the *ERCC1 rs3212986* polymorphism and ischemic stroke susceptibility were analyzed using odds ratios (ORs) and 95% confidence intervals (CIs) calculations for the allele contrast (A vs. C), dominant (AA+CA vs. CC), recessive (AA vs. CC+CA), and additive (AA+CC vs. CA) genetic models. SPSS version 17.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis, and a two-sided *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics of the study population

The clinical characteristics of the study population were summarized in Table 1. The mean age was 68.23 ± 10.16 years for the patients with ischemic stroke (289 male patients and 224 female patients) and 67.23 ± 7.56 years for the control subjects (289 male patients and 261 female patients). There was no significant difference in age and gender between the patients with ischemic stroke and the control subjects ($P > 0.05$).

Hypertension, coronary heart disease, hyperlipidemia, diabetes, BMI> 30, and former/current drinking were more commonly seen in the patients with ischemic stroke than in the controls ($P < 0.05$). The levels of WBC, neutrophil percentage, TG, and VLDL in patients with ischemic stroke were higher than those in the control group ($P < 0.05$). Nevertheless, the levels of PLT, HDLC, RBC, and HGB in patients with ischemic stroke were lower than in the control group ($P < 0.05$). The characteristics of the study population for analyzing *ERCC1* expression levels are seen in Supplementary Table S1.

TABLE 1 Characteristics of the study population.

Characteristics	Ischemic stroke patients ($N = 513$)	Controls ($N = 550$)	*P*-value
Demographics			
Male, *N* (%)	289(56.34%)	289(52.55%)	0.218
Age, mean (SD), years	68.23 ± 10.16	67.23 ± 7.56	0.07
Past medical history, *N* (%)			
Prior ischemic stroke	115(22.42%)		
Hypertension	328(63.94%)	179(32.55%)	<0.000
Coronary heart disease	54(10.53%)	29(5.27%)	0.002
Hyperlipidemia	117(22.81%)	60(10.91%)	<0.000
Diabetes	87(15.59%)	50(9.09%)	<0.000
BMI>30	93(18.13%)	46(8.36%)	<0.000
Former/current smoking	86(16.76%)	92(16.73%)	1.000
Former/current drinking	190(37.04%)	106(19.27%)	<0.000
Main laboratory data, mean (SD)			
WBC, 10^9 /L	7.29 ± 2.64	5.67 ± 1.52	<0.000
Neutrophil percentage, %	66.53 ± 13.06	61.41 ± 8.80	<0.000
PLT, 10^{12} /L	155.71 ± 56.71	169.06 ± 51.41	<0.000
TG, mmol/L	1.60 ± 0.94	1.24 ± 0.78	<0.000
TC, mmol/L	4.43 ± 1.02	4.46 ± 0.67	0.576
HDLC, mmol/L	1.13 ± 0.37	1.33 ± 0.45	<0.000
LDLC, mmol/L	2.60 ± 0.95	2.63 ± 0.60	0.494
VLDL, mmol/L	0.79 ± 0.59	0.62 ± 0.46	<0.000
RBC, 10^{12}/L	4.35 ± 0.58	4.90 ± 2.70	<0.000
HGB, g/L	127.93 ± 24.59	141.06 ± 17.62	<0.000
TOAST classification, *N* (%)			
LAA	97(18.91%)		
SAA	300(58.48%)		
CE	53(10.33%)		
SOE	29(5.65%)		
SUE	34(6.63%)		

SD, standard deviation; BMI, body mass index; WBC, white blood cells; PLT, platelet; TC, total cholesterol; TG, triglyceride; LDLC, low density lipoprotein cholesterol; HDLC, high density lipoprotein cholesterol; VLDL, very low density lipoproteinet; RBC, red blood cell; HGB, hemoglobin; TOAST, Trial of Org 10172 in Acute Stroke Treatment; LAA, large artery atherosclerosis; CE, cardioembolism; SAA, small artery occlusion; SOE, stroke of other determined etiology; SUE, stroke of undetermined etiology.

3.2. *ERCC1* mRNA and protein expression levels

The melting curves of *ERCC1* and *ß-actin* showed a single peak, and the melting temperatures (Tm) were 85.9 and 82.0°C, respectively. Additionally, the slopes of the standard curve of *ERCC1* and *ß-actin* were −3.331 and −3.337, respectively. The amplification efficiencies of *ERCC1* and *ß-actin* were 0.995 and 0.994, respectively, according to the conversion formula. Consequently, the amplification specificity and efficiency complied with the requirements of the qPCR assay.

The *ERCC1* mRNA relative expression levels in patients with ischemic stroke and controls were 1.02 ± 0.18 and 1.60 ± 0.19, respectively. The *ERCC1* mRNA's relative expression level in patients with ischemic stroke was lower than that in the control

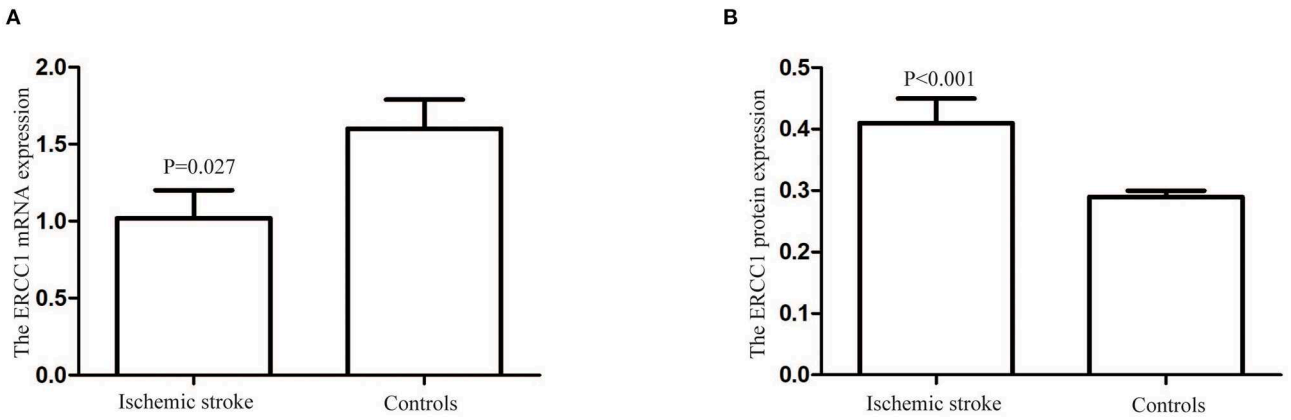

FIGURE 1
The *ERCC1* mRNA and protein levels in patients with ischemic stroke and controls. **(A)** The *ERCC1* mRNA relative expression level in patients with ischemic stroke was lower than in the controls ($P = 0.027$). **(B)** The *ERCC1* protein levels in patients with ischemic stroke were higher than in the controls ($P < 0.001$).

TABLE 2 Association between *ERCC1 rs3212986* polymorphism and ischemic stroke susceptibility.

	Allele/ genotype	Ischemic stroke patients ($N = 513$)	Controls ($N = 550$)	Crude OR (95 % CI)	Crude *P*	Adjust OR (95 % CI)[a]	Adjust *P*[a]
	Allele				0.006		
	A	384	349	1.287(1.076–1.540)			
	C	642	751	1 (Ref)			
Genetic model	Genotype						
Recessive model	AA	80	36	2.638(1.744–3.989)	<0.001	2.015(1.087–3.704)	0.026
	CC+CA	433	514	1 (Ref)		1 (Ref)	
Dominant model	AA+CA	304	313	1.101(0.863–1.406)	0.456	1.124(0.769–1.639)	0.554
	CC	209	237	1 (Ref)		1 (Ref)	
Additive model	AA+CC	289	273	1.309(1.028–1.667)	0.031	1.494(0.982-2.273)	0.061
	CA	224	277	1 (Ref)		1 (Ref)	

Ref, reference; OR, odds ratio; CI, confidence interval. [a]Adjusted for demographics information (age and gender), past medical history (hypertension, coronary heart disease, hyperlipidemia and diabetes), laboratory data (WBC, neutrophil percentage, TG, VLDL, PLT, HDLC, RBC and HGB), BMI>30 and former/current drinking.

group ($P = 0.027$, Figure 1A). However, the level of *ERCC1* protein in ischemic stroke patients (0.41 ± 0.04 ng/mL) was higher than that in controls (0.29 ± 0.01 ng/ml) ($P < 0.001$, Figure 1B).

3.3. *ERCC1 rs3212986* polymorphism and ischemic stroke susceptibility

A total of 513 patients with ischemic stroke and 550 healthy controls were successfully genotyped. The allele and genotype distributions of the *ERCC1 rs3212986* polymorphism were summarized in Table 2. The distribution of genotype *rs3212986* was not in accordance with the Hardy–Weinberg equilibrium (HWE) in the control group ($P < 0.05$).

The A allele of *rs3212986* significantly was associated with an increased risk of ischemic stroke (OR = 1.287, 95% CI = 1.076–1.540, $P = 0.006$). The association between the *ERCC1 rs3212986* polymorphism and ischemic stroke susceptibility was found in both recessive (OR = 2.638, 95% CI = 1.744–3.989, $P < 0.001$) and additive (OR = 1.309, 95% CI = 1.028–1.667, $P = 0.031$) models, respectively.

After adjusting for demographic information (age and gender), medical history (hypertension, coronary heart disease, hyperlipidemia, and diabetes), main laboratory data (WBC, neutrophil percentage, TG, VLDL, PLT, HDLC, RBC, and HGB), and other variables (BMI>30 and former/current drinking), the significant associations between *ERCC1 rs3212986* polymorphism and ischemic stroke susceptibility were also obtained in the recessive model (OR = 2.015, 95% CI = 1.087–3.704, $P = 0.026$), as shown in Table 2.

3.4. *ERCC1 rs3212986* polymorphism and its expression

The above data showed that the *ERCC1 rs3212986* polymorphism was associated with ischemic stroke susceptibility. However,

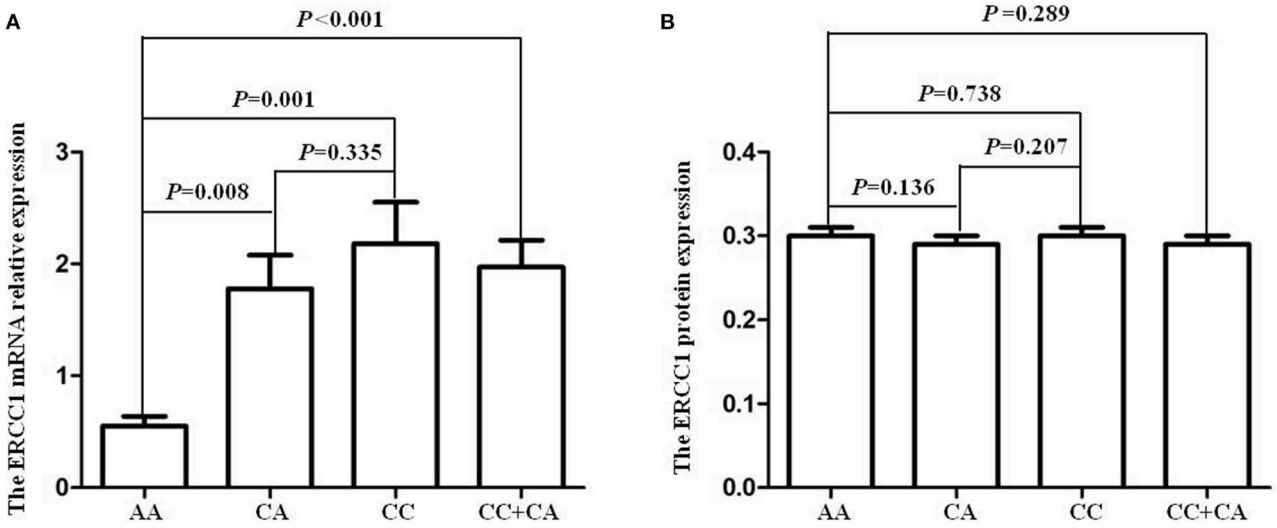

FIGURE 2
ERCC1 rs3212986 polymorphism and its expression levels. **(A)** The individuals carrying with C allele (CC/CA genotypes) of *ERCC1 rs3212986* polymorphism showed higher mRNA expression levels than those carrying the AA genotype (CC vs. AA, $P = 0.001$; CA vs. AA, $P = 0.008$; CC/CA vs. AA, $P < 0.001$). **(B)** None of the genotypes affected *ERCC1* protein expression levels ($P > 0.05$).

the effects of the *rs3212986* polymorphism on gene expression were unclear.

The results of this study showed that the *ERCC1* mRNA levels in individuals with AA, CA, CC, and CC/CA genotypes were 0.55 ± 0.09, 1.78 ± 0.30, 2.18 ± 0.37, and 1.97 ± 0.24, respectively. The *ERCC1* mRNA levels in individuals carrying the C allele (CC/CA genotypes) of the *rs3212986* polymorphism were higher than in those with the AA genotype ($P < 0.05$, Figure 2A). However, there was no significant difference in *ERCC1* plasma protein levels among different genotypes of *ERCC1 rs3212986* ($P > 0.05$, Figure 2B).

4. Discussion

This study indicated that the A allele of *ERCC1 rs3212986* was associated with increased ischemic stroke risk, and the altered *ERCC1* mRNA expression level caused by the *ERCC1 rs3212986* polymorphism might participate in the pathophysiological process of ischemic stroke.

There was the first evidence that A allele of the *ERCC1 rs3212986* polymorphism was associated with increased ischemic stroke risk. Similar results were observed after adjusting for clinical data, including demographic information, medical history, and main laboratory data. It was suggested that A allele of the *ERCC1 rs3212986* polymorphism might be a risk factor for ischemic stroke. The *ERCC1 rs3212986* polymorphism, located on the 3'-UTR of the *ERCC1* gene, has been widely studied in previous studies (16, 22, 23) and might be involved in the potential pathophysiological mechanism in ischemic stroke. *ERCC1 rs3212986* polymorphism might affect the DNA repair capacity and interfere with the NER pathway by regulating *ERCC1* transcription and translation and/or altering *ERCC1* biological activity (24, 25). The *rs3212986* polymorphism associated with altered *ERCC1* mRNA expression was found in different tissues through the GTEx database, such as skin, arteries, fibroblasts, testis, and adipose subcutaneous tissue. The site of *rs3212986* in the column "QTLhits" indicated that the site may be the expression quantitative trait loci (eQTLs) of the *ERCC1* gene in the HaploReg database, which may regulate the expression of *ERCC1*. In addition, Yu et al. predicted the *ERCC1* mRNA secondary structure between two genotypes of *rs3212986* by bioinformatics software and found that *ERCC1 rs3212986* genetic variation may affect DNA repair capacity by altering the folded stem-loop structure consisting of six repeats of "GCT", which could impact the 18-base sequence in the 3′-UTR of *ERCC1* (25). Furthermore, *ERCC1* mRNA expression may be regulated by miRNAs, which could also be affected by the *rs3212986* polymorphism in the target complementary sequence (16, 26). There were some candidate miRNAs in the *ERCC1* 3′-UTR region identified by online websites (miRNASNP-v3, miRBase, and TargetScan Release 7.2), including 4 miRNAs of target gain (hsa-miR-3185, hsa-miR-7-5p, hsa-miR-10522-5p, and hsa-miR-6077) and 2 miRNAs of target loss (hsa-miR-6828-5p and hsa-miR-671-5p) in 3'-UTR of *ERCC1*. The binding of *rs3212986* with 4 miRNAs of target gain in the 3'-UTR of *ERCC1* might result in lower *ERCC1* mRNA expression with the AA genotype. Therefore, the *ERCC1 rs3212986* polymorphism may alter its mRNA and protein expression and promote endothelial cell dysfunction, vascular senescence, and plaque rupture, which eventually contribute to accelerated vascular stiffness and increased ischemic stroke risk.

In the current study, a lower relative expression level of *ERCC1* mRNA in patients with ischemic stroke was observed. Unfortunately, a similar alteration of the expression level of the *ERCC1* protein failed to be identified. To investigate whether the *ERCC1 rs3212986* polymorphism alters *ERCC1* gene expression, the mRNA and protein expression levels between different genotypes were analyzed in this study. We found that *ERCC1* mRNA levels were higher in individuals with the C allele of the *ERCC1 rs3212986* polymorphism (CC/CA genotype) than in individuals with the AA genotype. The findings confirmed the mechanism of *ERCC1 rs3212986* polymorphism in regulating *ERCC1* transcription. In addition, genetic mutations might cause the activity of some proteins or enzymes to decrease

or even disappear (27). The previous *in vitro* studies examined the structural stability of proteins by molecular dynamics (MD) to understand the structure-function relationship and found that some non-synonymous single nucleotide polymorphisms limited the activity of proteins (28). However, no significant differences in *ERCC1* protein levels were found among subjects carrying different *ERCC1 rs3212986* genotypes. The translation or biological activity of *ERCC1* protein in different *ERCC1 rs3212986* genotypes needs to be confirmed *in vitro* studies in the future.

The discordance between the transcriptome and the proteome was also observed in a previous study and was considered to be strongly affected by the lack of temporal synchronization between the transcriptional and translational regulation levels (29, 30). In mammals, the correlation between expression levels of mRNA and protein was relatively weak, with a correlation coefficient of ∼0.40, which indicated that ∼40% of the variation in protein levels can be explained by mRNA abundances (31, 32). Therefore, transcription and translation were far from having a linear and simple relationship. The complicated and changeable mechanisms of transcription and translation generated a big regulator control system of gene expression that enhanced or repressed the synthesis of proteins from a certain copy number of mRNA molecules. Some proteins maximized all these processes with high and stable translation rates, but the transcription rates were relatively low and opposed mRNA stability. Initial anchoring of the ribosome onto the mRNA depended on the complementary binding of the Shine-Dalgarno (SD) sequence, which was considered an important factor to impact the efficiency of protein biosynthesis that contributed to 1.9–3.8% of the total variation of the mRNA-protein correlation (31, 33). Many details of the complicated biological processes of transcription and translation still need further investigation. Additionally, transient transcription of genes was limited, especially when genetic dysfunction is encountered (34). The transcription might be weak, even would not be induced or activated due to *ERCC1* deficiency, if there were severe ischemia, hypoxia, and stress stimulation in patients with ischemic stroke. Finally, the body failed to transcribe plenty of *ERCC1* mRNAs to provide the template for translation. Certainly, we cannot ignore that the sample heterogeneity may be the cause of the expression difference between mRNA in peripheral blood mononuclear cells and protein in plasma. Importantly, *ERCC1* expression levels in peripheral blood only relatively reflect the repair situation of patients with ischemic stroke to a certain extent, and future studies are needed to detect *ERCC1* expression in atherosclerotic plaque or edema area, ischemic penumbra, or ischemic core in ischemic stroke animal models and clarify the detailed function of the *ERCC1* gene in the pathophysiology of ischemic stroke.

In addition, the results indicated that neutrophils, as important laboratory indexes for inflammation, were integral to the pathology in patients with ischemic stroke, which was consistent with previous studies (35). In murine models of ischemic stroke, neutrophils were the primary cells recruited to the core area and penumbra area at the onset of ischemic stroke, accompanied by local production of cytokines or chemokines (36). Local inflammatory immune responses initiated in brain tissue might further exacerbate tissue damage and disrupt the brain-blood barrier (BBB) (37). Neutrophils might play a key role in the induction or promotion of inflammation-induced tissue damage in the presence of inappropriate and/or overactivation (38, 39).

However, there were some limitations in the study, which must be taken into account when interpreting the results. First, the results were only from a single hospital population of Han nationality, which might result in selection bias and limit the applicability to other ethnic groups. Second, some important clinical data, such as lifestyle, working conditions, or economic pressure, and some other ischemic stroke-related risk factors were lacking due to a retrospective study, which limited our evaluation of gene-environment interactions. Third, the plasma and PBMC samples from patients were selected to evaluate the biological function of the polymorphism rather than cell lines, which might influence experimental results. Fourth, the distribution of genotype *rs3212986* was not in accordance with the Hardy-Weinberg equilibrium (HWE) in the control group. As everyone knows, insufficient samples, genotyping error, and sample selection bias are the main factors of an unbalanced distribution of HWE. However, insufficient samples and genotyping errors were excluded by the GAS Power Calculator and by rechecking genotypes, respectively. Therefore, we carefully analyzed the clinical characteristics of the control group. We found that older, healthy people were included in the control group to match the age of patients with ischemic stroke. The sample selection bias was likely to be the source of the unbalanced distribution of genotype *rs3212986* in the control group. Nevertheless, the significant associations between *ERCC1 rs3212986* polymorphism and ischemic stroke susceptibility still remained after adjusting for the age factor. Furthermore, the results need to be verified by multicenter, randomized, and large-sample prospective studies.

5. Conclusion

The findings indicated that *ERCC1 rs3212986* polymorphism was associated with ischemic stroke susceptibility in a Chinese Han population, and A allele of *rs3212986* might be related to increasing ischemic stroke risk. The altered *ERCC1* mRNA expression level caused by the *ERCC1 rs3212986* polymorphism might participate in the pathophysiological process of ischemic stroke. Further prospective studies with a larger sample from multiple centers might enhance our results.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of North Sichuan Medical College. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication

of any potentially identifiable images or data included in this article.

Author contributions

X-DD, YL, and YM contributed to the conception and design of the study. J-LK, T-YC, QG, Z-LZ, M-LX, and L-ZW collected samples and performed the related experimental operations. X-DD, WZ, and HL performed the statistical analysis. X-DD and J-LK wrote the first draft of the manuscript. X-DD was mainly responsible for manuscript revision. All authors read and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.998428/full#supplementary-material

SUPPLEMENTARY FIGURE S1
The sequence maps of *ERCC1 rs3212986* polymorphism. **(A)** The sequence map of CC genotype. **(B)** The sequence map of CA genotype. **(C)** The sequence map of AA genotype.

SUPPLEMENTARY TABLE S1
Characteristics of the study population for analyzing ERCC1 expression levels.

References

1. Powers W, Rabinstein A, Ackerson T, Adeoye O, Bambakidis N, Becker K, et al. Guidelines For The Early Management Of Patients With Acute Ischemic Stroke: 2019 Update To The 2018 Guidelines For The Early Management Of Acute Ischemic Stroke: A Guideline For Healthcare Professionals From The American Heart Association/American Stroke Association. *Stroke*. (2019) 50:E344–418. doi: 10.1161/STROKEAHA.119.026917

2. Benjamin E, Virani S, Callaway C, Chamberlain A, Chang A, Cheng S, et al. Heart Disease And Stroke Statistics-2018 Update: A Report From The American Heart Association. *Circulation*. (2018) 137:E67–492. doi: 10.1161/CIR.0000000000000573

3. Zhang Q, Liao Y, Liu Z, Dai Y, Li Y, Li Y, et al. Interleukin-17 and ischemic stroke. *Immunology*. (2021) 162:179–93. doi: 10.1111/imm.13265

4. De Majo F, Martens L, Hegenbarth J, Ruhle F, Hamczyk M, Nevado Rm, et al. Genomic instability in the naturally and prematurely aged myocardium. *Proc Natl Acad Sci U S A*. (2021) 118:E2022974118. doi: 10.1073/pnas.2022974118

5. Mahmoudi M, Mercer J, Bennett M. Dna damage and repair in atherosclerosis. *Cardiovasc Res*. (2006) 71:259–68. doi: 10.1016/j.cardiores.2006.03.002

6. Durik M, Kavousi M, Van Der Pluijm I, Isaacs A, Cheng C, Verdonk K, et al. Nucleotide excision dna repair is associated with age-related vascular dysfunction. *Circulation*. (2012) 126:468–78. doi: 10.1161/CIRCULATIONAHA.112.104380

7. Bautista-Nino P, Portilla-Fernandez E, Rubio-Beltran E, Van Der Linden J, De Vries R, Van Veghel R, et al. Local endothelial dna repair deficiency causes aging-resembling endothelial-specific dysfunction. *Clin Sci (Lond)*. (2020) 134:727–46. doi: 10.1042/CS20190124

8. Smith SR. Niedernhofer L, Hetman M. Downregulation of cholesterol biosynthesis genes in the forebrain of Ercc1-deficient mice. *Neurobiol Dis*. (2012) 45:1136–44. doi: 10.1016/j.nbd.2011.12.036

9. Lee YB, Koo M, Noh E, Hwang SY, Kim JA, Roh E, et al. Myocardial infarction, stroke, and all-cause mortality according to low-density lipoprotein cholesterol level in the elderly, a nationwide study. *Diabetes Metab J*. (2022) 46:722–32. doi: 10.4093/dmj.2021.0225

10. Ghosh S, Canugovi C, Yoon J, Wilson D, Croteau D, Mattson M, et al. Partial loss of the DNA repair scaffolding protein, Xrcc1, results in increased brain damage and reduced recovery from ischemic stroke in mice. *Neurobiol Aging*. (2015) 36:2319–30. doi: 10.1016/j.neurobiolaging.2015.04.004

11. Faridounnia M, Folkers G, Boelens R. Function and interactions of ercc1-Xpf In Dna damage response. *Molecules*. (2018) 23:3205. doi: 10.3390/molecules23123205

12. He KY, Shen D, Zhang L, Lu S, Sun F. Excision repair cross-complementing 1 expression protects against ischemic injury following middle cerebral artery occlusion in the rat brain. *Gene Ther*. (2009) 16:840–8. doi: 10.1038/gt.2009.48

13. Ma Y, Deng X, Feng Y, Zhang W, Wang S, Liu Y, et al. Association of Xpf levels and genetic polymorphism with susceptibility to ischemic stroke. *J Mol Neurosci*. (2016) 59:168–76. doi: 10.1007/s12031-016-0729-4

14. Liu X, Wang Q, Zhu R. Association of Gwas-susceptibility loci with ischemic stroke recurrence in a Han Chinese population. *J Gene Med*. (2021) 23:E3264. doi: 10.1002/jgm.3264

15. Keene KH. Bis J, Kittner S, Mitchell B, Cheng Y, et al. Genome-wide association study meta-analysis of stroke in 22 000 individuals of African descent identifies novel associations with stroke *Stroke*. (2020) 51:2454–63. doi: 10.1161/STROKEAHA.120.029123

16. Xue P, Zhang G, Zhang H, Cui S, Zhang L, Yu T, et al. A Mir-15a related polymorphism affects Nsclc prognosis via altering Ercc1 repair to platinum-based chemotherapy. *J Cell Mol Med*. (2022) 26:5439–51. doi: 10.1111/jcmm.17566

17. Zhang Y, Cao S, Zhuang C, Chen J, Chen X, Sun H, et al. Ercc1 Rs11615 polymorphism and chemosensitivity to platinum drugs in patients with ovarian cancer: a systematic review and meta-analysis. *J Ovarian Res*. (2021) 14: 80. doi: 10.1186/s13048-021-00831-y

18. Koutsoukos K, Andrikopoulou A, Dedes N, Zagouri F, Bamias A, Dimopoulos M. Clinical perspectives of Ercc1 in bladder cancer. *Int J Mol Sci*. (2020) 21:8829. doi: 10.3390/ijms21228829

19. Yin M, Yan J, Voutsina A, Tibaldi C. Christiani Dc, Heist Rs, et al. No evidence of an association of Ercc1 And Ercc2 polymorphisms with clinical outcomes of platinum-based chemotherapies in non-small cell lung cancer: a meta-analysis. *Lung Cancer*. (2011) 72:370–7. doi: 10.1016/j.lungcan.2010.10.011

20. Liao W, Ho C, Tsai T, Chen K, Shih J, Yu C. Combined effect of Ercc1 And Ercc2 polymorphisms on overall survival in non-squamous non-small-cell lung cancer patients treated with first-line pemetrexed/platinum. *Lung Cancer*. (2018) 118:90–6. doi: 10.1016/j.lungcan.2018.01.011

21. Chen P, Wiencke J, Aldape K, Kesler-Diaz A, Miike R, Kelsey K, et al. Association of an ERCC1 polymorphism with adult-onset glioma cancer. *Cancer Epidemiol Biomarkers Prev*. (2000) 9:843–7.

22. Iwata H, Umeyama Y, Liu Y, Zhang Z, Schnell P, Mori Y, et al. Evaluation of the association of polymorphisms with palbociclib-induced neutropenia: pharmacogenetic analysis of paloma-2/-3. *Oncologist*. (2021) 26:E1143–55. doi: 10.1002/onco.13811

23. Zhang S. Wang Xb, Han Yd, Xiong Cl, Zhou Y, Wang C, et al. Polymorphism In Ercc1 confers susceptibility of coronary artery disease and severity of coronary artery atherosclerosis in a Chinese Han Population. *Sci Rep*. (2017) 7:6407. doi: 10.1038/s41598-017-06732-9

24. Formica V, Doldo E. Antonetti Fr, Nardecchia A, Ferroni P, Riondino S, et al. Biological and predictive role of Ercc1 polymorphisms in cancer. *Crit Rev Oncol Hematol*. (2017) 111:133–43. doi: 10.1016/j.critrevonc.2017.01.016

25. Alters Dna repair capacity via regulating ercc1 expression. *Cancer Med*. (2018) 7:6317–30. doi: 10.1002/cam4.1842

26. Zhang Q, Zheng X, Li X, Sun D, Xue P, Zhang G, et al. The polymorphisms of Mirna-binding site in Mlh3 And Ercc1 were linked to the risk of colorectal cancer in a case-control study. *Cancer Med*. (2018) 7:1264–74. doi: 10.1002/cam4.1319

27. Pinson A, Yarbrough A, Bush J, Cabanlong C, Shoeib A, Jackson B, et al. Metabolism, Cb1 cannabinoid receptor binding and in vivo activity of synthetic cannabinoid 5f-Akb48: implications for toxicity. *Pharmacol Biochem Behav*. (2020) 195:172949. doi: 10.1016/j.pbb.2020.172949

28. Madero Ayala PM, Ramos Ibarra M. A molecular dynamics approach on the Y393c variant of protein disulfide isomerase A1. *Chem Biol Drug Des*. (2020) 96:1341–7. doi: 10.1111/cbdd.13700

29. Kristensen A, Gsponer J, Foster L. Protein synthesis rate is the predominant regulator of protein expression during differentiation. *Mol Syst Biol*. (2013) 9:689. doi: 10.1038/msb.2013.47

30. Perl K, Ushakov K, Pozniak Y, Yizhar-Barnea O, Bhonker Y, Shivatzki S, et al. Reduced changes in protein compared to Mrna levels across non-proliferating tissues. *Bmc Genomics*. (2017) 18:305. doi: 10.1186/s12864-017-3683-9

31. Sousa AD, Penalva L, Marcotte E, Vogel C. Global signatures of protein and Mrna expression levels. *Mol Biosyst*. (2009) 5:1512–26. doi: 10.1039/b908315d

32. Maier T, Guell M, Serrano L. Correlation of Mrna and protein in complex biological samples. *FEBS Lett*. (2009) 583:3966–73. doi: 10.1016/j.febslet.2009.10.036

33. Nie L, Wu G, Zhang W. Correlation of Mrna expression and protein abundance affected by multiple sequence features related to translational efficiency in Desulfovibrio vulgaris: a quantitative analysis. *Genetics*. (2006) 174:2229–43. doi: 10.1534/genetics.106.065862

34. Tsai C. Sauna Ze, Kimchi-Sarfaty C, Ambudkar Sv, Gottesman Mm, Nussinov R. Synonymous Mutations And Ribosome Stalling Can Lead To Altered Folding Pathways And Distinct Minima. *J Mol Biol*. (2008) 383:281–91. doi: 10.1016/j.jmb.2008.08.012

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

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

37. Shi Y, Zhang L, Pu H, Mao L, Hu X, Jiang X, et al. Rapid endothelial cytoskeletal reorganization enables early blood-brain barrier disruption and long-term ischemic reperfusion. *Brain Injury Nat Commun*. (2016) 7:10523. doi: 10.1038/ncomms10523

38. He L, Wang J, Wang F, Zhang L, Zhang L, Zhao W. Increased neutrophil-to-lymphocyte ratio predicts the development of post-stroke infections in patients with acute ischemic stroke. *BMC Neurol*. (2020) 20:328. doi: 10.1186/s12883-020-01914-x

39. Weisenburger-Lile D, Dong Y, Yger M, Weisenburger G. Polara Gf, Chaigneau T, et al. Harmful neutrophil subsets in patients with ischemic stroke. *Neurol Neuroimmunol Neuroinflamm*. (2019) 6:E571. doi: 10.1212/NXI.0000000000000571

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Development and external validation of a prognostic model for occult atrial fibrillation in patients with ischemic stroke

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Objective: Currently, the risk of occult atrial fibrillation (AF) could not be predicted in patients with acute ischemic stroke (AIS) using a simple scoring system. Therefore, in this study, we developed and externally validated a nomogram to predict occult AF in patients with AIS.

Methods: In this study, we prospectively conducted a development cohort study with data collected at our stroke center from July 2017 to February 2018, and an external validation cohort from March 2019 to December 2019.

Results: Follow-up data were collected from 177 participants (56.5% older than 65 years, 29.4% female) for generating the nomogram model. Multivariate logistic regression analysis was performed with AF as the dependent variable indicated that age >65 years, heart rate >100, C-reactive protein (CRP), N-terminal pro-B-type natriuretic peptide (NT-proBNP) >270, hemorrhagic transformation (HT) as independent variables for predicting the development of AF, and a nomogram was generated based on these factors. The area under the receiver operating characteristic curve (AUC-ROC) for the model was 0.937, the C-index was 0.926, and the AUC-ROC for the validation cohort was 0.913.

Conclusion: To our knowledge, this is the first nomogram developed and externally validated in a stroke center cohort for individualized prediction of risk of developing AIS in patients with occult AF. This nomogram could provide valuable information for the screening of occult AF after a stroke.

KEYWORDS

acute ischemic stroke, occult atrial fibrillation, nomogram, secondary prevention, cardioembolism

Introduction

Atrial fibrillation (AF) increases the risk of stroke by four to five times (1), and according to early estimates of the prevalence of AF, 15%−20% of strokes are caused by AF (2). AF-related stroke generally has a poor clinical outcome, although anticoagulation is largely preventable (64% lower risk of stroke and 25% lower mortality) (3). Currently, 12 million ischemic stroke cases are reported each year, and one in four strokes and half of transient ischemic attacks (TIAs) have no established cause after standard diagnostic

testing and are marked as "cryptogenic" (4). Undiagnosed AF is believed as the cause of many cryptogenic strokes (CSs); however, anticoagulation is not indicated unless AF is proven. Because patients with AF have an increased risk of recurrent stroke, improved detection and treatment strategies for AF are expected to reduce the burden of recurrent stroke. The detection of AF has a substantial impact on the treatment decisions of patients with ischemic stroke. Subclinical AF is one of the pathogenesis of embolic stroke of unknown origin (ESUS) (5). According to statistics, AF accounts for approximately 8%−15% of ESUS cases (5, 6); even with direct oral anticoagulation, empirical anticoagulation is no better than aspirin in preventing recurrent strokes without documented AF (7, 8). Therefore, in the absence of documented AF, aspirin remains the standard antithrombotic therapy (9). Nevertheless, in patients with ESUS, the annual recurrence rate with standard antithrombotic therapy remains as high as 5% (10). Therefore, early etiological classification of patients with acute ischemic stroke (AIS) can select a reasonable secondary prevention program for the patients. However, the screening of some types of AF is not easy, and accurate etiological classification is highly dependent on the results of complete auxiliary examinations. Occult AF is often transient and asymptomatic, so there are challenges in clinical screening work (11, 12). The Chinese National Stroke Registry data showed that the proportion of stroke or TIA patients with AF in China is only 6.7% (13), while a large-scale registration study conducted in Europe showed that the proportion is 38% (14); thus, it can be seen that the detection rate of AF in China is significantly lower than that in Europe. Similarly, in terms of secondary prevention in patients with AIS complicated by AF, the situation in China is also significantly worse than that in developed countries. Compared with foreign countries, in China, the diagnosis rate of AF is low, and the missed diagnosis rate is high. Screening for this risk factor makes the diagnosis of occult AF difficult. If a reliable screening method can be found to preliminarily predict the possibility of cerebral embolism caused by occult AF in patients with AIS, it can guide further targeted examinations to determine whether it is occult AF, which will greatly improve the diagnosis rate of occult AF. At present, there is no well-recognized and very effective primary screening method for differential diagnosis. Therefore, the importance of screening AF is obvious. Screening high-risk patients, examining them more accurately, and improving the etiological diagnosis of occult AF will not only affect the direction of treatment but also guide the choice of stroke prevention programs in this group.

Methods

Study design and patient enrollment

This is a longitudinal study aimed at developing and validating prediction models. A total of 401 patients with AIS who were hospitalized in the Stroke Center of the Tenth People's Hospital affiliated with Tongji University in Shanghai from July 2017 to February 2019 were consecutively recruited. Participants met the following criteria: (1) 18 years of age or older and (2) diagnosed with AIS based on diffusion-weighted magnetic resonance imaging (MRI) within 1 week, and patients were excluded if key data were missing. The validation cohort included 65 stroke subjects without previous documented AF who were admitted from the same stroke center between March 2019 and December 2019. The inclusion/exclusion criteria for the development cohort also apply to the external validation cohort. AF was defined as a prior history or diagnosis of AF after hospital discharge. A history of a previous stroke was defined as a history of a previous ischemic stroke or TIA. According to our stroke center clinical reference value range, a high heart rate on admission was defined as a heart rate >100 beats/min, high TnT was defined as a TnT >0.014 ng/ml, and high NT-proBNP was defined as an NT-proBNP >270 pg/ml. Age and AF-related ROC curves showed an optimum sensitivity and specificity trade-off at age 65 years, so patients were grouped according to the optimal age cutoff of 65 years, the elderly patients are defined as those of age >65 years. HT is defined as the first head CT/MRI after AIS without bleeding, and the second head CT/MRI examination hemorrhagic infarction can be identified by the finding of intracranial hemorrhage or by the first CT/MRI findings. Multiple ischemic lesions in MRI are defined as infarcts caused by the occlusion of two or more different cerebral vessels in the blood supply system. Shanghai Tenth People's Hospital Ethics Committee approved this study, and all participants and their carers provided written informed permission.

Data collection

Demographic data such as sex and age, current medical history, vascular risk factors, and the National Institutes of Health Stroke Scale (NIHSS) admission scores were collected. Laboratory test data such as routine blood count, blood biochemistry, and coagulation function were collected from all patients based on medical records during hospitalization. Relevant imaging data, including brain imaging, routine electrocardiogram, transthoracic echocardiography, and 24-h Holter heart rate test were also collected. All patients included in the external examination of the model were examined by a 24-h dynamic electrocardiogram. Based on whether AF was present or not, patients were divided into AF and non-AF groups.

Statistical analysis

Continuous quantitative variables are shown as mean and standard deviation, while categorical variables are shown as frequencies and ratios. First, the normal distribution of

TABLE 1 Baseline and procedural characteristics.

Characteristics	Non-AF ($n = 110$)	AF ($n = 67$)	*P*-values
Age>65	44 (40%)	56 (83.6%)	0.0001
Female, n (%)	25 (22.7%)	27 (40.3%)	0.013
NIHSS on admission, means (SD)	2.51 (2.79)	5.07 (4.72)	0.0001
Vascular risk factors, n (%)			
Hypertension	87 (79.1%)	44 (65.7%)	0.048
Diabetes mellitus	48 (43.6%)	23 (34.3%)	0.220
Previous stroke	16 (14.5%)	19 (28.4%)	0.025
Heart rate	74.25 (13.54)	87.48 (24.28)	0.0001
Heart rate >100	21 (19.1%)	23 (34.3%)	0.023
Aortic sinus inner diameter	35.05 (4.31)	33.70 (3.82)	0.037
Left atrial diameter	37.41 (4.25)	42.73 (6.95)	0.0001
Laboratory findings			
C-reactive protein, means (SD)	5.80 (8.53)	15.57 (24.09)	0.002
WBC, means (SD)	7.50 (7.72)	7.79 (3.30)	0.724
Platelet count (×109/L)	212.62 (50.97)	196.46 (53.54)	0.046
PT, means (SD)	11.29 (1.02)	11.52 (1.11)	0.149
INR, means (SD)	0.97 (0.09)	1.39 (2.24)	0.131
APTT, means (SD)	28.09 (3.26)	28.95 (6.62)	0.323
Fibrinogen (g/L)	3.02 (0.69)	3.15 (0.75)	0.239
D-Dimer, means (SD)	0.78 (1.16)	1.52 (2.59)	0.025
Triglyceride (mmol/L)	1.61 (1.00)	1.22 (0.62)	0.004
Total cholesterol (mmol/L)	4.16 (1.16)	4.02 (1.08)	0.429
HDL-C (mmol/L)	1.05 (0.23)	1.13 (0.31)	0.025
LDL-C (mmol/L)	2.62 (0.97)	2.37 (0.97)	0.102
TnT (ng/mL)	0.02 (0.03)	0.03 (0.04)	0.024
TnT > 0.014, n (%)	35 (31.8%)	44 (65.7%)	0.0001
NT-proBNP (pg/ml)	278.18 (710.79)	1,811.60 (1,952.17)	0.0001
NT-proBNP > 270, n (%)	21 (19.1%)	61 (91%)	0.0001
Hemorrhagic transformation, n (%)	2 (1.8%)	12 (17.9%)	0.0001
Multiple ischemic lesions in MRI, n (%)	51 (46.4%)	42 (62.7%)	0.035

NIHSS, National Institute of Health Stroke; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TnT, troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

TABLE 2 Multivariate analysis for predictors of AF-related stroke ($n = 177$).

Variable	OR	95%CI	*P*-values
Age>65	4.95	1.18–20.76	0.029
Female	3.41	0.81–14.32	0.094
NIHSS on admission	1.05	0.88–1.25	0.574
Hypertension	0.30	0.08–1.16	0.080
Previous stroke	1.48	0.39–5.59	0.563
Heart rate>100	8.04	1.99–32.48	0.003
Aortic sinus inner diameter	1.00	0.84–1.18	0.975
Left atrial diameter	1.11	0.99–1.25	0.078
CPR	1.06	1.00–1.11	0.042
Platelet count	1.01	1.00–1.02	0.173
D-Dimer	0.92	0.55–1.53	0.743
Triglyceride	0.75	0.32–1.78	0.516
HDL-C	1.28	0.17–9.40	0.810
TnT > 0.014	3.02	0.74–12.29	0.124
Pro-BNP > 270	20.01	4.27–93.74	0.0001
Multiple ischemic lesions in MRI	1.06	0.32–3.46	0.924
Hemorrhagic transformation	32.24	2.47–420.79	0.008

NIHSS, National Institute of Health Stroke; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; TnT, troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

quantitative variables was determined using the Kolmogorov–Smirnov test. Student's *t*-test was then used to compare normally distributed quantitative data, while the chi-square test was employed to compare qualitative variables. The Mann–Whitney *U*-test was used to compare non-normally distributed variables. Based on the odds ratio (OR) and 95% confidence interval (CI), we conducted a logistic regression analysis with a probability of entry set to 0.05 and a probability of removal set to 0.10. To build the prediction model, variables with a *p*-value of <0.05 in the multivariate analysis were incorporated into the R language. AUC-ROC curves were used to calculate the predictive accuracy of nomogram models to differentiate patients with AF. Then, we used the bootstrap method (1,000 resamplings) for internal verification and to calculate a revised C-index, which is equivalent to a range of 0.5–1.0 for AUC-ROC. The higher the score, the more accurate the prediction. Models built from the development cohort were then applied to an external validation cohort, and performance was evaluated by AUC-ROC. Calibration of the risk prediction model in the development cohort was performed by comparing the observed AF probabilities according to the nomogram-based total score with the nomogram-based predicted probabilities and assessed using the Hosmer–Lemeshow test whether event rates observed

in patients with AIS match expected rates. In all cases, a *P*-value of <0.05 is considered statistically significant. We used R software version 3.6.2 (2019 R Foundation for Statistical Computing platform), SPSS 24 (IBM Corporation, New York, USA), and GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA) for the analysis.

Results

Development cohort characteristics

Of all 401 patients, 224 were excluded from data analysis due to incomplete examination and lack of baseline data; finally, 177 patients were analyzed. Compared with included patients, those excluded due to the lack of baseline data had no significant difference in terms of age >65 (59.8 vs. 56.5%), female gender (30.8 vs. 29.4%), NIHSS on admission (3.53 ± 3.88 vs. 3.48 ± 3.84), hypertension (66.1 vs. 74%), diabetes mellitus (37.5 vs. 40.1%), AF (29.0 vs. 37.9%), and p >0.05 for all (Supplementary Table S1). As shown in Table 1, the mean age was 68.7 ± 12.1 years, 52 patients (29.3%) were women, and the mean NIHSS score at presentation was 3.5 ± 3.1. A total of 67 patients (49 were previously diagnosed, and 18 were diagnosed after discharge) were identified as having AF. Univariate analysis found that age >65, sex, NIHSS score, hypertension, history of the previous stroke, heart rate >100, aortic sinus diameter, left atrial diameter, CRP, platelet count, D-D dimer, triglyceride, high-density lipoprotein, TnT > 0.014, NT-proBNP > 270, HT, and multiple ischemic lesions in MRI were significantly different between AF and non-AF groups ($P < 0.05$ for all). Multivariate logistic regression was conducted with these factors.

The development of an individualized prediction model

In binary logistic analysis, sex, NIHSS score, hypertension, history of previous stroke, heart rate, aortic sinus diameter, left atrial diameter, platelet count, D-D dimer, triglyceride, high-density lipoprotein, TnT > 0.014, and multiple ischemic lesions in MRI were excluded because they were not statistically significant. As shown in Table 2, for the development of the model, five potential predictors were generated by multivariate logistic regression (LR method): age > 65 (OR, 4.95, 95% CI, 1.18–20.76; $p = 0.029$), heart rate > 100 (OR, 8.04, 95% CI, 1.99–32.48; $p = 0.003$), CRP (OR, 1.06, 95% CI, 1.00–1.11; $p = 0.042$), NT-proBNP > 270 (OR, 20.01, 95% CI, 4.27–93.74; $p = 0.0001$), and HT (OR, 32.24, 95% CI, 2.47–420.79; $p = 0.008$). The nomogram was used to build a prediction model. The point values of each factor used to calculate the total score are summarized as shown in Figure 1. The area under the ROC curve for the developed validation was 0.937, with a sensitivity of 95.5% and a specificity of 85.5% (Figure 2).

External validation and apparent performance of the occult AF risk nomogram

According to Figure 3, the calibration curve of the nomogram for predicting occult AF in patients with AIS in this cohort showed good agreement. The resulting estimated AUC value of 1,000 bootstrap samples is 0.937, indicating that the model has good discrimination. The validation cohort's area under the ROC curve was 0.913, as illustrated in Figure 2, with a sensitivity of 86.7% and a specificity of 87.8%. Therefore, in both the internal and external variations, our nomogram had excellent prediction performance, suggesting the nomogram based on the five existing risk factors has a high degree of generalizability.

Discussion

In this study, the risk factors associated with AF in patients with stroke were identified using logistic regression analysis. In addition, we developed and externally validated a screening model for predicting occult AF in patients with stroke to facilitate the screening of high-risk patients with occult AF.

Studies have shown that cryptogenic stroke (CS) accounts for about 35% of all ischemic strokes (15), and the literature shows that approximately 67% of patients initially diagnosed with CS are found to have evidence of underlying cardioembolic stroke after a period of follow-up (16). Meanwhile, the main cause of cardioembolic stroke is AF. Compared with paroxysmal AF, persistent AF and permanent AF are easier to diagnose. We have observed from clinical work that paroxysmal AF is more likely to be missed during hospitalization. Many studies have reported that the risk of AIS due to paroxysmal AF is not significantly different from persistent AF and permanent AF (17). In addition, ECG monitoring for patients with AIS lasting >48 h can increase the detection rate of AF by 17.5–21.3% (18). However, the detection of AF, especially paroxysmal AF, remains limited due to the uneven distribution of medical resources.

Hence, we established a nomogram based on age, heart rate, CRP, NT-proBNP, and HT to accurately predict the probability of occult AF in patients with AIS. By combining readily available clinical information with this model, clinicians can predict occult AF in patients with AIS rapidly and personally.

Both our findings and earlier research indicate that the association of AF with stroke increases with age (14, 19). In clinical work, the CHA2DS2-VASc score is widely used clinically to evaluate whether patients with a definite diagnosis of AF

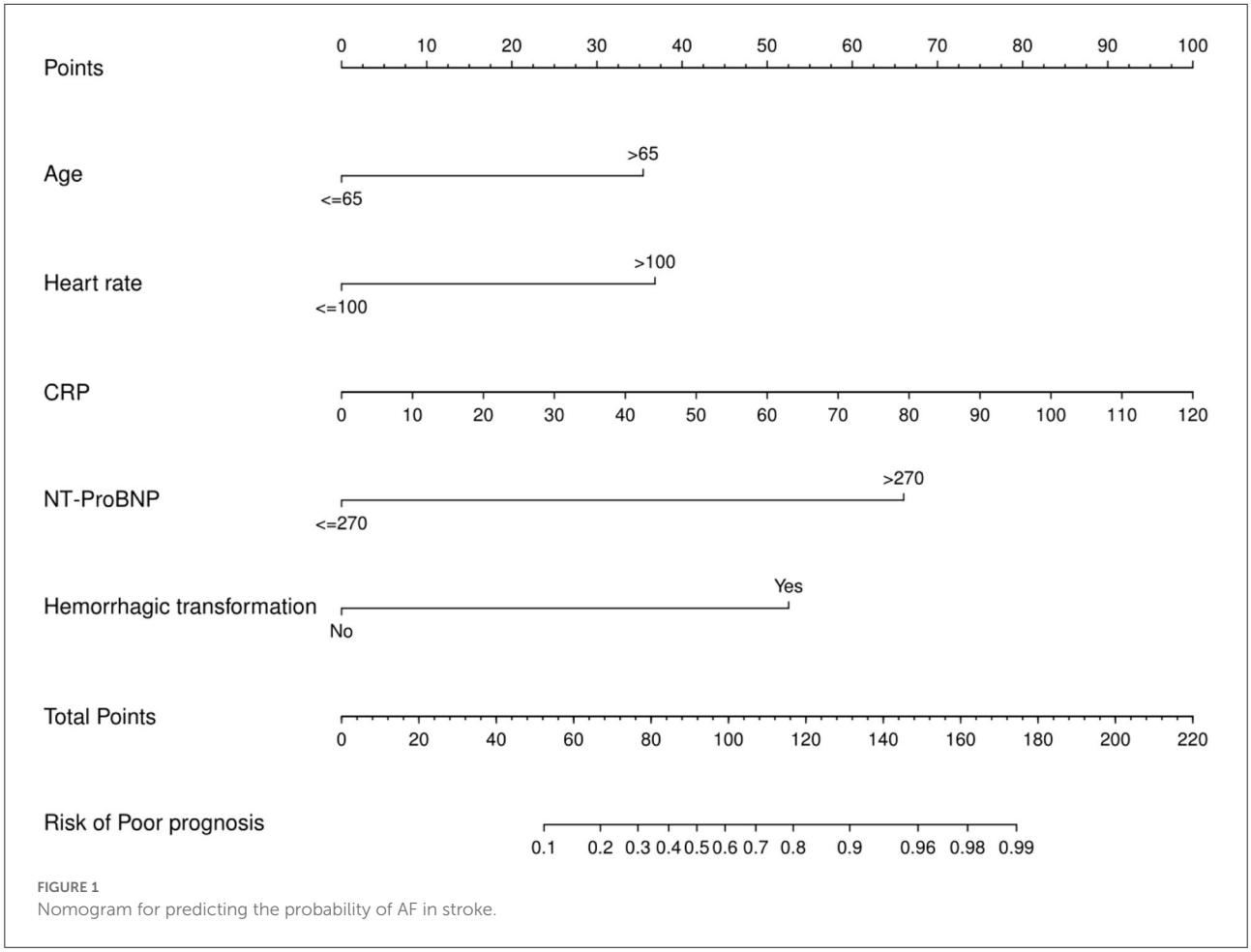

FIGURE 1
Nomogram for predicting the probability of AF in stroke.

need anticoagulation, and factors such as age >65 years in the scoring system are considered to be relatively higher risk. Apart from advanced age, some studies have shown that NT-proBNP is also an independent predictor of paroxysmal AF (20). NT-proBNP has the advantages of a long half-life and good stability and is more suitable for clinical use. Serum NT-proBNP levels were considerably greater in patients with AF than in sinus rhythm controls (21). Lucie Garnie et al. (22) suggested that post-stroke AF was independently predicted by NT-proBNP. Our data further demonstrate the close association of NT-proBNP with AF in patients with AIS. The STAF scoring system developed by Suissa et al. (19) found that higher NIHSS scores, left atrial enlargement, asymptomatic extracranial stenosis ($I > 50\%$), or cavitary infarction syndrome were associated with AF in patients with stroke. However, a prospective study from China showed that the STAF score has only moderate sensitivity and specificity in detecting AF in patients with stroke, with a relatively limited ability to predict AF, especially paroxysmal and new-onset AF (23).

In addition to the aforementioned factors, higher CRP at admission was also a strong modifiable predictor of AF. A previous study found that atrial inflammation is an important factor in the pathogenesis of AF. In long-term persistent/permanent AF, inflammatory infiltration and CRP levels in blood were significantly increased, and it was found that the degree of atrial inflammation was closely related to CRP blood levels (24), and the significance of CRP in predicting occult AF in individuals with AIS is further supported by our investigation. In our model, the strongest predictor of AF was HT. In fact, few studies have explored the relationship between HT and stroke complicated with AF, whereas a recent article published in *Stroke* pointed out that AF is associated with cerebral parenchymal hematoma and symptomatic intracranial hemorrhage after AIS (25). In multivariate analysis, wide 95% confidence intervals of HT and NT-proBNP suggest that these patients have a lower overall incidence of events and a higher incidence of AF. The blood biochemical, ultrasonography, imaging, and other indicators used for screening AIS complicated with occult AF have the characteristics of non-invasiveness, simple detection operation, and low price, which are more convenient for clinical application and promotion, and provide

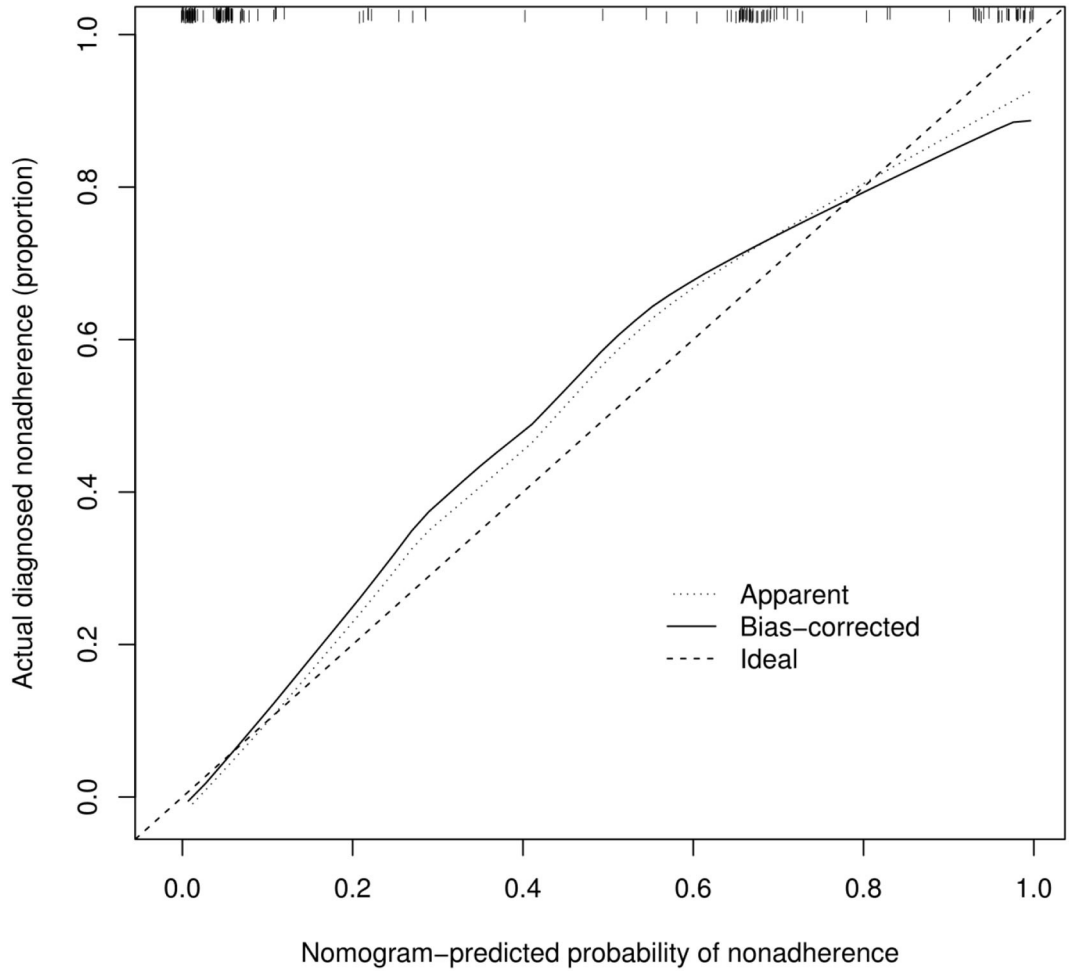

FIGURE 2
Predictive model based on logistic analysis for early diagnosis of AF in stroke in development and validation cohorts.

a new alternative diagnostic method for the diagnosis of cardiogenic embolism. Therefore, for high-risk groups, more accurate examinations such as long-term electrocardiography can be carried out in a targeted manner, observe the condition closely, repeat multiple examinations, increase the detection rate of AF, choose a more appropriate antithrombotic plan, thereby reducing the risk of cardioembolic stroke and death.

There are several limitations that need to be emphasized. First, in the developed validation, we included all patients with stroke in the study, not just those with cryptogenic stroke. We did this because the sample size is small and the number of ESUS is relatively small, and it is difficult to establish a validation model in the development cohort if we focus only on patients with ESUS, but external validation of the predictive model for occult AF in the ESUS patient cohort further confirmed the reliability of the model, which may compensate for the shortcomings introduced by our experimental design. Second, due to the limited conditions and the number of days the patients were in the hospital, we only performed 24-h dynamic electrocardiogram and not long-term ECG monitoring for all patients in the validation cohort, which leaves our prediction model somewhat deficient in detecting occult atrial AF. However, due to the limited conditions at our stroke center and the number of days the patients were in the hospital, it was difficult for us to complete long-term ECG monitoring such as 14 vs. 30 days of external cardiac monitoring during hospitalization, and the long-term ECG follow-up will be refined as much as possible in future clinical work. Third, compared with included patients, those excluded due to lack of baseline data showed a trend toward lower hypertension and AF (both $p < 0.10$), and there may be an inadvertent selection bias in place. Finally, this study is a single-center study with the problems of a single population and a small sample size, which may cause natural bias. If possible, external validation should be considered in a multicenter cohort with a large number of patients.

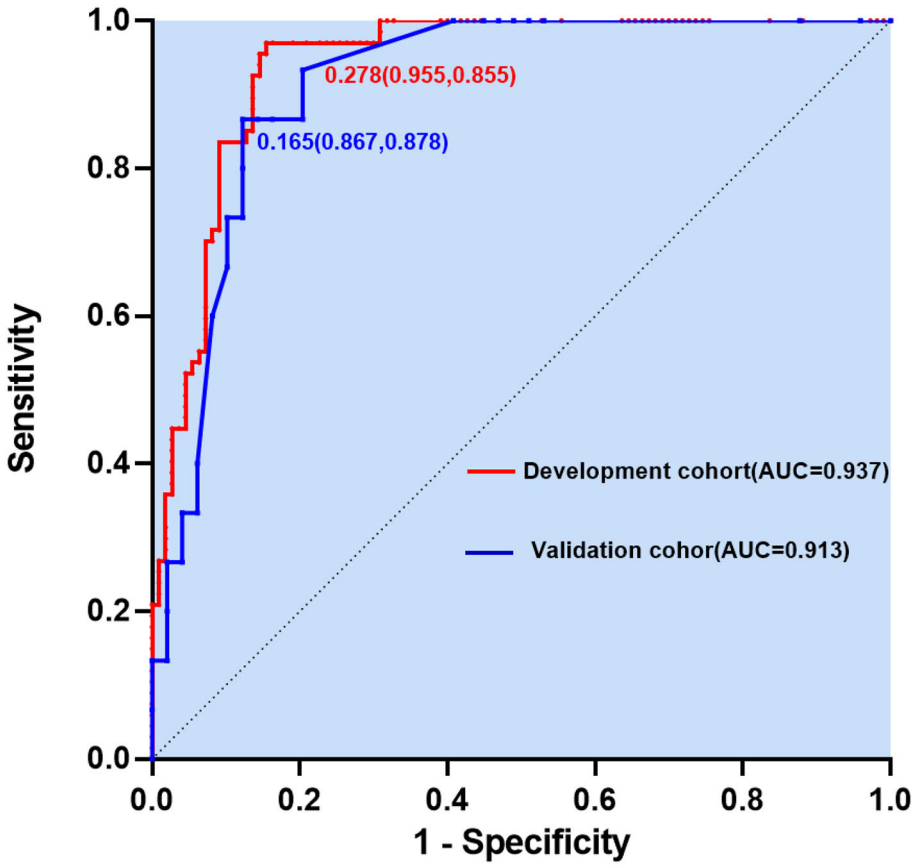

FIGURE 3
Calibration curve for the nomogram-predicted probability of AF in stroke.

Conclusion

This study provides a simple screening model for occult AF in acute stroke patients. This externally validated nomogram provides clinicians with a new tool that is more useful than traditional patient consultation risk scores because it makes it easy and fast to visualize each stroke survivor's risk.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Shanghai Tenth People's Hospital, Tongji University School of Medicine, 301 Middle Yanchang Road, Shanghai 200072, China. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YZ and XL conceived and designed the study. XW collected and analyzed the data. LM and XW drafted the article. All authors read and approved the version of the final manuscript.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1034350/full#supplementary-material

References

1. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham study. *Stroke.* (1991) 22:983–8. doi: 10.1161/01.STR.22.8.983

2. Ericson L, Bergfeldt L, Bjorholt I. Atrial fibrillation: the cost of illness in Sweden. *Eur J Health Econ.* (2011) 12:479–87. doi: 10.1007/s10198-010-0261-3

3. Saposnik G, Gladstone D, Raptis R, Zhou L, Hart RG, Investigators of the Registry of the Canadian Stroke N, et al. Atrial fibrillation in ischemic stroke: predicting response to thrombolysis and clinical outcomes. *Stroke.* (2013) 44:99–104. doi: 10.1161/STROKEAHA.112.676551

4. Bal S, Patel SK, Almekhlafi M, Modi J, Demchuk AM, Coutts SB. High rate of magnetic resonance imaging stroke recurrence in cryptogenic transient ischemic attack and minor stroke patients. *Stroke.* (2012) 43:3387–8. doi: 10.1161/STROKEAHA.112.671172

5. Gladstone DJ, Spring M, Dorian P, Panzov V, Thorpe KE, Hall J, et al. Atrial fibrillation in patients with cryptogenic stroke. *N Engl J Med.* (2014) 370:2467–77. doi: 10.1056/NEJMoa1311376

6. Sanna T, Diener HC, Passman RS, Di Lazzaro V, Bernstein RA, Morillo CA, et al. Cryptogenic stroke and underlying atrial fibrillation. *N Engl J Med.* (2014) 370:2478–86. doi: 10.1056/NEJMoa1313600

7. Diener HC, Sacco RL, Easton JD, Granger CB, Bernstein RA, Uchiyama S, et al. Dabigatran for prevention of stroke after embolic stroke of undetermined source. *N Engl J Med.* (2019) 380:1906–17. doi: 10.1056/NEJMoa1813959

8. Hart RG, Sharma M, Mundl H, Kasner SE, Bangdiwala SI, Berkowitz SD, et al. Rivaroxaban for stroke prevention after embolic stroke of undetermined source. *N Engl J Med.* (2018) 378:2191–201. doi: 10.1056/NEJMoa1802686

9. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. 2018 Guidelines for the Early Management of Patients with Acute Ischemic Stroke: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke.* (2018) 49:e46–e110. doi: 10.1161/STR.0000000000000158

10. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics–2015 update: a report from the American Heart Association. *Circulation.* (2015) 131:e29–e322. doi: 10.1161/cir.0000000000000152

11. Yoshida Y, Jin Z, Russo C, Homma S, Nakanishi K, Ito K, et al. Subclinical left ventricular systolic dysfunction and incident stroke in the elderly: long-term findings from cardiovascular abnormalities and brain lesions. *Eur Heart J Cardiovasc Imaging.* (2022). doi: 10.1093/ehjci/jeac145

12. Camm AJ, Corbucci G, Padeletti L. Usefulness of continuous electrocardiographic monitoring for atrial fibrillation. *Am J Cardiol.* (2012) 110:270–6. doi: 10.1016/j.amjcard.2012.03.021

13. Wang Y, Jing J, Meng X, Pan Y, Wang Y, Zhao X, et al. The Third China National Stroke Registry (Cnsr-Iii) for patients with acute Ischaemic stroke or transient Ischaemic attack: design, rationale and baseline patient characteristics. *Stroke Vasc Neurol.* (2019) 4:158–64. doi: 10.1136/svn-2019-000242

14. Bjorck S, Palaszewski B, Friberg L, Bergfeldt L. Atrial fibrillation, stroke risk, and warfarin therapy revisited: a population-based study. *Stroke.* (2013) 44:3103–8. doi: 10.1161/STROKEAHA.113.002329

15. Carrazco C, Golyan D, Kahen M, Black K, Libman RB, Katz JM. Prevalence and risk factors for paroxysmal atrial fibrillation and flutter detection after cryptogenic ischemic stroke. *J Stroke Cerebrovasc Dis.* (2018) 27:203–9. doi: 10.1016/j.jstrokecerebrovasdis.2017.08.022

16. Yaghi S, Kamel H, Elkind MSV. Atrial cardiopathy: a mechanism of cryptogenic stroke. *Expert Rev Cardiovasc Ther.* (2017) 15:591–9. doi: 10.1080/14779072.2017.1355238

17. Furie KL, Kasner SE, Adams RJ, Albers GW, Bush RL, Fagan SC, et al. Guidelines for the prevention of stroke in patients with stroke or transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2011) 42:227–76. doi: 10.1161/STR.0b013e3181f7d043

18. Fujii S, Shibazaki K, Kimura K, Sakai K, Aoki J. A simple score for predicting paroxysmal atrial fibrillation in acute ischemic stroke. *J Neurol Sci.* (2013) 328:83–6. doi: 10.1016/j.jns.2013.02.025

19. Suissa L, Bertora D, Lachaud S, Mahagne MH. Score for the targeting of atrial fibrillation (Staf): a new approach to the detection of atrial fibrillation in the secondary prevention of Ischemic stroke. *Stroke.* (2009) 40:2866–8. doi: 10.1161/STROKEAHA.109.552679

20. Patton KK, Ellinor PT, Heckbert SR, Christenson RH, DeFilippi C, Gottdiener JS, et al. N-Terminal pro-B-type natriuretic peptide is a major predictor of the development of atrial fibrillation: the cardiovascular health study. *Circulation.* (2009) 120:1768–74. doi: 10.1161/CIRCULATIONAHA.109.873265

21. Shelton RJ, Clark AL, Goode K, Rigby AS, Cleland JG. The diagnostic utility of N-terminal pro-B-type natriuretic peptide for the detection of major structural heart disease in patients with atrial fibrillation. *Eur Heart J.* (2006) 27:2353–61. doi: 10.1093/eurheartj/ehl233

22. Garnier L, Duloquin G, Meloux A, Benali K, Sagnard A, Graber M, et al. Multimodal approach for the prediction of atrial fibrillation detected after stroke: Safas study. *Front Cardiovasc Med.* (2022) 9:949213. doi: 10.3389/fcvm.2022.949213

23. Liu XY Li YX, Fu YG, Cai YY, Zhang YS, Min JY, et al. The value of the score for the targeting of atrial fibrillation (Staf) screening in acute stroke patients. *J Stroke Cerebrovasc Dis.* (2017) 26:1280–6. doi: 10.1016/j.jstrokecerebrovasdis.2017.01.022

24. Wu L, Emmens RW, van Wezenbeek J, Stooker W, Allaart CP, Vonk ABA, et al. Atrial inflammation in different atrial fibrillation subtypes and its relation with clinical risk factors. *Clin Res Cardiol.* (2020) 109:1271–81. doi: 10.1007/s00392-020-01619-8

25. van Kranendonk KR, Treurniet KM, Boers AMM, Berkhemer OA, van den Berg LA, Chalos V, et al. Clinical and imaging markers associated with hemorrhagic transformation in patients with acute ischemic stroke. *Stroke.* (2019) 50:2037–43. doi: 10.1161/STROKEAHA.118.024255

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Identifying vulnerable plaques: A 3D carotid plaque radiomics model based on HRMRI

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Background: Identification of vulnerable carotid plaque is important for the treatment and prevention of stroke. In previous studies, plaque vulnerability was assessed qualitatively. We aimed to develop a 3D carotid plaque radiomics model based on high-resolution magnetic resonance imaging (HRMRI) to quantitatively identify vulnerable plaques.

Methods: Ninety patients with carotid atherosclerosis who underwent HRMRI were randomized into training and test cohorts. Using the radiological characteristics of carotid plaques, a traditional model was constructed. A 3D carotid plaque radiomics model was constructed using the radiomics features of 3D T_1-SPACE and its contrast-enhanced sequences. A combined model was constructed using radiological and radiomics characteristics. Nomogram was generated based on the combined models, and ROC curves were utilized to assess the performance of each model.

Results: 48 patients (53.33%) were symptomatic and 42 (46.67%) were asymptomatic. The traditional model was constructed using intraplaque hemorrhage, plaque enhancement, wall remodeling pattern, and lumen stenosis, and it provided an area under the curve (AUC) of 0.816 vs. 0.778 in the training and testing sets. In the two cohorts, the 3D carotid plaque radiomics model and the combined model had an AUC of 0.915 vs. 0.835 and 0.957 vs. 0.864, respectively. In the training set, both the radiomics model and the combination model outperformed the traditional model, but there was no significant difference between the radiomics model and the combined model.

Conclusions: HRMRI-based 3D carotid radiomics models can improve the precision of detecting vulnerable carotid plaques, consequently improving risk classification and clinical decision-making in patients with carotid stenosis.

KEYWORDS

carotid atherosclerosis (AS), radiomics, 3D reconstruction, vulnerable plaque, high-resolution magnetic resonance imaging, stroke

Introduction

The most common type of cerebrovascular disease is ischemic stroke and 15–20% of these are caused by carotid artery stenosis (1, 2). By 2020, the global prevalence of carotid plaque among people between the ages of 30 and 79 years was about 20%, and 816 million patients were reported with carotid stenosis (3). The current guidelines account for the patient's clinical presentation and the degree of carotid stenosis to determine a need for surgical intervention (4). However, as radiographic methods have limited accuracy in identifying the degree of histological stenosis in carotid arteries, imaging screening is not recommended in the general population (5). Numerous studies have demonstrated that it is essential to identify vulnerable plaques

by analyzing their composition, as it has implications for a patient's clinical presentation and future cerebral ischemic events (6). Recently, magnetic resonance imaging (MRI) has been effective for identifying intraplaque hemorrhage (IPH) and lipid-rich necrotic cores (LRNC), but an atherosclerotic plaque is complex in structure and necessitates specialized knowledge of MRI for assessing plaque composition (7). Even the most advanced high-resolution magnetic resonance imaging (HRMRI) can only demonstrate the qualitative and subjective identification of lesions' structural characteristics. In the majority of previous studies, plaque composition was qualitatively quantified (8).

Radiomics is a computational technique for extracting and statistically assessing vast quantities of image texture information from medical images (9). It has been proven to be effective in oncology owing to its numerous applications in the diagnosis, grading, and staging of cancer, evaluating therapeutic efficacy, and predicting clinical outcomes (10). Computed tomography (CT) or ultrasound-based radiomics models reportedly have a potential clinical application for diagnosing carotid plaque vulnerability, whereas HRMRI-based radiomics models have received less attention and have extracted radiomics features of the carotid plaque only at the most stenotic level (11–13). In addition, it has been shown that radiomics models based on 3D HRMRI can accurately identify high-risk intracranial plaques (14). HRMRI has clear advantages in assessing vessel wall composition, and its three-dimensional T_1 weighted sampling perfection with application-optimized contrasts by using different flip angle evolutions (3D T_1-SPACE) sequence can provide three-dimensional, large-area, high-spatial-resolution imaging of the arterial wall (15). This study aims to develop a 3D HRMRI-based carotid radiomics model to investigate the importance of radiomics in analyzing carotid atherosclerotic plaques, improving the accuracy of vulnerable plaque identification and furthering data on the management of asymptomatic carotid stenosis.

TABLE 1 Clinical and radiological characteristics of patients.

Parameters	Asymptomatic ($n = 42$)	Symptomatic ($n = 48$)	p
Age (year)	58.43 ± 10.62	59.27 ± 11.16	0.716
Sex (female)	19 (45.23)	13 (27.08)	0.082
BMI (kg/m^2)	24.72 ± 2.83	24.78 ± 2.66	0.649
Clinical features			
CAD	4 (9.52)	9 (18.75)	0.245
Hypertension	22 (52.38)	31 (64.58)	0.286
Diabetes	9 (21.43)	19 (39.58)	0.072
Current smoking	10 (23.81)	18 (37.50)	0.290
Current medications			
Aspirin	9 (21.43)	6 (12.50)	0.274
Clopidogrel	5 (11.90)	3 (6.25)	0.465
Statin	9 (21.43)	4 (8.33)	0.131
Serum lipid (mmol/L)			
Total cholesterol	3.77 ± 0.93	3.63 ± 1.07	0.521
Triglycerides	1.64 ± 1.05	1.58 ± 0.92	0.783
HDL-C	1.08 ± 0.36	1.03 ± 0.31	0.508
LDL-C	2.14 ± 0.78	2.01 ± 0.86	0.453
Plaque characteristics			
IPH	4 (9.52)	14 (29.17)	0.026
RMP (Positive)	6 (14.29)	15 (31.25)	0.063
PE (Apparent)	3 (7.14)	6 (12.50)	0.404
Degree of luminal stenosis	67.49 ± 9.71	76.09 ± 5.21	0.001

Data were expressed as means ± SD for continuous variables and number (percentage) for dichotomous variables. CAD, coronary artery disease; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; IPH, Intraplaque hemorrhage; RMP, Remodeling pattern; PE, Plaque enhancement.

Materials and methods

Study population

In this retrospective study, patients with carotid artery stenosis who underwent HRMRI at the First Affiliated Hospital of Zhengzhou University between June 2021 and June 2022 were enrolled. All patients ($N = 90$) had 50% carotid stenosis. Patients ($n = 42$) who were noted with carotid stenosis upon physical examination but had never experienced a transient ischemic attack (TIA) or stroke in the past 6 months and any radiological finding of cerebral infarction were grouped into the asymptomatic group. In symptomatic atherosclerosis, plaque enhancement subsides over time after an ischemic stroke (16). In addition, to approximate the state of carotid plaque at the time of rupture, we included patients in the symptomatic plaque group whose MRI demonstrated the presence of an acute phase (<4 weeks) of cerebral infarction in the ipsilateral blood supply area of carotid stenosis. All patients with symptomatic carotid stenosis met the Trial of Org 101072 in Acute Stroke Treatment (TOAST) criteria for "atherosclerotic TIA/ischemic stroke" before their inclusion in the study (17). Patients were excluded if they had subacute or old infarct foci on MRI, combined intracranial vascular lesions such as severe stenosis or occlusion of the anterior or middle cerebral artery, Moyamoya disease, poor HRMRI imaging, or absence of 3D T_1-SPACE sequences.

The clinical characteristics of the patients were gathered for both groups (Table 1), and they were then randomly split into a training set ($n = 63$) and a testing set ($n = 27$) in a 7:3 ratio. The training set was used to build the radiomics model whereas the testing set was utilized to validate the model's diagnostic performance. This study protocol was reviewed and approved by our institution's ethics committee, and all patients provided informed permission.

MRI acquisition

All patients were scanned with a 3T MRI (Magnetom Verio, Siemens Healthineers) with a 64-channel coil. Subjects were reminded to avoid swallowing and neck movements before the examination. The 3D T_1- SPACE and its contrast-enhanced sequence (3D T_1-SPACE-CE) scan were performed in the oblique coronal position. The following parameters were applied to these image sequences for diffusion-weighted imaging: 4,000 ms Repetition Time (TR), 60 ms echo time (TE), the field of view (FOV) 200×200 mm,

matrix size 150 ×150, and slice thickness 2 mm; and for 3D T_1-SPACE: TR 900 ms, TE 15 ms, FOV 200 × 224 mm, and slice thickness 0.63 mm. Before the acquisition of the 3D T_1-SPACE-CE sequence, 0.1 mL/kg of a Gadopentetate Dimeglumine was administered to the patient.

Image analysis and segmentation

A radiologist with 4 years of experience in vascular wall imaging performed conventional measures and segmentation while blinded to the clinical information. On 3D T_1-SPACE and its enhanced sequence, the HRMRI characteristics, including intraplaque hemorrhage (IPH), plaque enhancement (PE), wall remodeling pattern, and lumen stenosis, were manually measured. The related methods to measure were as follows: (1) IPH: On T_1WI, the signal intensity of plaque exceeded 150% of normal brain parenchyma.; (2) PE was classified as mild and apparent enhancement based on a comparison of plaque enhancement with the pituitary funnel stalk; (3) The wall remodeling pattern was divided into positive and negative patterns according to the ratio of the vessel area at the site of maximum luminal stenosis to the reference vessel area; (4) Diameter stenosis rate = [1 – narrow lumen area/reference lumen area] × 100% (18).

Utilizing the free program 3D Slicer (version 4.13.0, www.slicer.org), plaque segmentation was carried out for radiomics investigation. After identifying all slices containing plaques, the same radiologist manually drew regions of interest (ROIs) around the plaque margins until the ROI contained the full 3D carotid plaque (Figure 1A).

Feature extraction, selection, and model development

To prevent data heterogeneity and bias, all MRI images were normalized and resampled (2 × 2 × 2 mm) before the radiomics features extraction. Following the guidelines of the Image Biomarker Normalization Initiative (19), this study used Python (version 3.7.0) to import the PyRadiomics (github.com/Radiomics/pyradiomics) toolkit to extract radiomics features, including shape features (2D and 3D), first-order features, gray level co-occurrence matrix (GLCM), gray level size zone matrix (GLSZM), gray level run length matrix (GLRLM), and gray level dependence matrix (GLDM) based original images and Gaussian and wavelet images. After 2 weeks, the same MRI physician randomly selected 20 carotid HRMRIs to re-segment 3D carotid ROIs and extracted radiomics features, and those featuring an interclass correlation coefficient (ICC) of ≥ 0.7 were included in the subsequent study, which was considered to be excellent robustness (20).

The extracted radiomics features typically contained redundancies, many of which were strongly correlated, so the following criteria were used to screen the features in this study. (1) 70 and 30% of the data were randomly divided into training and test sets, respectively. We utilized Z-Score ([value-mean]/standard deviation) distribution to normalize all radiomics features. (2) Univariate analysis was used to select radiomics features with $p < 0.01$. And then, the LASSO (least absolute shrinkage and selection operator) algorithm was used to further reduce the number of features. Using the LASSO algorithm, the most significant features with the smallest deviation were chosen as the final features (Figure 1B). Due to its improved screening of high-dimensional data, the LASSO technique is widely employed in radiomics. (3) In the training cohort, we constructed a multivariate logistic regression radiomics model using the final features, and in the test cohort, we assessed its performance. (4) In this radiomics model, the probability of vulnerability for each patient is determined using a regression-weighted algorithm.

According to previous studies, the traditional radiological models were constructed based on IPH, PE, wall remodeling pattern, and lumen stenosis rates (18, 21). Finally, a combined model was built based on the conventional radiological and radiomics characteristics, and the corresponding nomogram was established by the R software.

Statistical analysis

All statistical analyses were performed using R 4.2.1 (www.Rproject.org) and python (www.python.org). All continuous variables were reported as mean ± standard deviations (SD), and categorical variables were depicted as count (%). We used the Shapiro-Wilk test to check for normal distribution. To compare clinical characteristics and traditional features between asymptomatic and symptomatic carotid stenosis patients, the student's t test/Mann–Whitney *U*-test was used for quantitative variables, and the chi-square test/Fisher's exact test was used for categorical variables. Univariate logistic regression and the LASSO algorithm were performed for the final screening of radiomics characteristics. Receiver operating characteristic (ROC) calculations were made for each model to evaluate the identification of vulnerable plaques. The ROCs were compared using Delong testing. The statistical significance was determined by the two-tailed *p*-value of < 0.05.

Results

Patient characteristics

Ninety patients with carotid stenosis who underwent HRMRI were included, 48 (53.33%) had MRI findings suggestive of acute phase cerebral infarction ipsilateral to the carotid stenosis. There were no significant differences between asymptomatic and symptomatic carotid stenosis patients in terms of gender, age, BMI, history, medication history, and lipids (Table 1). A median of 8.0 days passed between an HRMRI and an ischemic cerebrovascular incident (interquartile range: 4.0–13.75 days).

Radiomics assessment of the carotid plaque

On both raw and filtered pictures, 4,170 features were initially collected from each ROI. In terms of the intraclass correlation coefficient, 1,563 characteristics (37.5%) showed outstanding robustness. After univariate analysis and LASSO feature screening, 13 features, including 5 features on 3D T_1-SPACE and 8 features on 3D T_1-SPACE-CE sequence, were ultimately chosen and used to

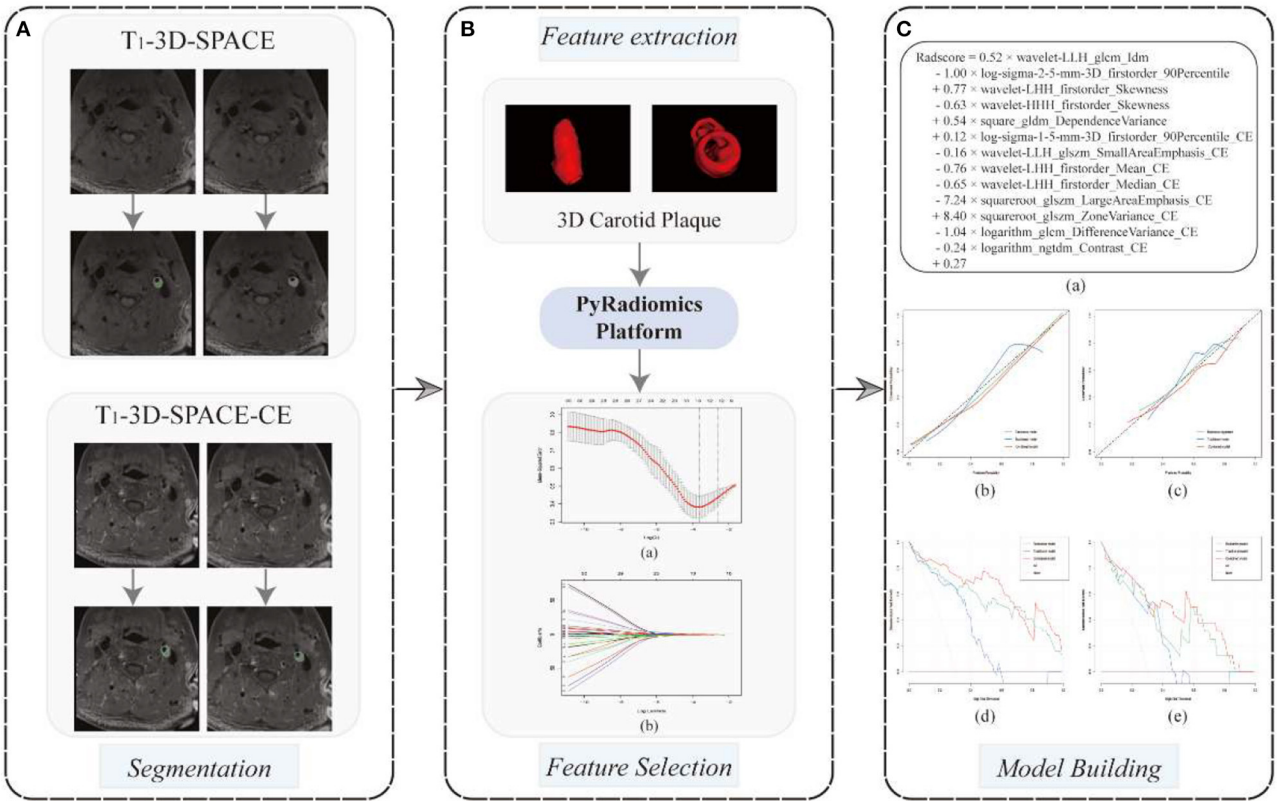

FIGURE 1
A flowchart of radiomics model development. **(A)** 3D carotid plaque segmentation in 3D T_1-SPACE and 3D T_1-SPACE- CE sequences, respectively; **(B)** PyRadiomics-based radiomics feature extraction, 13radiomics features were screened at the minimum mean square error of the LASSO regression and used to build radiomics models (a, b); **(C)** The formulation of radiomics signature (a); The calibration curves (b, c) and decision curves analysis (d, e) for the training and testing cohorts were employed to evaluate the traditional model, the radiomics model and the combined model, respectively.

create the multifactorial logistic radiomics model. The formulation of the radiomics signature is as Figure 1C.

Five-fold cross-validation was used to simulate the discriminatory power of the 3D carotid radiomics model, which had AUC values of 0.915 (95% CI: 0.85–0.98) for the training set and 0.835 (95% CI: 0.68–0.99) for the testing set. And it had the desirable discrimination ability with a specificity of 63.6% and a sensitivity of 93.8% in the testing set (Table 2, Figure 2).

Traditional and combined assessment models

IPH and luminal stenosis rate were found to be linked with symptomatic plaques according to the univariate analysis results ($p < 0.05$, Table 2). Although there were no significant differences in PE and wall remodeling patterns between the two groups, we created models employing IPH, PE, wall remodeling pattern, and lumen stenosis to improve the performance of the traditional model. With a sensitivity of 90.6% and a specificity of 74.2%, the traditional model produced an AUC of 0.816 (95% CI: 0.70–0.93) for the training set and an AUC of 0.778 (95% CI: 0.57–0.99) for the testing set. The final combined model has an AUC in the training set of 0.957 (95% CI: 0.92–1.00) and the testing set of 0.864 (95% CI: 0.72–1.00) (Table 2, Figure 2). The related nomograms for estimating the risk of ischemic cerebrovascular episodes are depicted in Figure 3.

The DeLong test revealed that the ROC curves of the combined model performed better than those of the traditional model in both the training and testing groups ($p = 0.005$ and $p = 0.037$, respectively). However, in both cohorts, there was no significant difference between the combined model and the radiomics model ($p > 0.05$). The calibration curve reveals a sufficient correlation between the diagnostic results of our radiomics and combined model and the actual results in the two sets (Figure 1Cb, c). The decision curves showed that, in the range of 0 to 1, decisions based on our radiomics and combined model achieved a net benefit over "no treatment" or "all treatment" (Figure 1Cd, e).

Discussion

In patients with carotid stenosis, high-risk plaque poses a significant risk of developing cerebrovascular embolic events. Numerous carotid plaque MRI investigations have been carried out to explore plaque components or characteristics linked to cerebral ischemic episodes. However, most of the early studies have emphasized only conventional qualitative assessments (8). In this study, we constructed a radiomics model to identify vulnerable carotid plaques by extracting 3D carotid plaque radiomics features from HRMRI using a

TABLE 2 Performance of the models in training and testing cohorts.

Assessment models	Cohort	AUC (95% CI)	Sensitivity	Specificity
Radiomics model	Training	0.915 (0.846–0.984)	0.812	0.903
	Testing	0.835 (0.677–0.993)	0.938	0.636
Traditional model	Training	0.816 (0.697–0.934)	0.906	0.742
	Testing	0.778 (0.567–0.990)	0.812	0.818
Combined model	Training	0.957 (0.915–0.999)	0.875	0.903
	Testing	0.864 (0.721–1.000)	0.875	0.727

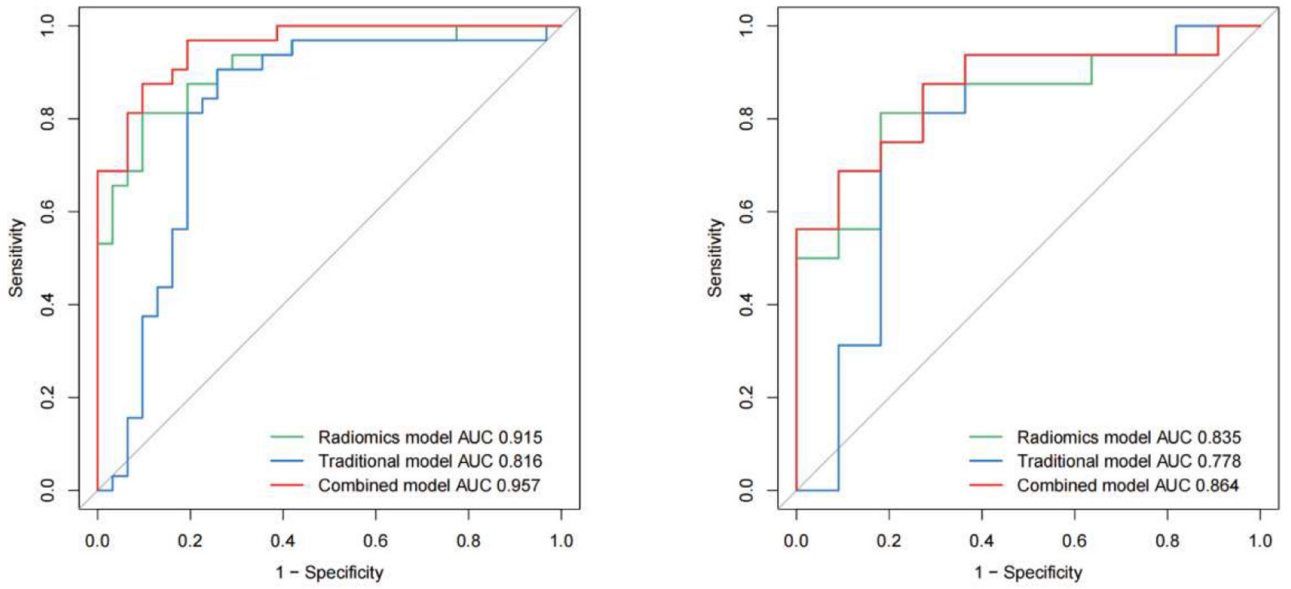

FIGURE 2
Receiver operating characteristic (ROC) curves of all the models (traditional model, radiomics model and combined model) in the training and test cohorts respectively.

radiomics technique. Excellent diagnostic performance was demonstrated in identifying vulnerable carotid plaques, allowing quantitative scoring of each plaque's vulnerability to indicate the risk of stroke.

To the authors' knowledge and literature review, this study is the first study to develop a 3D carotid plaque radiomics diagnostic model for identifying vulnerable carotid plaques based on 3D T_1-SPACE. Previous research has only extracted radiomics features at the MRI level, where the plaque area is the biggest. However, the generalizability of the previous model is diminished due to the model's extensive radiomics content. In addition, although the previous combined model outperforms our model, its AUCs per sequence were 0.846, 0.826, 0.857, and 0.816, which were inferior to our radiomics model (AUC of 0.915) (13). Compared to single-layer carotid plaques, 3D plaques can more accurately indicate plaque vulnerability and rupture risk. Arna et al. used ultrasonography to create a model of 3D plaque ultrasound texture paired with plaque volume, which was considerably more accurate at predicting future cerebrovascular events than the conventional risk classification techniques (22). Saba et al. discovered that with an increase in plaque volume, lipid levels and proportion of calcification also increase, in addition to a correlation between the volume of lipid component and plaque surface ulceration, and is a significant risk factor for cerebrovascular events (23). Cai et al. studied 63 patients for up to 55.1 months and discovered that advancement in carotid plaque volume was independently linked to recurrent ischemic cerebrovascular episodes (24). Therefore, feature extraction and evaluation of 3D carotid plaques may enhance the diagnostic performance of vulnerable plaques.

The latest guidelines published by the European Society of Vascular Surgery currently recommend optimal medication treatment for asymptomatic patients with 60% stenosis, favor revascularization for patients at average surgical risk with 60–99% stenosis, and affirm the importance of radiological evaluation in decision-making (25). However, a meta-analysis by Joseph et al. revealed that about 26.5% of patients with asymptomatic carotid stenosis had coupled vulnerable plaques, and this correlated with a greater incidence of ipsilateral ischemic cerebrovascular episodes. However, the prevalence of high-risk plaques was unrelated to the degree of stenosis (26). Therefore, regardless of the level of stenosis, it is essential to identify plaques at risk of causing cerebrovascular events using radiological techniques. Previous studies have shown that the

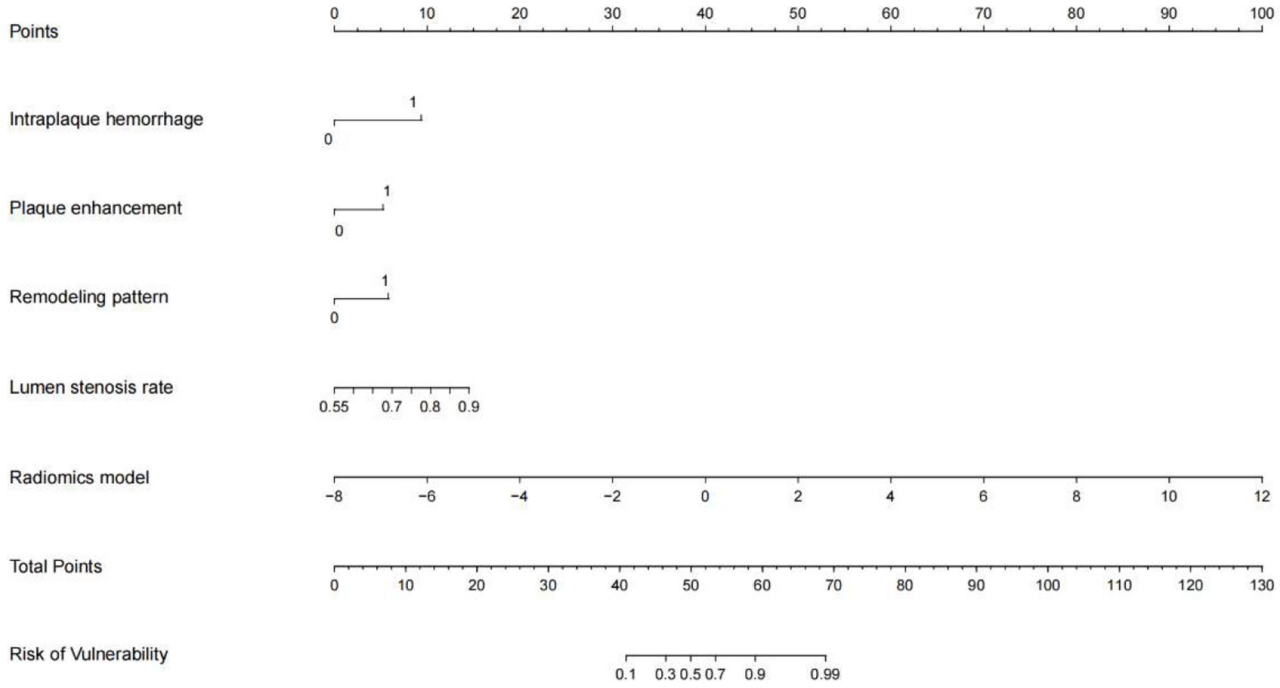

FIGURE 3
A nomogram integrating the radiomics scores and traditional features of the training sets.

HRMRI features of symptomatic plaque mainly include IPH, PE, wall remodeling pattern, and lumen stenosis. However, it only allows for qualitative judgments and requires a specialist with radiological expertise and substantial work experience. Similar to previous findings in coronary and cerebral plaque studies, we discovered that the radiomics model outperformed the conventional model in diagnosing plaque state (AUC = 0.915 vs. 0.816) (14, 27). What's more, optimal diagnostic performance can be achieved when conventional radiological and radiomics features are combined. The decision curves depicted demonstrate that the combined model caused a net benefit for patients in both the training and testing sets. Therefore, the nomogram developed in this study is a useful tool in clinical practice (Figure 3).

However, there are some limitations to this study. Our study had a limited sample size. Although cross-validation was performed to enhance our model's performance, it may still be susceptible to over- or under-fitting. In addition, using single-center data analysis, the same scanning instrument, and set MRI parameters might have limited the generalizability of the model. Also, the ROI could be manually segmented before the extraction of radiomics features. Although the hand-drawn method is regarded as the "gold standard" for image segmentation now, the process is tedious and time-consuming. Despite rapid advancements in deep learning semantic segmentation algorithms for automatic segmentation of ROIs in recent years, its clinical use requires the development of accurate and interpretable algorithmic codes. Thus, future studies might integrate multi-omics techniques that would include clinical data, radiomics, gene proteomics, and hemodynamics to further enhance the prediction of cerebrovascular ischemia risk in patients with carotid stenosis.

Conclusion

HRMRI-based 3D carotid radiology models can improve the performance of traditional radiology in identifying vulnerable carotid plaques. One major advantage of radiomics analysis is its ability to extract quantitative data from images which enhances the diagnostic performance beyond traditional evaluation. Future prospective studies could further enhance radiomics in predicting ischemic cerebrovascular episodes in individuals with carotid artery stenosis.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of First Affiliated Hospital of Zhengzhou University, approval number 2022-KY-0068, and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XZ, ZH, ZJ, RC, and ZL: conception, design, analysis, and interpretation. XZ, ZH, RC, ZJ, JS, and ZL: data collection and statistical analysis. XZ, ZH, CL, and ZL: writing the article. XZ, ZH, ZJ, RC, CL, and ZL: critical revision of the article. XZ, ZH, RC, ZJ,

JS, CL, and ZL: final approval of the article. All authors approved the final version to be submitted for publication.

Conflict of interest

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

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References

1. Chaturvedi S, Bruno A, Feasby T, Holloway R, Benavente O, Cohen SN, et al. Carotid endarterectomy–an evidence-based review: report of the therapeutics and technology assessment subcommittee of the American Academy of Neurology. *Neurology.* (2005) 65:794–801. doi: 10.1212/01.wnl.0000176036.07558.82

2. Flaherty ML, Kissela B, Khoury JC, Alwell K, Moomaw CJ, Woo D, et al. Carotid artery stenosis as a cause of stroke. *Neuroepidemiology.* (2013) 40:36–41. doi: 10.1159/000341410

3. Song P, Fang Z, Wang H, Cai Y, Rahimi K, Zhu Y, et al. Global and regional prevalence, burden, and risk factors for carotid atherosclerosis: a systematic review, meta-analysis, and modelling study. *Lancet Glob Health.* (2020) 8:e721–9. doi: 10.1016/S2214-109X(20)30117-0

4. AbuRahma AF, Avgerinos ED, Chang RW, Darling RC, Duncan AA, Forbes TL, et al. Society for vascular surgery clinical practice guidelines for management of extracranial cerebrovascular disease. *J Vasc Surg.* (2022) 75:4S—22S. doi: 10.1016/j.jvs.2021.04.073

5. Meschia JF, Klaas JP, Brown RD, Brott TG. Evaluation and management of atherosclerotic carotid stenosis. *Mayo Clin Proc.* (2017) 92:1144–57. doi: 10.1016/j.mayocp.2017.02.020

6. Nardi V, Benson J, Bois MC, Saba L, Larson AS, Özcan I, et al. Carotid plaques from symptomatic patients with mild stenosis is associated with intraplaque hemorrhage. *Hypertension.* (2022) 79:271–82. doi: 10.1161/HYPERTENSIONAHA.121.18128

7. Liu Q, Jiang P, Jiang Y, Ge H, Li S, Jin H, et al. Prediction of aneurysm stability using a machine learning model based on PyRadiomics-derived morphological features. *Stroke.* (2019) 50:2314–21. doi: 10.1161/STROKEAHA.119.025777

8. Bos D, Arshi B, van den Bouwhuijsen QJA, Ikram MK, Selwaness M, Vernooij MW, et al. Atherosclerotic carotid plaque composition and incident stroke and coronary events. *J Am Coll Cardiol.* (2021) 77:1426–35. doi: 10.1016/j.jacc.2021.01.038

9. Gillies RJ, Kinahan PE, Hricak H. Radiomics: images are more than pictures, they are data. *Radiology.* (2016) 278:563–77. doi: 10.1148/radiol.2015151169

10. Liu Z, Wang S, Dong D, Wei J, Fang C, Zhou X, et al. The applications of radiomics in precision diagnosis and treatment of oncology: opportunities and challenges. *Theranostics.* (2019) 9:1303–22. doi: 10.7150/thno.30309

11. Huang Z, Cheng XQ, Liu HY, Bi XJ, Liu YN, Lv WZ, et al. Relation of carotid plaque features detected with ultrasonography-based radiomics to clinical symptoms. *Transl Stroke Res.* (2021) 13:970–82. doi: 10.1007/s12975-021-00963-9

12. Cilla S, Macchia G, Lenkowicz J, Tran EH, Pierro A, Petrella L, et al. CT angiography-based radiomics as a tool for carotid plaque characterization: a pilot study. *Radiol med.* (2022) 127:743–53. doi: 10.1007/s11547-022-01505-5

13. Zhang R, Zhang Q, Ji A, Lv P, Zhang J, Fu C, et al. Identification of high-risk carotid plaque with MRI-based radiomics and machine learning. *Eur Radiol.* (2021) 31:3116–26. doi: 10.1007/s00330-020-07361-z

14. Li H, Liu J, Dong Z, Chen X, Zhou C, Huang C, et al. Identification of high-risk intracranial plaques with 3D high-resolution magnetic resonance imaging-based radiomics and machine learning. *J Neurol.* (2022) 269:6494–503. doi: 10.1007/s00415-022-11315-4

15. Lu SS, Ge S, Su CQ, Xie J, Mao J, Shi HB, et al. MRI of plaque characteristics and relationship with downstream perfusion and cerebral infarction in patients with symptomatic middle cerebral artery stenosis. *J Magn Reson Imaging.* (2018) 48:66–73. doi: 10.1002/jmri.25879

16. Yang WJ, Abrigo J, Soo YO, Wong S, Wong KS, Leung TW, et al. Regression of plaque enhancement within symptomatic middle cerebral artery atherosclerosis: a high-resolution MRI study. *Front Neurol.* (2020) 11:755. doi: 10.3389/fneur.2020.00755

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

18. Zhao JJ, Lu Y, Cui JY, Ma LQ, Zhang RP, Xu Z. Characteristics of symptomatic plaque on high-resolution magnetic resonance imaging and its relationship with the occurrence and recurrence of ischemic stroke. *Neurol Sci.* (2021) 42:3605–13. doi: 10.1007/s10072-021-05457-y

19. Zwanenburg A, Vallières M, Abdalah MA, Aerts HJWL, Andrearczyk V, Apte A, et al. The image biomarker standardization initiative: standardized quantitative radiomics for high-throughput image-based phenotyping. *Radiology.* (2020) 295:328–38. doi: 10.1148/radiol.2020191145

20. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med.* (2016) 15:155–63. doi: 10.1016/j.jcm.2016.02.012

21. Dieleman N, van der Kolk AG, Zwanenburg JJM, Harteveld AA, Biessels GJ, Luijten PR, et al. Imaging intracranial vessel wall pathology with magnetic resonance imaging: current prospects and future directions. *Circulation.* (2014) 130:192–201. doi: 10.1161/CIRCULATIONAHA.113.006919

22. van Engelen A, Wannarong T, Parraga G, Niessen WJ, Fenster A, Spence JD, et al. Three-dimensional carotid ultrasound plaque texture predicts vascular events. *Stroke.* (2014) 45:2695–701. doi: 10.1161/STROKEAHA.114.005752

23. Saba L, Sanfilippo R, Sannia S, Anzidei M, Montisci R, Mallarini G, et al. Association between carotid artery plaque volume, composition, and ulceration: a retrospective assessment with MDCT. *AJR Am J Roentgenol.* (2012) 199:151–6. doi: 10.2214/AJR.11.6955

24. Lu M, Peng P, Cui Y, Qiao H, Li D, Cai J, et al. Association of progression of carotid artery wall volume and recurrent transient ischemic attack or stroke: a magnetic resonance imaging study. *Stroke.* (2018) 49:614–20. doi: 10.1161/STROKEAHA.117.019422

25. Naylor AR, Rantner B, Ancetti S, de Borst GJ, De Carlo M, Halliday A, et al. European society for vascular surgery (ESVS) 2023 Clinical practice guidelines on the management of atherosclerotic carotid and vertebral artery disease. *Eur J Vasc Endovasc Surg* (2022) 65:7–111. doi: 10.1016/j.ejvs.2022.04.011

26. Kamtchum-Tatuene J, Noubiap JJ, Wilman AH, Saqqur M, Shuaib A, Jickling GC. Prevalence of high-risk plaques and risk of stroke in patients with asymptomatic carotid stenosis: a meta-analysis. *JAMA Neurol.* (2020) 77:1524. doi: 10.1001/jamaneurol.2020.2658

27. Kolossváry M, Park J, Bang JI, Zhang J, Lee JM, Paeng JC, et al. Identification of invasive and radionuclide imaging markers of coronary plaque vulnerability using radiomic analysis of coronary computed tomography angiography. *Eur Heart J Cardiovasc Imaging.* (2019) 20:1250–8. doi: 10.1093/ehjci/jez033

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Systemic inflammation response index predicts 3-month outcome in patients with mild acute ischemic stroke receiving intravenous thrombolysis

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Introduction: A crucial aspect of stroke progression is the inflammatory response. As novel inflammatory and prognostic markers, the systemic immune inflammation index (SII) and the systemic inflammation response index (SIRI) have recently been studied. The objective of our study was to evaluate the prognostic value of SII and SIRI in mild acute ischemic stroke (AIS) patients following intravenous thrombolysis (IVT).

Methods: Our study screened the clinical data of patients with mild AIS admitted to the Minhang Hospital of Fudan University for retrospective analysis. The SIRI and SII were examined by the emergency laboratory before IVT. Functional outcome was evaluated 3 months after the onset of stroke using the modified Rankin Scale (mRS). mRS $\geq$ 2 was defined as an unfavorable outcome. The relationship between SIRI and SII and the 3-month prognosis was determined using both univariate and multivariate analysis. Receiver operating characteristic curve was performed to evaluate the predictive value of SIRI for AIS prognosis.

Results: A total of 240 patients were included in this study. Both SIRI and SII were higher in the unfavorable outcome group than in the favorable outcome group [1.28 (0.70–1.88) vs. 0.79 (0.51–1.08), $P < 0.001$ and 531.93 (377.55–797.12) vs. 397.23 (263.32–577.65), $P < 0.001$]. Multivariate logistic regression analyses showed that SIRI was significantly associated with 3-month unfavorable outcome of mild AIS patients [odds ratio (OR) = 2.938, 95% confidence interval (CI) = 1.805–4.782, $P < 0.001$], conversely, SII had no prognostic value. When SIRI combined with the established clinical factors, the area under the curve (AUC) showed a significant improvement (0.773 vs. 0.683, P for comparison = 0.0017).

Conclusions: Higher SIRI could be valuable in predicting poor clinical outcomes for patients with mild AIS following IVT.

KEYWORDS

systemic immune inflammation index, systemic inflammation response index, mild acute ischemic stroke, intravenous thrombolysis, 3-month outcome

Introduction

Worldwide, acute ischemic stroke (AIS) is a frequent condition characterized by high morbidity, disability, and mortality. Previous studies reported that more than half of AIS patients present with mild stroke (1, 2). Currently, there is no formal definition for mild AIS, but the majority of studies describe it as a National Institutes of Health stroke scale (NHISS) score $\leq$5 at admission (3, 4). In the clinical guidelines published in December 2019, intravenous

thrombolysis (IVT) was recommended for mild AIS patients with disabling symptoms (5). However, ∼30% of mild AIS patients have an unfavorable outcome after IVT (6, 7). Therefore, identification of risk factors linked to poor outcomes in patients with mild AIS after IVT is crucial. Age, diabetes, the baseline NHISS, and large vessel occlusion are some of the factors that have been mentioned in prior investigations (3, 8, 9). Moreover, it has been proposed that serum biomarkers may play an important role in the prognosis of mild AIS in recent years. Serum biomarkers, however, have received little research attention.

The blood-brain barrier disruption, oxidative stress, and the direct induction of neurocyte death that occurs as a result of inflammation response (IR) have all been identified as being important pathogenic processes of AIS (10, 11). Systemic immune inflammation index (SII) and systemic inflammation response index (SIRI), which are composed of platelets and three subtypes of white blood cells, have recently been reported as new inflammatory biomarkers that can reflect IR (12). Several studies have investigated the association between serum inflammatory indicators and functional outcomes in AIS patients (13–15). Additionally, it has been noted that the neutrophil-to-lymphocyte ratio (NLR) is a helpful inflammatory biomarker for predicting a bad short-term outcome in individuals with mild AIS following IVT, but their predictive effectiveness is not clarified (9).

Recently, SII and SIRI have been proven to predict the prognosis of some diseases such as ischemic stroke, coronary artery disease, subarachnoid hemorrhage and several cancers (16–19). However, the prognostic importance of SII and SIRI in mild AIS patients who have undergone thrombolysis has not yet been reported. Therefore, we aimed to systematically investigate the association of SII and SIRI with functional outcomes in mild AIS patients who received thrombolysis.

Materials and methods

Patients recruitment

This retrospective observational study was conducted from January 2017 to May 2022. All AIS patients receiving IVT therapy alone were consecutively included in the study and collected from the Minhang Hospital of Fudan University. An admission NHISS score ≤ 5 was considered to be mild AIS. The following were the inclusion requirements: (1) aged 18 years or older; (2) NHISS score ≤ 5 at admission; (3) stroke symptoms appearing within 4.5 h and receiving IVT treatment. Patients were disqualified if they fulfilled the following requirements: (1) score on the modified Rankin Scale (mRS) ≥ 2 before the stroke; (2) patients with malignant tumor, autoimmune disease and hepatic or renal diseases; (3) patients with acute infection, including pneumonia or other active concomitant infections. This study was reviewed and approved by the Ethical Review Board of Minhang Hospital of Fudan University.

Data collection

Onset to treatment time (ONT), NHISS score and stroke subtype were evaluated by experienced clinicians. The Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria were used to classify stroke subtypes. Stroke nurses gathered demographic and baseline information on patients with age, gender, smoking, diabetes, hypertension, and atrial fibrillation. Before IVT, initial counts for platelets, monocytes, neutrophils, and white blood cells were also obtained. We calculated the SIRI and SII as follows: SIRI = NEUT × Mo/TLC, SII = PLT × NEUT/TLC, where PLT is the platelet count, NEUT is the neutrophil count, TLC is the total lymphocyte count and Mo is the monocyte count.

Laboratory tests

Before thrombolysis, 2 mL of venous blood was collected with EDTA-K2 anticoagulant vacuum tube. Within 30 min after reversing evenly, Complete blood counts including white blood cells, neutrophils, lymphocytes, platelets, and other parameters were carried out using an automatic Mindray cal 8000 blood cell analyzer.

Clinical outcome

Functional outcome was evaluated by mRS score at 3 months after the onset of stroke. There was a neurologist in charge of the follow-up using the phone and face-to-face follow-up. Favorable outcome was considered to be an mRS score ≤ 1, while an unfavorable outcome was considered an mRS score ≥ 2. Safety outcomes included hemorrhagic transformation (HT) and symptomatic intracerebral hemorrhage (sICH). HT was confirmed by computed tomography (CT) scan within 7 days after IVT therapy. Any intracranial hemorrhage that raises the overall NIHSS score by 4 points is considered to be a sICH.

Imaging analysis

To determine the location of the infarction, all patients underwent brain magnetic resonance imaging (MRI). Ipsilateral severe vessel stenosis (ISVS) and ipsilateral severe vessel occlusion (ISVO) were evaluated by computed tomographic angiography (CTA). For patients with incomplete CTA, brain magnetic resonance angiography (MRA) examination was performed after admission. An intracranial or extracranial artery on the same side of the infarction with a diameter loss higher than 70% was categorized as ISVS. ISVO was defined as the absence of a blood flow signal from the ipsilateral infarction. Blinded to the clinical data, two seasoned MRI professional neuroradiologists separately assessed each subject's imaging manifestations, including the degree of artery stenosis and HT.

Statistical analysis

Statistical analyses were performed using SPSS (version 26.0, IBM Corp, Armonk, NY, USA). Receiver operating characteristic (ROC) analysis was conducted to identify the optimal cutoff values of SII and SIRI for predictive clinical outcomes in mild AIS patients after IVT. All subjects were divided into two groups according to the mRS score at 3 months (favorable 0–1 vs. unfavorable 2–6).

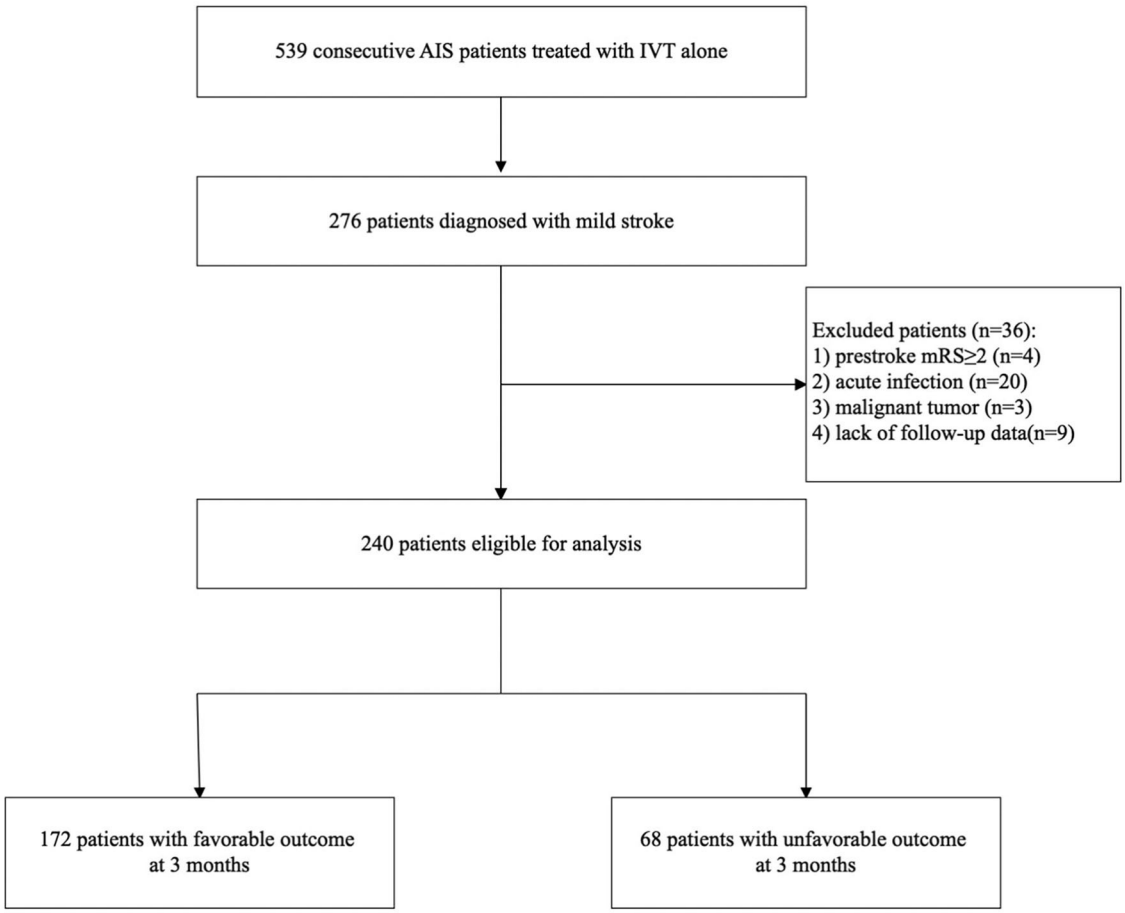

FIGURE 1
Diagram of the study recruitment.

Normal-distribution continuous data were reported as mean ± SD and compared using the *t*-test, whereas non-normal-distribution continuous variables were expressed as median (interquartile range) and compared using the Mann–Whitney *U* test. Categorical variables were presented numerically (percentages, %) and compared between the groups using the relevant Fisher exact or Pearson χ^2 tests. A subsequent multivariate logistic regression analysis included the variables for which $P < 0.1$ in the univariate analysis. $P < 0.05$ (two-sided) was used to determine statistical significance.

Results

Clinical characteristics of patients

During the study period, we collected 539 consecutive AIS patients treated with IVT alone. Of these patients, 276 patients were diagnosed with mild stroke. Finally, 240 patients were involved in our analysis after excluding those with pre-stroke mRS ≥ 2 ($n = 4$), acute infection ($n = 20$) including 18 patients with pneumonia and 2 patients with cholecystitis, malignant tumor ($n = 3$), lack of follow-up data ($n = 9$). Figure 1 shows a diagram depicting study recruitment.

There were 159 men and 81 women, aged 32–92 (median 66) years. Vascular risk factors included diabetes mellitus ($n = 74$, 30.8%), atrial fibrillation ($n = 41$, 17.1%), hypertension ($n = 140$, 58.3%), and smoking ($n = 73$, 30.4%). All patients were categorized into two subgroups according to the mRS score at 3 months. Compared with the patients in the favorable outcome group, the patients in the unfavorable group were older (66.00 vs. 65.00, $P = 0.039$). In terms of laboratory findings, the white blood cell level (7.42 vs. 6.51, $P < 0.001$), neutrophil count level (4.97 vs. 3.96, $P < 0.001$), monocyte count level (0.42 vs. 0.38, $P < 0.001$) were significantly increased in the unfavorable outcome group. The unfavorable outcome group had significantly higher SIRI (1.28 vs. 0.79, $P < 0.001$) and SII (531.93 vs. 397.23, $P < 0.001$) than the favorable outcome group and exhibited more serious neurological deficits on admission (NIHSS score 4.0 vs. 3.0, $P = 0.002$). The proportion of ISVS (25.0 vs. 13.4%, $P = 0.029$), ISVO (11.8 vs. 4.7%, $P = 0.047$), and ISVS/ISVO (36.8 vs. 18.0%, $P = 0.002$) in unfavorable outcome group were higher than in the favorable outcome group. Demographic features and risk factors are summarized in Table 1.

Multivariate analysis of factors related to unfavorable outcome

In mild AIS patients who were only receiving IVT, univariate and multivariate logistic regression analyses were run to assess the prognostic significance of SIRI and SII. We included age, NHISS

TABLE 1 Baseline characteristics of mild AIS stroke patients receiving IVT therapy based on favorable vs. unfavorable outcome at 3 months.

Variables	All patients ($n = 240$)	Favorable outcome ($n = 178$)	Unfavorable outcome ($n = 62$)	P-value
Demographics				
Age (year)	66.00 (60.00–73.35)	65.00 (58.35–72.00)	68.00 (62.00–75.75)	0.039*
Men, *n* (%)	81 (33.8%)	60 (34.9%)	21 (30.9%)	0.555
Medical history				
Hypertension, *n* (%)	140 (58.3%)	99 (57.6%)	41 (60.3%)	0.698
Diabetes mellitus, *n* (%)	74 (30.8%)	51 (29.7%)	23 (33.8%)	0.528
Atrial fibrillation, *n* (%)	41 (17.1%)	31 (18.0%)	10 (14.7%)	0.538
Current smoking, *n* (%)	73 (30.4%)	49 (28.5%)	24 (35.3%)	0.302
OTT (min)	145.00 (110.25–182.25)	143.00 (110.00–179.50)	155.00 (116.25–195.75)	0.143
NHISS score at admission	3.00 (2.00–4.00)	3.00 (2.00–4.00)	4.00 (3.00–4.75)	0.002*
Stroke subtype, *n* (%)				0.410
LAA	86 (35.8%)	56 (32.6%)	29 (42.6%)	
CE	35 (14.6%)	26 (15.1%)	9 (13.2%)	
SAO	89 (37.1%)	64 (37.2%)	25 (36.8%)	
SAE	3 (1.3%)	3 (1.7%)	1 (1.5%)	
SUE	27 (11.3%)	23 (13.4%)	4 (5.9%)	
HT, *n* (%)	20 (8.3%)	12 (7.0%)	8 (11.8%)	0.227
sICH, *n* (%)	4 (1.7%)	2 (1.2%)	2 (2.9%)	0.332
Vascular stenosis, *n* (%)				
ISVS	40 (16.7%)	23 (13.4%)	17 (25.0%)	0.029*
ISVO	16 (6.7%)	8 (4.7%)	8 (11.8%)	0.047*
ISVS/ISVO	56 (23.3%)	31 (18.0%)	25 (36.8%)	0.002*
Laboratory measures				
WBC counts (10^9/L)	6.95 (5.67–8.10)	6.51 (5.45–7.95)	7.42 (6.62–8.75)	<0.001*
Neutrophil counts (10^9/L)	4.18 (3.10–5.37)	3.93 (2.98–4.86)	5.11 (4.08–6.17)	<0.001*
Lymphocyte counts (10^9/L)	1.90 (1.40–2.51)	1.97 (1.48–2.51)	1.77 (1.27–2.54)	0.195
Monocyte counts (10^9/L)	0.39 (0.32–0.50)	0.38 (0.31–0.47)	0.42 (0.36–0.58)	<0.001*
Platelet counts (10^9/L)	197.50 (164.24–236.75)	194.00 (159.50–237.25)	202.50 (174.50–236.75)	0.443
Hemoglobin (g/L)	142.00 (132.00–153.00)	142.00 (133.00–150.75)	140.00 (127.50–156.75)	0.800
SIRI	0.90 (0.55–1.34)	0.79 (0.51–1.08)	1.28 (0.70–1.88)	<0.001*
SII	434.51 (285.30–650.60)	397.23 (263.32–577.65)	531.93 (377.55–797.12)	<0.001*

*Means $P < 0.05$.

LAA, large-artery atherosclerosis; CE, cardioembolism; SAO, small-artery occlusion; SOE, stroke of other determined etiology; SOE, stroke of undetermined etiology; ISVS, ipsilateral severe vessel stenosis; ISVO, ipsilateral severe vessel occlusion; HT, hemorrhagic transformation; sICH, symptomatic intracerebral hemorrhage; SIRI, systemic inflammation response index; SII, systemic immune-inflammation index.

score at admission, diabetes mellitus, ISVS/ISVO, SIRI and SII into the multivariate analysis.

In multivariate analysis, the NHISS score at admission [OR = 1.430, 95% confidence interval (CI) = 1.114–1.835, $P = 0.005$], ISVS/ISVO (OR = 2.347, 95% CI = 1.173–4.696, $P = 0.016$), and SIRI (OR = 2.938, 95% CI = 1.805–4.782, $P < 0.001$) were the predictors of an unfavorable outcome (Table 2).

Association of SIRI with clinical outcome

According to the ROC analysis (Figure 2), the optimal cut-off threshold of SIRI was 1.00×10^9/L [area under the curve (AUC) = 0.714, 95% CI = 0.652–0.770, $P < 0.001$]. The ROC curve showed that the AUC of NHISS score combined with ISVS/ISVO to predict the adverse outcome after thrombolysis in mild stroke was 0.683 (95% CI = 0.620–0.742, $P < 0.001$). When SIRI combined the above indicators, the AUC increased to 0.773 (95% CI = 0.715–0.825, $P <$

TABLE 2 Multivariate regression analysis of factors related to 3-month unfavorable outcome.

Variables	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (year)	1.029 (1.004–1.055)	0.024	–	0.119
NHISS score at admission	1.424 (1.130–1.795)	0.003	1.430 (1.114–1.835)	0.005
Diabetes mellitus	0.825 (0.453–1.502)	0.529	–	0.977
ISVS/ISVO	2.644 (1.411–4.954)	0.002	2.347 (1.173–4.696)	0.016
SIRI	3.101 (1.918–5.015)	<0.001	2.938 (1.805–4.782)	<0.001
SII	1.002 (1.001–1.003)	0.001	–	0.918

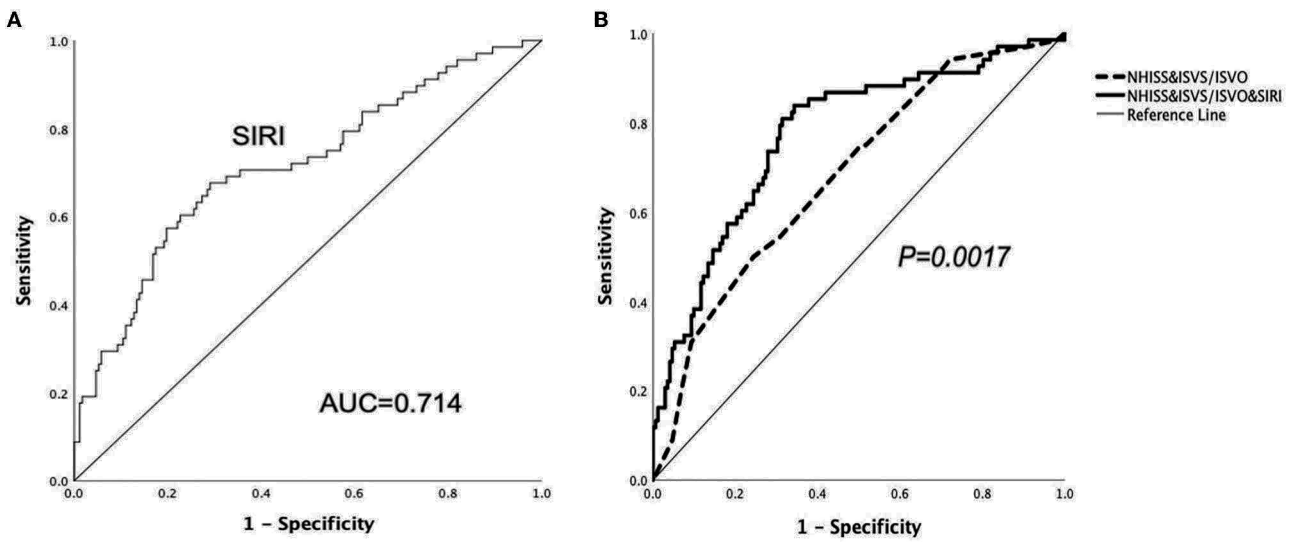

FIGURE 2
ROC curves demonstrate the predictive ability of SIRI and show SIRI improves the predictive ability of ISVS/ISVO&NHISS for unfavorable outcomes in mild AIS.

0.001). There was a significant statistical difference between the two ROC curves ($P = 0.0017$).

Discussion

For the first time, the current study examined the relationship between SIRI and SII and the 3-month functional outcome of mild AIS after IVT therapy alone. Our analysis demonstrated that SIRI and SII in patients with 3-month unfavorable outcomes of mild AIS treated with IVT are higher than those in patients with favorable outcomes. SIRI was an independent predictor for unfavorable outcome. Most importantly, when SIRI was combined, it can enhance the prognostic usefulness of traditional risk variables in people who had mild stroke after IVT.

It has been proved that the IR after AIS is related to the secondary brain damage after infarction (20, 21). The inflammatory mediators, cytokines, adhesion molecules, and chemokines released by immune inflammatory cells exacerbate tissue damage. According to previous studies, IR can be activated immediately after stroke event has occurred and is associated with poor prognosis (22–24). The mentioned mechanisms explain why the biomarkers now being researched are based on numerous inflammatory variables linked to stroke.

SIRI is a novel inflammation index, including peripheral neutrophils, lymphocytes and monocytes. Peripheral circulating neutrophils are regarded as the first inflammatory cells to penetrate the ischemic parenchyma after the onset of AIS, which exacerbate brain injury by releasing particles containing antibacterial enzymes and chemicals (25, 26). Higher neutrophil levels in early AIS were associated with larger infarction size. This may be because the increase in neutrophil concentration will promote the enhanced expression of matrix metalloproteinase-9 (MMP-9), a protein related to blood brain barrier damage (27, 28). In addition, monocytes are another important type of inflammatory cell after AIS, which can infiltrate infarcted areas and aggravate brain injury (29–31). Nevertheless, in contrast to neutrophils and monocytes, certain lymphocytes largely serve a protective role in the IR following AIS, controlling and reducing local IR (32). As a result, high SIRI (an increase in neutrophils, monocytes, and a decrease in lymphocytes) may accurately reflect adaptive immune response and IR, which are crucial processes for the incidence of stroke and may be effective biomarkers for prognosis.

Several earlier research has shown that SIRI is an effective marker for assessing the prognosis of varieties of inflammation-related diseases, which was consistent with our research results. According to Qi et al. (33), SIRI can be used to evaluate survival in pancreatic cancer patients receiving chemotherapy. Recently, Yun et al. (34) analyzed 680 aneurysmal subarachnoid hemorrhage (aSAH) patients and concluded that SIRI is an independent risk factor for unfavorable outcomes in aSAH patients. In addition, a retrospective study found that SIRI may serve as a predictive index for patients undergoing mechanical thrombectomy (MT) due to large artery occlusion (12). However, no study has found an association between SIRI and mild AIS treated with IVT. To the best of our knowledge, this is the first study to show a potential correlation between SIRI and the outcome of mild AIS after IVT.

Inflammatory markers based on platelets as well as leukocyte-based markers have been investigated in AIS patients. During AIS, platelets aggregate rapidly after blood vessel damage and play an important role in thrombosis. Furthermore, platelets also participate in immune inflammatory reaction. By altering the surface expression of *P*-selectin, platelets can directly interact with circulating leukocytes to create platelet-leukocyte aggregates and activate innate immunity in ischemic organs (35). Recently, the prognosis of AIS patients is thought to be reflected by SII, which is based on a combination of platelets, neutrophils, and lymphocytes (13). Even though the SII level in the group with a poor prognosis was higher than that in the group with a good prognosis in our study of thrombolytic patients with mild stroke, we did not discover that the SII was directly associated with the unfavorable outcome at 3 months. As it might be a viable clinical tool for determining mild AIS and its potential consequences, a more thorough and well-designed study on large populations of patients is required.

It has been demonstrated that the NHISS score at admission is a reliable predictor of a poor outcome in moderate AIS following IVT (3, 6). Our findings are consistent with those of earlier research. Additionally, we found that ISVS/ISVO was a predictor of a worse clinical outcome at 3 months, which is similar to a finding from a prior study (9). It reflects the bad condition of intracranial vessels and extracranial vessels.

There are some strengths in our study. First off, to the best of our knowledge, it is the first article that has specifically addressed the correlation between the short-term unfavorable outcome in patients with mild AIS following IVT and novel inflammation index, including SIRI and SII. Second, our study has a benefit over many earlier studies in that we investigated the predictive potency for the prognosis of mild AIS. However, our study also has some limitations that need to be considered. First of all, this analysis was performed retrospectively and without blinding, which has a chance of selection bias. Second, the absence of dynamic SIRI and SII data prevented us from making predictions that might be more accurate. Third, given the limited sample size, the results should be confirmed in future, larger populations.

Conclusion

In conclusion, the current investigation shows for the first time that mild AIS patients with poor outcomes after IVT had increased SIRI and SII. Also, SIRI was an independent predictor for unfavorable outcomes at 3 months. Our work provides an economically convenient way to refine risk stratification for adverse outcomes in mild AIS. Further large-scale prospective studies are needed for dynamically monitoring SIRI and SII to understand the value of the index better.

Data availability statement

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

Ethics statement

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

Author contributions

MC and JZ designed the study. YaL and YW collected the data. MC, YuL, and DaW performed the statistical analysis and wrote the paper. DeW and JZ revised the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

References

1. Reeves M, Khoury J, Alwell K, Moomaw C, Flaherty M, Woo D, et al. Distribution of national institutes of health stroke scale in the Cincinnati/Northern Kentucky Stroke study. *Stroke.* (2013) 44:3211–3. doi: 10.1161/STROKEAHA.113.002881

2. Wang Y, Li Z, Wang Y, Zhao X, Liu L, Yang X, et al. Chinese stroke center alliance: a national effort to improve healthcare quality for acute stroke and transient ischaemic attack: rationale, design and preliminary findings. *Stroke Vascul Neurol.* (2018) 3:256–62. doi: 10.1136/svn-2018-000154

3. Tang H, Yan S, Wu C, Zhang Y. Characteristics and outcomes of intravenous thrombolysis in mild ischemic stroke patients. *Front Neurol.* (2021) 12:744909. doi: 10.3389/fneur.2021.744909

4. Duan H, Cheng Z, Yun H, Cai L, Tong Y, Han Z, et al. Serum bilirubin associated with stroke severity and prognosis: preliminary findings on liver function after acute ischemic stroke. *Neurol Res.* (2022) 2022:1–8. doi: 10.1080/01616412.2022.2119724

5. Powers W, Rabinstein A, Ackerson T, Adeoye O, Bambakidis N, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2019) 50:e344–418. doi: 10.1161/STR.0000000000000211

6. Romano J, Smith E, Liang L, Gardener H, Camp S, Shuey L, et al. Outcomes in mild acute ischemic stroke treated with intravenous thrombolysis: a retrospective analysis of the get with the guidelines-stroke registry. *JAMA Neurol.* (2015) 72:423–31. doi: 10.1001/jamaneurol.2014.4354

7. Yu A, Hill M, Coutts S. Should minor stroke patients be thrombolyzed? A focused review and future directions. *Int J Stroke Off J Int Stroke Soc.* (2015) 10:292–7. doi: 10.1111/ijs.12426

8. Kenmuir C, Hammer M, Jovin T, Reddy V, Wechsler L, Jadhav A. Predictors of outcome in patients presenting with acute ischemic stroke and mild stroke scale scores. *J Stroke Cerebrovasc Dis Off J Natl Stroke Assoc.* (2015) 24:1685–9. doi: 10.1016/j.jstrokecerebrovasdis.2015.03.042

9. Liu Y, Wu Z, Qu J, Qiu D, Luo G, Yin H, et al. High neutrophil-to-lymphocyte ratio is a predictor of poor short-term outcome in patients with mild acute ischemic stroke receiving intravenous thrombolysis. *Brain Behav.* (2020) 10:e01857. doi: 10.1002/brb3.1857

10. Macrez R, Ali C, Toutirais O, Le Mauff B, Defer G, Dirnagl U, et al. Stroke and the immune system: from pathophysiology to new therapeutic strategies. *Lancet Neurol.* (2011) 10:471–80. doi: 10.1016/S1474-4422(11)70066-7

11. Jenny N, Callas P, Judd S, McClure L, Kissela B, Zakai N, et al. Inflammatory cytokines and ischemic stroke risk: the regards cohort. *Neurology.* (2019) 92:e2375–e84. doi: 10.1212/WNL.0000000000007416

12. Yi HJ, Sung JH, Lee DH. Systemic inflammation response index and systemic immune-inflammation index are associated with clinical outcomes in patients treated with mechanical thrombectomy for large artery occlusion. *World Neurosurg.* (2021) 153:e282–e9. doi: 10.1016/j.wneu.2021.06.113

13. Huang L. Increased systemic immune-inflammation index predicts disease severity and functional outcome in acute ischemic stroke patients. *Neurologist.* (2022). doi: 10.1097/NRL.0000000000000464

14. Zhou Y, Zhang Y, Cui M, Zhang Y, Shang X. Prognostic value of the systemic inflammation response index in patients with acute ischemic stroke. *Brain Behav.* (2022) 12:e2619. doi: 10.1002/brb3.2619

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

16. Li J, Yuan Y, Liao X, Yu Z, Li H, Zheng J. Prognostic significance of admission systemic inflammation response index in patients with spontaneous intracerebral hemorrhage: a propensity score matching analysis. *Front Neurol.* (2021) 12:718032. doi: 10.3389/fneur.2021.718032

17. Dziedzic E, Gasior J, Tuzimek A, Paleczny J, Junka A, Dabrowski M, et al. Investigation of the associations of novel inflammatory biomarkers-systemic inflammatory index (Sii) and systemic inflammatory response index (Siri)-with the severity of coronary artery disease and acute coronary syndrome occurrence. *Int J Mol Sci.* (2022) 23:9553. doi: 10.3390/ijms23179553

18. Pacheco-Barcia V, Mondéjar Solís R, France T, Asselah J, Donnay O, Zogopoulos G, et al. A systemic inflammation response index (Siri) correlates with survival and predicts oncological outcome for mfolfirinox therapy in metastatic pancreatic cancer. *Pancreatol Off J Int Assoc Pancreatol.* (2020) 20:254–64. doi: 10.1016/j.pan.2019.12.010

19. Wang J, Zhang X, Tian J, Li H, Tang H, Yang C. Predictive values of systemic inflammatory responses index in early neurological deterioration in patients with acute ischemic stroke. *J Integ Neurosci.* (2022) 21:94. doi: 10.31083/j.jin2103094

20. Wu F, Liu Z, Zhou L, Ye D, Zhu Y, Huang K, et al. Systemic immune responses after ischemic stroke: from the center to the periphery. *Front Immunol.* (2022) 13:911661. doi: 10.3389/fimmu.2022.911661

21. Sadeghi F, Sarkady F, Zsóri K, Szegedi I, Orbán-Kálmándi R, Székely E, et al. High neutrophil-lymphocyte ratio and low lymphocyte-monocyte ratio combination after thrombolysis is a potential predictor of poor functional outcome of acute ischemic stroke. *J Personal Med.* (2022) 12:1221. doi: 10.3390/jpm12081221

22. Feng Y, Bai X, Li W, Cao W, Xu X, Yu F, et al. Postoperative neutrophil-lymphocyte ratio predicts unfavorable outcome of acute ischemic stroke patients who achieve complete reperfusion after thrombectomy. *Front Immunol.* (2022) 13:963111. doi: 10.3389/fimmu.2022.963111

23. Wu F, Wang Q, Qiao Y, Yu Q, Wang F. A new marker of short-term mortality and poor outcome in patients with acute ischemic stroke: mean platelet volume-to-lymphocyte ratio. *Medicine.* (2022) 101:e30911. doi: 10.1097/MD.0000000000030911

24. Stuckey S, Ong L, Collins-Praino L, Turner R. Neuroinflammation as a key driver of secondary neurodegeneration following stroke? *Int J Mol Sci.* (2021) 22:101. doi: 10.3390/ijms222313101

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

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

27. Buck BH, Liebeskind DS, Saver JL, Bang OY, Yun SW, Starkman S, et al. Early neutrophilia is associated with volume of ischemic tissue in acute stroke. *Stroke.* (2008) 39:355–60. doi: 10.1161/STROKEAHA.107.490128

28. Garau A, Bertini R, Colotta F, Casilli F, Bigini P, Cagnotto A, et al. Neuroprotection with the Cxcl8 inhibitor repertaxin in transient brain ischemia. *Cytokine.* (2005) 30:125–31. doi: 10.1016/j.cyto.2004.12.014

29. Kaito M, Araya S, Gondo Y, Fujita M, Minato N, Nakanishi M, et al. Relevance of distinct monocyte subsets to clinical course of ischemic stroke patients. *PLoS ONE.* (2013) 8:e69409. doi: 10.1371/journal.pone.0069409

30. Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. *J Leukoc Biol.* (2010) 87:779–89. doi: 10.1189/jlb.1109766

31. Ray MJ, Walters DL, Bett JN, Cameron J, Wood P, Aroney CN. Platelet-monocyte aggregates predict troponin rise after percutaneous coronary intervention and are inhibited by abciximab. *Int J Cardiol.* (2005) 101:249–55. doi: 10.1016/j.ijcard.2004.03.033

32. Liesz A, Zhou W, Na SY, Hämmerling GJ, Garbi N, Karcher S, et al. Boosting regulatory T cells limits neuroinflammation in permanent cortical stroke. *J Neurosci.* (2013) 33:17350–62. doi: 10.1523/JNEUROSCI.4901-12.2013

33. Qi Q, Zhuang L, Shen Y, Geng Y, Yu S, Chen H, et al. A novel systemic inflammation response index (Siri) for predicting the survival of patients with pancreatic cancer after chemotherapy. *Cancer.* (2016) 122:2158–67. doi: 10.1002/cncr.30057

34. Yun S, Yi H, Lee D, Sung J. Systemic inflammation response index and systemic immune-inflammation index for predicting the prognosis of patients with aneurysmal subarachnoid hemorrhage. *J Stroke Cerebrovasc Dis Off J Natl Stroke Assoc.* (2021) 30:105861. doi: 10.1016/j.jstrokecerebrovasdis.2021.105861

35. Maugeri N, Rovere-Querini P, Evangelista V, Covino C, Capobianco A, Bertilaccio M, et al. Neutrophils phagocytose activated platelets *in vivo*: a phosphatidylserine, p-selectin, and integrin-dependent cell clearance program. *Blood.* (2009) 113:5254–65. doi: 10.1182/blood-2008-09-180794

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The development of neurocritical care in China from the perspective of evaluation and treatment of critical neurological diseases

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Objective: To understand the varieties, evaluation, treatment, and prognosis of severe neurological diseases using the third NCU survey in China.

Design: A cross-sectional questionnaire study. The study was completed in three main steps: filling in the questionnaire, sorting out the survey data, and analyzing the survey data.

Results: Of 206 NCUs, 165 (80%) provided relatively complete information. It was estimated that 96,201 patients with severe neurological diseases were diagnosed and treated throughout the year, with an average fatality rate of 4.1%. The most prevalent severe neurological disease was cerebrovascular disease (55.2%). The most prevalent comorbidity was hypertension (56.7%). The most prevalent complication was hypoproteinemia (24.2%). The most common nosocomial infection was hospital-acquired pneumonia (10.6%). The GCS, APACHE II, EEG, and TCD were the most commonly used (62.4–95.2%). The implementation rate of the five nursing evaluation techniques reached 55.8–90.9%. Routinely raising the head of the bed by 30°, endotracheal intubation and central venous catheterization were the most

prevalent treatment strategies (97.6, 94.5, and 90.3%, respectively). Traditional tracheotomy, invasive mechanical ventilation and nasogastric tube feeding (75.8, 95.8, and 95.8%, respectively) were more common than percutaneous tracheotomy, non-invasive mechanical ventilation and nasogastric tube insertion (57.6, 57.6, and 66.7%, respectively). Body surface hypothermia brain protection technology was more commonly used than intravascular hypothermia technology (67.3 > 6.1%). The rates of minimally invasive hematoma removal and ventricular puncture were only 40.0 and 45.5%, respectively.

Conclusion: In addition to traditional recognized basic life assessment and support technology, it is necessary to the use of promote specialized technology for neurological diseases, according to the characteristics of critical neurological diseases.

KEYWORDS

critical neurological disease, evaluation, treatment, NCCU, investigation

Highlights

- Question: What is the current state of the Neurocritical Care Unit (NCU) in China?
- Findings: In addition to traditional recognized basic life assessment and support technology, it is necessary to promote the use of specialized technology for neurological diseases, according to the characteristics of critical neurological diseases.
- Meaning: To understand the varieties, evaluation, treatment, and prognosis of severe neurological diseases in China.

Introduction

At the beginning of the twenty-first century, the neurocritical care committee (NCC) of the China Neurology Association (CNA) conducted national neurocritical care unit (NCCU) surveys in 2010, 2015, and 2020 (1). The results of these surveys were used to promote the rapid and orderly development of the neurocritical care specialty. The third survey was different from the first and second surveys. To align with the goal of "improving the prognosis of severe neurological diseases" in the "Neurocritical Care Society Global Partners Program," six survey parts related to prognosis/outcome were added: types of severe neurological diseases, comorbidities, complications, nosocomial infection, evaluation technology, and treatment technology. The purpose is to guide the development of severe neurological diseases in the next 5–10 years.

Methods

Survey organization and form

The NCC chairman was responsible for designing the questionnaire. The NCC members were responsible for distributing and collecting the NCCU questionnaires and then verifying the first data collected in the region. The NCC survey working group (NCC-SWG) was responsible for sorting the questionnaires, checking the second data and performing the statistical analysis. The survey area covered the 31 provinces of mainland China, the autonomous regions and municipalities directly under the central government.

Objects and methods of investigation

The NCCUs of tertiary hospitals employed with full-time doctors were included in the survey. The survey was a cross-sectional survey. The investigation process and steps included three stages: filling out the questionnaire, data collection and sorting, data verification and confirmation, and statistical analysis. First stage (October 1 to December 31, 2020): the NCC-SWG sent the self-report questionnaire to the regional principals (NCC members) by e-mail, and each member sent the questionnaire to the NCCU of the hospital in the region according to the survey inclusion conditions. The person in charge of the NCCU to be surveyed completed the questionnaire. Second stage (from January 1 to March 31, 2021): the person in charge of the NCCU in each region submitted a questionnaire to the NCC members. After each member received and completed the questionnaire, the questionnaire was submitted to the NCC-SWG by email. Third stage (from April 1 to June 30, 2021): the NCC-SWG first collated and verified the questionnaire (verified the query information or data by telephone or e-mail) and then performed statistical analysis on the survey results (Figure 1).

Investigation content

The questionnaire adopted closed questions to reflect the actual situation in 2020. The survey included the types of neurological diseases, comorbidities, complications, prevalence of nosocomial infection, use of evaluation technology and treatment technology observed in the NCCU, and the questionnaire had a total of 6 parts and 57 items.

Statistical analysis

SPSS 19.0 statistical software was used for statistical analyses. Descriptive statistics were used. The enumeration data were expressed as the number of cases (percentage), and the measurement data were expressed as the mean and range (minimum and maximum). To reflect seasonal changes, the data of the 1st month (January, April, July, and October) of each quarter in 2020 were

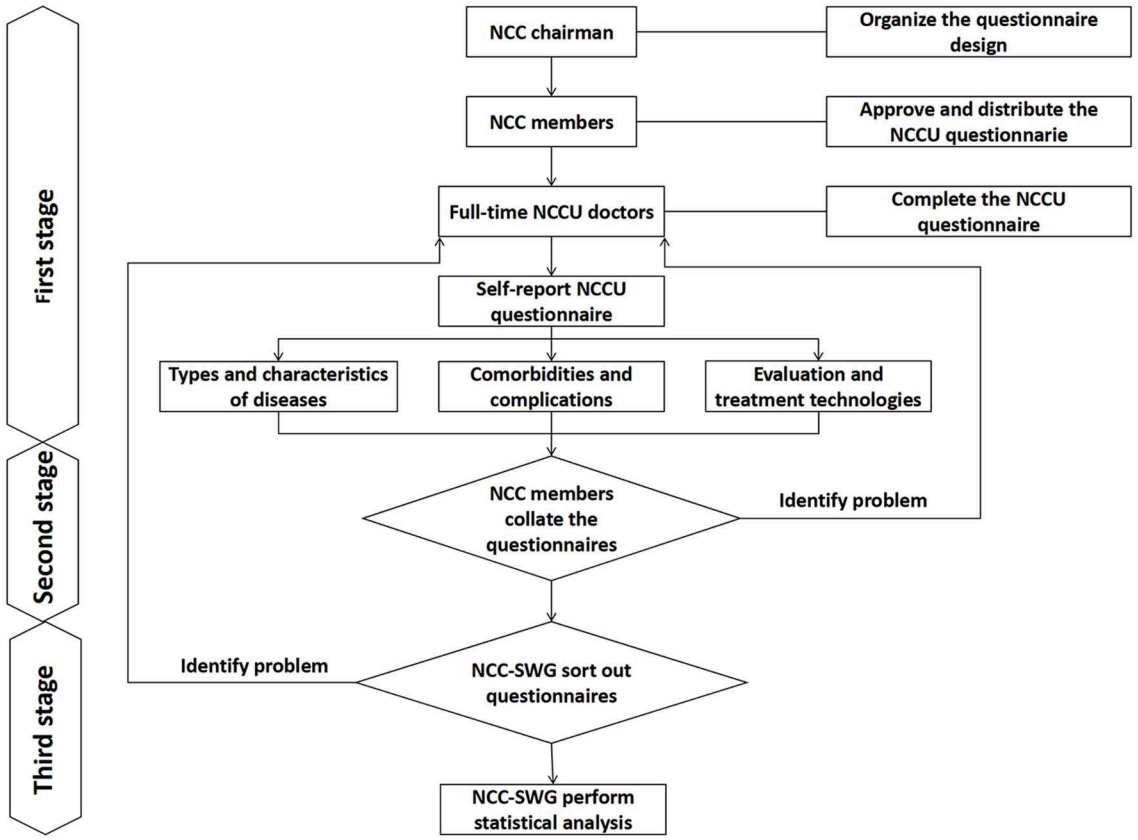

FIGURE 1
Investigation process. NCC, neurocritical care; NCCU, neurocritical care unit.

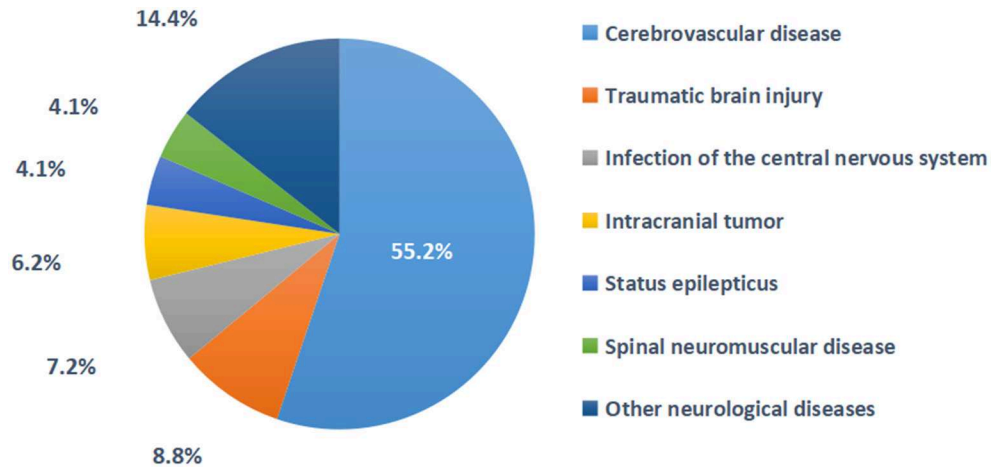

FIGURE 2
The proportion of diseases in 165 NCCU patients in 2020. Other neurological diseases include demyelinating disease, metabolic encephalopathy, postcranial/spinal surgery, etc.

selected, and the annual data were estimated according to the formula of monthly average sum/4*12.

Results

In 2020, 165 NCCUs in 152 tertiary hospitals in 29 provinces provided relatively complete information on severe neurological diseases, comorbidities, complications, nosocomial infections and mortality. It was estimated that 96,201 patients with severe neurological diseases were diagnosed and treated throughout the year (an average of 582 cases per NCCU), with an average case fatality rate of 4.1%. Among all seven types of severe neurological diseases, the three most prevalent were cerebrovascular diseases, traumatic brain injury and central nervous system infection, among which cerebrovascular diseases accounted for the highest proportion (55.2%), which was higher than the sum of all of the other

TABLE 1 Disease proportion and mortality of 165 NCCU patients in 2020[a].

Item	January	April	July	October	Annual estimation[d]
Number of cases (average), *n*[b]	7,764 (47)	7,560 (46)	8,144 (49)	8,599 (52)	96,201 (582)
Number of diseases (average and proportion), *n* (*n*, %)[c]					
Cerebrovascular disease	0–320 (26, 55%)	0–297 (25, 54.3%)	1–307 (27, 55.1%)	2–309 (29, 55.8%)	9–3699 (321, 55.2%)
Traumatic brain injury	0–120 (4, 8.5%)	0–140 (4, 8.7%)	0–150 (4, 8.2%)	0–140 (5, 9.6%)	0–1650 (51, 8.8%)
Infection of the central nervous system	0–27 (3, 6.4%)	0–28 (3, 6.5%)	0–32 (4, 8.2%)	0–35 (4, 7.7%)	0–366 (42, 7.2%)
Intracranial tumor	0–80 (3, 6.4%)	0–96 (3, 6.5%)	0–102 (3, 6.1%)	0–100 (3, 5.8%)	0–1134 (36, 6.2%)
Status epilepticus	0–16 (2, 4.3%)	0–25 (2, 4.4%)	0–20 (2, 4.1%)	0–18 (2, 3.8%)	0–237 (24, 4.1%)
Spinal neuromuscular disease	0–12 (2, 4.3%)	0–12 (2, 4.4%)	0–18 (2, 4.1%)	0–14 (2, 3.8%)	0–168 (24, 4.1%)
Other neurological diseases	0–90 (7, 14.8%)	0–127 (7, 15.2%)	0–119 (7, 14.2%)	0–118 (7, 13.5%)	0–633 (84, 14.4%)
Number of deaths (average, mortality), *n* (*n*, %)	0–22 (2, 4.3%)	0–18 (2, 4.3%)	0–21 (2, 4.1%)	0–20 (2, 3.8%)	0–243 (24, 4.1%)

[a]Cases from 165 NCCUs in the first month of each quarter (January, April, July, and October) in 2020 were selected to estimate the disease types and mortality of the whole year. Other neurological diseases include demyelinating disease, metabolic encephalopathy, postcranial/spinal surgery, etc.
[b]Average number of cases: number of cases/number of NCCUs.
[c]Average number of cases/average number of cases of diseases/average number of cases of death: the number of cases, cases of diseases and cases of death divided by the number of NCCUs. Proportion of diseases: the average number of diseases divided by the average total number of cases.
[d]Annual estimation: January, April, July, and October represent four quarterly sampling, respectively, take the average value and multiply by 12.

severe neurological diseases (Figure 2). The different types of diseases changed in the different time periods, but they did not change significantly (Table 1). Of all six comorbidities, the three most prevalent were hypertension, diabetes and coronary heart disease (56.7, 33, and 22.7%, respectively; Supplementary material). Among the 10 complications, the four most prevalent were hypoproteinemia, hyperglycemia, acute pulmonary dysfunction and acute gastrointestinal dysfunction (24.2, 21.1, 17.5, and 14.8%, respectively). The types of comorbidities and complications changed in the different time periods, but they did not change significantly (Supplementary material). Among the four types of nosocomial infections observed, the highest proportion was hospital-acquired pneumonia (10.6%), but there was little change within the different time periods. The percent of NCCUs that had doctors and nurses in charge of nosocomial infections was as high as 87.3 and 67.3%, respectively (Supplementary material). Among the evaluation techniques, the Glasgow Coma Scale (GCS) and Acute Physiology and Chronic Health Evaluation II (APACHE II) were the most commonly used (95.2 and 62.4%). Electroencephalography (EEG) and transcranial Doppler (TCD) were the most used brain function evaluation techniques (84.2 and 67.9%). The implementation rate of the five nursing evaluation techniques reached 55.8–90.9% (Figure 3). Routinely raising the head of the bed by 30° was the most used treatment technique (97.6%). Endotracheal intubation and central venous catheterization were the second most commonly used treatment technique (94.5 and 90.3%, respectively). Traditional tracheotomy was performed more often than percutaneous tracheotomy (75.8 > 57.6%). Invasive mechanical ventilation was used more often than non-invasive mechanical ventilation (95.8 > 57.6%). Nasogastric tubes were used more often than nasointestinal tubes (95.8 > 66.7%). Body surface hypothermia was induced more often than intravascular hypothermia (67.3 > 6.1%). Minimally invasive brain hematoma removal and ventriculocentesis were performed 40.0 and 45.5%, respectively (Figure 4).

Discussion

After the first and second surveys were administered in 2010 and 2015 in China, the third survey was administered in 2020. Unfortunately, COVID-19 was prevalent in 2020. Although NCCUs in some areas were affected by the temporary epidemic (the closure of Wuhan for 2.5 months), NCCUs in most areas still received patients with severe neurological diseases, so the size, characteristics and treatment and diagnostic ability of those NCCUs in mainland China that remained in operation was noted.

NCCU case volume

In this study, the average volume of patients per NCCU was large (582 cases) and exceeded expectations. We speculated that this was mainly related to the high proportion of patients with cerebrovascular diseases (55.2%). The morbidity of cerebrovascular disease in China is 1,114.8 per 100,000, which is the highest in the world, and the mortality rate is 149.49 per 100,000 and is only inferior to malignant tumors and heart disease (2). As a result, the burdens on NCCU patients with severe cerebrovascular disease and the doctors and nurses who treat these patients have increased. In addition, in recent years, the use of intravascular treatment for acute stroke macrovascular occlusion has been popularized in China (3). The number of patients who received intensive monitoring in the NCCU for a short time (24–72 h) after treatment has increased, and the increase has become the main reason for the increase in the volume of NCCU patients. Therefore, the NCC suggested the expansion of NCCUs and the allocation of medical staff to allow the setup of a special area for patients with cerebrovascular diseases in the NCCU when necessary. Additionally, the NCC also suggested strengthening the monitoring and treatment of patients with severe cerebrovascular diseases to improve their short-term and long-term neurological prognosis.

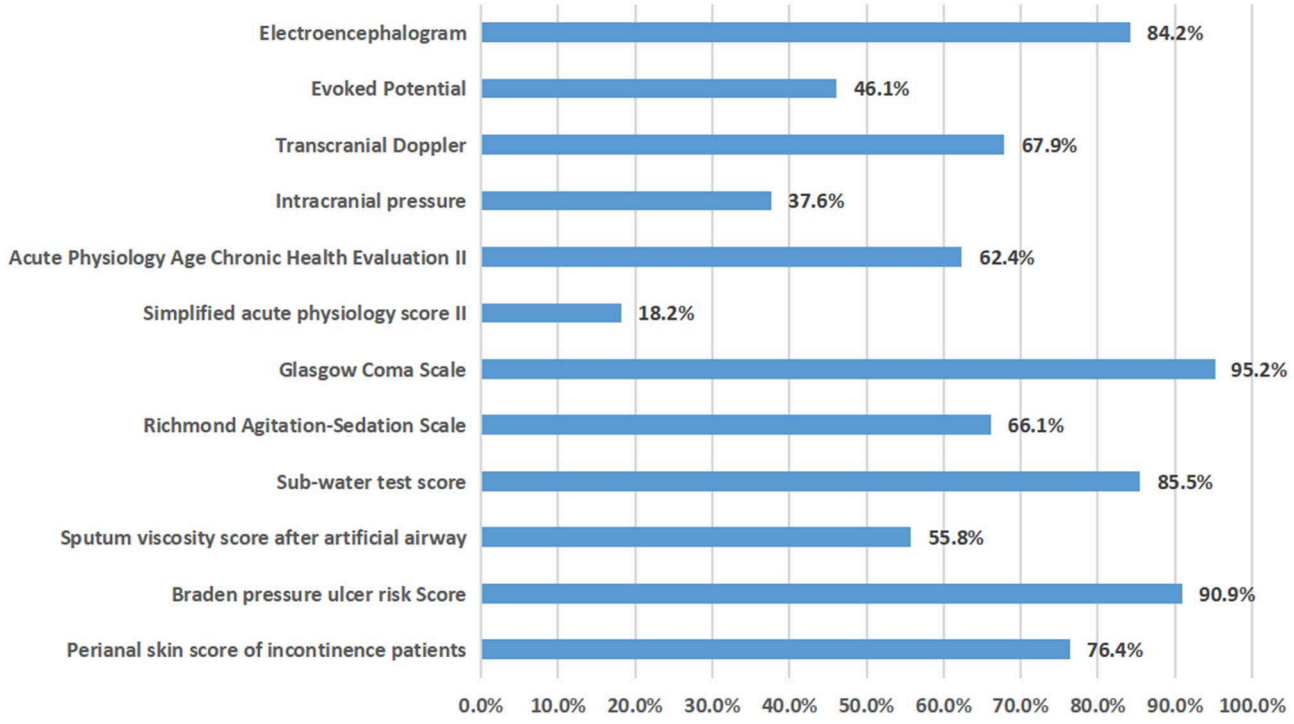

FIGURE 3
Proportion of 165 NCCU evaluation technologies in 2020.

NCCU disease characteristics

Among the severe neurological diseases in this study, cerebrovascular diseases, traumatic brain injury and central nervous system infection accounted for the highest proportion (55.2, 8.8, and 7.2%), resulting in a high proportion of acute brain diseases and severe brain injury. This feature makes the focus of medical care and clinical research clearer.

In this study, hypertension, diabetes and coronary heart disease were characterized by high proportions (56.7, 33, and 22.7%, respectively). This feature was related to the high proportion of cerebrovascular diseases in the NCCU. China's national stroke statistics report showed that hypertension, diabetes and coronary heart disease were the most prevalent comorbidities that affected the prognosis (2). Therefore, in the diagnosis and treatment of primary diseases, we should pay attention to the diagnosis and treatment of comorbidities.

Among the complications observed in this study, acute pulmonary dysfunction (pulmonary infection), acute gastrointestinal dysfunction (gastric motility deficiency and acute gastric mucosal lesions), hypoproteinemia and hyperglycemia were highly prevalent (13.9, 13.4, 24.2, and 21.1%, respectively). These high prevalences were related to the high proportion of acute severe brain injury patients. Brain and multiple organ system injuries not only increase the complexity of patient monitoring and treatment but also increase the risk of death and poor prognosis regarding the patient's neurological function (4). Therefore, predicting and reducing the risk of complications have become an important part of improving prognosis.

In the NCCU, due to coma, paralysis, dysphagia, and immune impairment in patients, nosocomial infections can occur and persist. Therefore, medical staff should be vigilant in preventing and combating infection. This study showed that most of the NCCUs had doctors and nurses specifically responsible for nosocomial infections (87.3 and 67.3%), so they had a more thorough understanding of the guidelines for isolation and prevention of nosocomial infections (5) and could better implement measures to prevent and treat nosocomial infections, which led to higher satisfaction with the control of the four nosocomial infection rates, such as hospital-acquired pneumonia (2.2–10.6%).

This study did not find significant changes in the characteristics of the above diseases in the different time periods. Therefore, the NCC suggested focusing on the high proportion of patients with primary neurological diseases, comorbidities, complications and nosocomial infections, and focusing on implementing high-intensity continuous control measures.

NCCU evaluation and treatment

In evaluating the treatment technology used for the cohort in this study, the implementation rate of EEG and TCD was very high (84.2 and 67.9%), which has further increased compared with the first survey 10 years ago (63.1 and 32.9%) (1), which was related to the change in NCCU doctors' concept of reversible or irreversible (brain death) coma evaluation and the promotion of brain death determination criteria in the past 10 years (6–8), followed by the acceptance and practice of consensus recommendations for early EEG monitoring and the treatment of status epilepticus (9). The high implementation rate of the GCS score (95.2%) was expected, and an increasing number of NCCU physicians were willing to use the APACHE II evaluation (62.4%), which allowed improvements

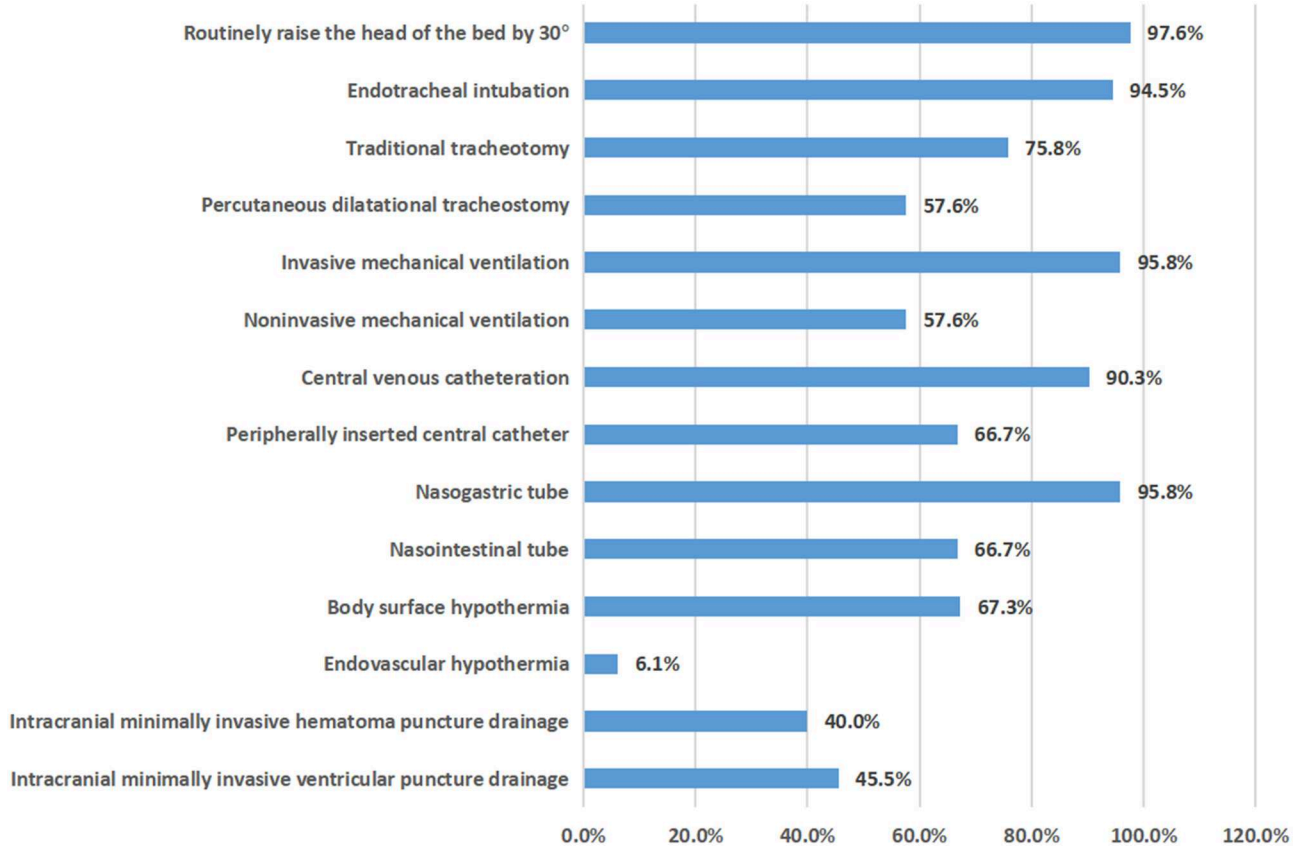

FIGURE 4
Proportion of 165 NCCU treatment technologies in 2020.

in the cognition of patients with a brain injury and the condition of those with multiple organ systems dysfunction (10). The high implementation rate of 4/5 nursing evaluation techniques (66.1–90.9%) reflected the intensity of the transformation and promotion of the NCCU nursing management concept.

In the basic treatment technology of the NCCU in this study, traditional technologies such as endotracheal intubation, central venous catheterization and nasogastric tube feeding had a high implementation rate (>90%), which currently plays an important role, has always played an important role in the past and has provided guarantees for basic life support. The implementation rate of new technologies, such as percutaneous tracheotomy, non-invasive mechanical ventilation and naso-intestinal feeding, was <67%. Although clinical studies have confirmed that patients might benefit from these techniques (11–13), the promotion and application of these techniques still need verification in clinical practice and accurate patient selection.

In this study, the implementation rate of intravascular hypothermia was significantly lower than that of body surface hypothermia (6.1 < 67.3%), and there was almost no change compared with the first survey 10 years ago. It has been reported that intravascular hypothermia treatment technology is more accurate and controllable and obtains a good neurological prognosis with fewer hypothermia complications and more reliable hypothermia efficacy (14). The implementation rate of microinvasive removal of cerebral hematoma was only 40.0%, and it has been reported that it could reduce surgical trauma and improve neurological prognosis (15). Obviously, these new technologies are not universal enough. Therefore, the NCC suggests promoting and applying advanced monitoring and evaluation technology to accurately guide treatment. Advanced life support technology and specialized neurotherapy technology should be expanded and implemented to achieve the ultimate goal of improving prognosis.

Conclusion

Through this investigation, we have a preliminary understanding of the case volume, disease characteristics, monitoring and treatment technology and outcome/prognosis of NCCU at this stage and have put forward suggestions for reference. However, there were also some problems in this survey, such as the lack of rigorous questionnaire design and missing data, which affected the accuracy of the results. For example, (1) the estimation of mortality (outcome index) being <4.1% comes from the data submitted by each NCCU. Although we speculated that part of the reason was related to the short-term admission of patients to the NCCU due to the need for intravenous thrombolytic therapy and intravascular therapy, the questionnaire lacked relevant information. (2) The investigation items of the short-term/long-term neurological function prognosis score (prognosis index) were absent, so the main influencing factors of prognosis could not be further analyzed. Therefore, to further investigate the "goal of improving the prognosis of neurological diseases," we also

need a more careful survey scale design, more reliable NCCU information and a more reasonable data analysis to achieve the purpose of promoting development through investigation and research. In addition, the statistical data in 2020 might be affected by the temporary prevalence of COVID-19 in some parts of China. We hope that the next survey (2025) will provide more accurate data.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University, Beijing. Written informed consent from the participants or 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

YS: conception and design of the study and drafting the article or revising it critically for important intellectual content. JT, FT, JJ, HHua, SP, WJ, FW, LeZhang, YZ, MZ, LL, JC, HHu, WL, CL, LM, XM, LT, CW, LW, YW, ZheW, ZhiW, ZX, MY, JY, CZ, LZe, LeiZhang, XZ, YoZ, BZ, SZ, and ZZ: acquisition of data. FT, JJ, and HHua: analysis and interpretation of data. All authors: final approval of the version to be submitted.

Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1114204/full#supplementary-material

References

1. Su Y-Y, Wang M, Feng H-H, Chen W-B, Ye H, Gao D-Q, et al. An overview of neurocritical care in China: A nationwide survey. *Chin Med J.* (2013) 126:3422–6.

2. Wang YJ, Li ZX, Gu HQ, Zhai Y, Jiang Y, Zhao XQ, et al. China Stroke Statistics 2019 Writing Committee. China Stroke Statistics 2019: A report from the National Center for Healthcare Quality Management in Neurological Diseases. China National Clinical Research Center for Neurological Diseases, the Chinese Stroke Association, National Center for Chronic and Non-communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention and Institute for Global Neuroscience and Stroke Collaborations. *Stroke Vasc Neurol.* (2020) 5:211–39. doi: 10.1136/svn-2020-000457

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

4. Koennecke WB, Belz W, Berfelde D, Endres M, Fitzek S, Hamilton F, et al. Factors influencing in-hospital mortality and morbidity in patients treated on a stroke unit. *Neurology.* (2011) 77:965–72. doi: 10.1212/WNL.0b013e31822dc795

5. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Health Care Infection Control Practices Advisory Committee. 2007 guideline for isolation precautions: Preventing transmission of infectious agents in health care settings. *Am J Infect Control.* (2007) 35(10 Suppl.2):S65–164. doi: 10.1016/j.ajic.2007.10.007

6. Brain Injury Evaluation Quality Control Center of National Health Commission, Neurocritical Care Committe of the Chinese Society of Neurology (NCC/CSN), Neurocritical Care Committe of China Neurologist Association (NCC/CNA). Criteria and practical guidance for determination of brain death in adults (2nd edition). *Chin Med J.* (2019) 132:329–35. doi: 10.1097/CM9.0000000000000014

7. Neurocritical Care Committee of the Chinese Society of Neurology (NCC/CSN). The Chinese expert consensus on evaluation of coma after cardiopulmonary resuscitation. *Chin Med J.* (2016) 129:2123–7. doi: 10.4103/0366-6999.189054

8. Huang H, Niu Z, Liu G, Jiang M, Jia Q, Li X, et al. Early consciousness disorder in acute large hemispheric infarction: An analysis based on quantitative EEG and brain network characteristics. *Neurocrit Care.* (2020) 33:376–88. doi: 10.1007/s12028-020-01051-w

9. Tian F, Su Y, Chen W, Gao R, Zhang Y, Zhang Y, et al. RSE prediction by EEG patterns in adult GCSE patients. *Epilepsy Res.* (2013) 105:174–82. doi: 10.1016/j.eplepsyres.2013.02.007

10. Su Y-Y, Li X, Li S-J, Luo R, Ding J-P, Wang L, et al. Predicting hospital mortality using APACHE II scores in neurocritically ill patients: A prospective study. *J Neurol.* (2009) 256:1427–33. doi: 10.1007/s00415-009-5129-z

11. Sahiner IT, Sahiner Y. Bedside percutaneous dilatational tracheostomy by Griggs technique: A single-center experience. *Med Sci Monit.* (2017) 23:4684–8. doi: 10.12659/MSM.907006

12. Neumann B, Angstwurm K, Mergenthaler P, Kohler S, Schönenberger S, Bösel J, et al. Myasthenic crisis demanding mechanical ventilation: A multicenter analysis of 250 cases. *Neurology.* (2020) 94:e299–313. doi: 10.1212/WNL.0000000000008688

13. Wan B, Fu H, Yin J. Early jejunal feeding by bedside placement of a nasointestinal tube significantly improves nutritional status and reduces complications in critically ill patients vs. enteral nutrition by a nasogastric tube. *Asia Pac J Clin Nutr.* (2015) 24:51–7.

14. Su Y, Fan L, Zhang Y, Zhang Y, Ye H, Gao D, et al. Improved neurological outcome with mild hypothermia in surviving patients with massive cerebral hemispheric infarction. *Stroke.* (2016) 47:457–63. doi: 10.1161/STROKEAHA.115.009789

15. Lian L, Xu F, Hu Q, Liang Q, Zhu W, Kang H, et al. No exacerbation of perihematomal edema with intraclot urokinase in patients with spontaneous intracerebral hemorrhage. *Acta Neurochir.* (2014) 156:1735–44. doi: 10.1007/s00701-014-2130-9

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Effect of intravenous thrombolysis on core growth rate in patients with acute cerebral infarction

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Objective: This study aimed to investigate the effects of recombinant tissue plasminogen activator intravenous thrombolysis (IVT) on the core growth rate of acute ischemic stroke.

Methods: Stroke patients with large vessel occlusion and non-recanalization from IVT treatment were retrospectively included in this study and divided into two groups: IVT and non-IVT. The core growth rate was estimated by the acute core volume on perfusion CT divided by the last known well time from stroke to CT perfusion. The primary endpoint was the core growth rate, the tissue outcome was 24 h-ASPECTS, and the clinical outcome was a 3-month modified Rankin score.

Results: A total of 94 patients were included with 53 in the IVT group and 41 in the non-IVT group. There was no significant difference in age, gender, hypertension, diabetes, atrial fibrillation, acute NIHSS, and last known well time from stroke to CT perfusion acquisition between the two groups. The core growth rate in the IVT group was lower than that in the non-IVT group, which was statistically significant after multivariate adjustment (coefficient: −5.20, 95% CI= [−9.85, −0.56], $p = 0.028$). There was a significant interaction between the IVT and the collateral index in predicting the core growth rate. The analysis was then stratified according to the collateral index, and the results suggested that IVT reduced the core growth rate more significantly after the worsening of collateral circulation (coefficient: 15.38, 95% CI= [−26.25, −4.40], $p = 0.007$). The 3-month modified Rankin score and 24 h-ASPECTS were not statistically significant between the two groups.

Conclusion: Intravenous thrombolysis reduces the core growth rate in patients with AIS, especially those with poor collateral status.

KEYWORDS

acute ischemic stroke, intravenous thrombolysis, core growth rate, collateral circulation, large vessel occlusion, reperfusion, therapy

1. Introduction

Intravenous thrombolysis (IVT) is an established treatment for acute ischemic stroke (AIS), and it can be rapidly initiated after clinical assessment and cranial CT scan (1–3). However, IVT also has significant limitations, such as the patients' need to receive IV tPA within 4.5 h of the onset, and the recanalization rates are low in patients with large vascular occlusion (LVO), that is, a meta-analysis reported approximately 35% for M1 MCA occlusions, 13% for ICA occlusions, and 13% for BA occlusions (4).

Since 2015, several clinical trials acknowledged the superiority of endovascular thrombectomy (EVT), which has a higher rate of recanalization of LVO and a longer treatment window (5–9). Therefore, the "bridge" therapy was proposed to use EVT to rescue patients with a lack of recanalization after IVT. Some clinical trials showed that early administration of alteplase can promote microvessel patency and that the rate of successful recanalization in bridge therapy was significantly higher than that in patients who only accept EVT (10, 11). However, IVT may increase the risk of bleeding (12) and promote thromboplastic migration (13). Hence, whether patients with LVO-AIS who arrive at the hospital within 4.5 h of the onset can benefit from the "bridge" therapy is a research hotspot (14, 15).

In patients with AIS, successful recanalization and core volume are the strongest predictors of outcome (16). Before vessel recanalization, the infarct core increases linearly within 6 h of stroke onset (17, 18). Assuming that the core at the time of stroke onset was 0, the infarction volume divided by the time from stroke onset to CTP can be used to estimate the speed of cerebral infarction progression. The core growth rate has been reported to be an independent predictor of clinical outcomes and is highly associated with the collateral status. Reducing the core growth rate may reduce the volume of core infarction and may have important therapeutic implications on AIS.

This study aimed to assess the efficacy of IVT on changing infarct core growth rate in patients with LVO-AIS who had not achieved recanalization.

2. Materials and methods

2.1. General information

We conducted a retrospective cohort study that involved 94 AIS patients with LVO between January 2017 and March 2021 at the Shanghai East Hospital—Department of Neurology. All the patients were divided into two groups according to whether or not they received IVT.

2.2. Inclusion criteria

Patients were selected based on the following criteria: (1) All the patients met the diagnostic criteria of acute cerebral infarction due to LVO; according to the guidelines, the IVT group patients met the indications of IVT, and the contraindications of IVT were excluded; (2) the last known well time from stroke to completion of CTP was <6 h; (3) the ischemic core volume on the CTP was <70 ml, penumbra ≥ 10 ml, and radio $>$ 1.2; and (4) the clinical information was complete.

2.3. Methods

The clinical data of patients included age, gender, and histories of hypertension, diabetes, heart issues, prior stroke, current smoking, and NIHSS recorded at the hospital arrival, as well as radiographic data. The DT collateral index was used to evaluate the collateral status (19).

The IVT group patients were given 0.9 mg/kg of rt-PA for thrombolysis: 10% of the total dose was given intravenously for 1 min, while the remaining dose was injected intravenously 1 h later.

2.4. Imaging acquisition and post-processing

Baseline CT imaging included brain non-contrast CT, CTP, and CTA, obtained with different CT scanners (64, 128, 256, or 320 detectors, with Toshiba [Tokyo, Japan], Siemens [Munich, Germany], or GE [Cleveland, OH, USA] scanners). The axial coverage ranged from 80 to 160 mm.

The CTP data were processed by commercial software MIStar (Apollo Medical Imaging Technology, Melbourne, Vic, Australia). CTP parameters were generated by applying the mathematical algorithm of singular value decomposition with delay and dispersion correction (20, 21). The following four CTP parameters were generated: cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT), and delay time (DT). The penumbra and core volume were measured on acute CTP with dual threshold setting (22): DT at the threshold of 3 s for whole ischemic lesion volume and CBF at the threshold setting of 30% for acute core volume. The collateral index was defined by the ratio of DT >6 s/DT >2 s volume.

2.5. Calculation of the ischemic core growth rate

The core growth rate was determined using the baseline core volume divided by the time between the symptom onset and the CTP. It was assumed that the core volume was zero just prior to symptom onset and would grow in a near-linear pattern within 6 h of stroke onset (23). This study calculated the core growth rate using the following approach:

Core growth rate = Acute core volume on CTP / Last known well time from stroke to CTP (19, 24).

2.6. Statistical analysis

Data were statistically analyzed using the statistical software SPSS 25.0. The normality of the continuous variables was examined using the Kolmogorov–Smirnov test. When the normality assumption was not met, a non-parametric test was used. The categorical variables were compared by the chi-square test. Multi-factor linear regression was used to analyze predictors of ischemic core growth rate. Variables with a $p < 0.25$ were included in the regression model. In addition, collateral circulation was included in the model as a significant predictor. A scatter diagram was used to describe the interaction between IVT and DT collateral index in predicting the core growth rate. Then, the core

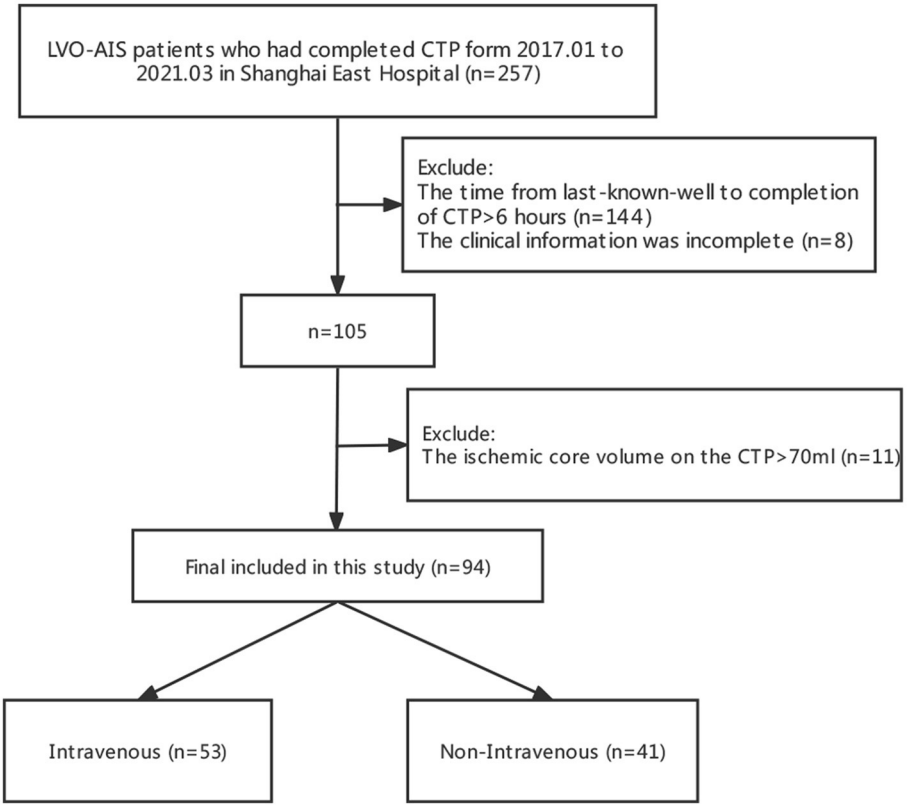

FIGURE 1
A flow diagram of the study.

growth rate of IVT vs. non-IVT patients was plotted across the collateral index (with a 0.100 increment). Within each collateral index category, the predictive power of IVT (vs. non-IVT) on the patient core growth rate was assessed by regression models.

2.7. Tissue outcomes

Alberta stroke program early CT score (ASPECTS) was used to evaluate the extent of anterior circulation infarction (25). The posterior circulation stroke was assessed by pc-ASPECTS (26). A normal CT scan has an ASPECTS value of 10 points. ΔASPECTS is defined as 24 h-ASPECTS minus baseline ASPECTS, which would be used to describe the change in brain tissue. Multi-factor logistic and linear regression was used to describe the relationship between intravenous therapy and clinical outcomes and tissue outcomes.

2.8. Patient outcomes

The primary endpoint was the core growth rate. The clinical outcome was the modified Rankin score (mRS) at three months. The good clinical outcome was defined by an mRS score of 0–2 vs. an mRS score of 3–6, and the poor clinical outcome was an mRS score of 5–6 vs. an mRS score of 0–4.

3. Results

The flow diagram of the study is given in Figure 1. A total of 94 patients met the inclusion criteria, of whom 53 patients received IVT and 41 patients were treated without IVT.

The baseline characteristics of the patients were similar in the two groups (Table 1). The average age of the patient was 39–98 years, and 57 patients (61%) were men. The median baseline NIHSS was 16 (IQR = 7). The median last known well time from stroke to CTP was 3.18 h (IQR, 2.27–3.98) in the IVT group and 3.4 h (IQR, 1.88–4.57) in the non-IVT group. There was no statistical difference between the two groups in age, gender, or AIS risk factors such as hypertension, diabetes mellitus, atrial fibrillation, previous history of stroke, or current smoking (P>0.05). Neither infarct volume nor collateral index showed a significant difference between the two groups ($p = 0.982$ and $p = 0.760$). No between-group differences were found at baseline ASPECTS or ΔASPECTS ($p = 0.698$ and $p = 0.682$). Although the difference was not statistically significant, 24 h-ASPECTS in the IVT group was slightly higher (7 vs. 6).

Delay time collateral index (coefficient: 30.65, 95% CI= [14.37, 46.93], p<0.001) and intravenous therapy (coefficient: −5.20, 95% CI = [−9.85, −0.56], $p = 0.028$) showed significant differences. Intravenous may decrease the core growth rate of 5 ml/h for patients with stroke (Table 2).

Figure 2 depicts the scatter plots for the association between the DT collateral index and the core growth rate in two groups.

TABLE 1 Characteristics of the intravenous group vs. the non-intravenous group.

	Intravenous ($n = 53$)	Non-intravenous ($n = 41$)	P-value
Age, median (IQR)	71 (63–83)	71 (65–79)	0.565
Men, % (*N*)	58.5 (31/53)	63.4 (26/41)	0.628
Atrial fibrillation, % (*N*)	43.4 (23/53)	48.4 (20/41)	0.603
Hypertension, % (*N*)	64.2 (34/53)	46.3 (19/41)	0.084
Diabetes, % (*N*)	26.4 (14/53)	19.5 (8/41)	0.433
current smoking, % (*N*)	20.8 (11/53)	22.0 (9/41)	0.888
Prior Stroke, % (*N*)	22.6 (12/53)	34.1 (14/41)	0.216
Acute NIHSS score, median (IQR)	15 (12–19)	16 (12–19)	0.472
CBF < 30, median (IQR)	14.0 (4.0–30.5)	18.0 (5.0–41.5)	0.235
Core growth, median (IQR)	4.52 (1.35–11.16)	5.91 (1.52–12.98)	0.430
Time from last-known-well to CTP acquisition (Hours), median (IQR)	2.83 (2.27–3.98)	3.98 (1.88–4.57)	0.064
DT > 6 s, median (IQR)	35 (16.5–61.0)	39 (13.5–55.5)	0.982
DT > 2 s, median (IQR)	176.0 (111.5–228.5)	203.0 (136.5–248.6)	0.595
Collateral index, median (IQR)	0.17 (0.12–0.32)	0.21 (0.12–0.31)	0.760
Baseline ASPECTS, median (IQR)	8 (8–9)	8 (7–9)	0.698
24 h-ASPECTS, median (IQR)	7 (6–8)	6 (5.75–7)	0.224
ΔASPECTS, median (IQR)	−1 (−2–0)	−1 (−2–0)	0.682
In-hospital mortality, % (*N*)	26.4% (14/53)	26.8% (11/41)	0.964
3-month modified Rankin score, median (IQR)	5 (1–6)	5 (2–6)	0.521
Good outcome rate, % (*N*)	34.0% (18/53)	26.8% (11/41)	0.314
Poor outcome rate, % (*N*)	50.9% (27/53)	56.1% (23/41)	0.581

IVT, intravenous thrombolysis; NIHSS, Scores on the National Institutes of Health Stroke Scale; IQR, interquartile range; CBF, cerebral blood flow; DT, delay time; AIS, acute ischemic stroke. Good outcomes and poor outcomes are defined by 3-month modified Rankin scores of 0–2 and 5–6, respectively. ΔASPECTS as 24 h-ASPECTS minus baseline ASPECTS.

TABLE 2 Intravenous effect for core growth rate.

	Adjusted p-value	Coefficient
Intravenous	0.028	−5.20
DT collateral index	<0.001	30.65
hypertension	0.598	−1.24
Prior stroke	0.700	−0.99

Delay time collateral index was classified into three categories based on the core growth rate of the IVT vs. the non-IVT group (Figure 2). For DT collateral index <0.100 and 0.100–0.250, there was no statistical significance in the effect of IVT on the core growth rate ($p = 0.616$ and $p = 0.426$). For DT collateral index >0.25, after adjusting for DT collateral index, hypertension, and prior stroke, the IVT showed a statistically significant result on the core growth rate (coefficient: 15.38, 95% CI= [−26.25, −4.40], $p = 0.007$) (Table 3). In other words, for patients with poor collateral index, IVT may significantly decrease the core growth rate (Figures 3, 4).

Univariate and multivariate regression analyses were used to explore the association between intravenous therapy and clinical outcomes. There was no statistical difference in the 3-month modified Rankin score (Table 1). After adjusting for hypertension, prior stroke, and DT collateral index, both the good outcome (OR = 0.60, 95% CI = [0.205, 1.760]) and poor outcome (OR = 0.83, 95% CI = [0.355,1.953]) showed no significant predictive power between the two groups (Table 4). No significant between-group differences were detected in 3-month mortality (26.4 vs. 26.8%; OR=0.77, 95% CI = [0.314, 1.886]).

After multivariate adjustments, the differences in 24 h-ASPECTS (coefficient: 0.451, 95% CI= [-0.281, 1.183], $p = 0.224$) and ΔASPECTS (coefficient: 0.183, 95% CI = [−0.501, 0.868], $p = 0.595$) were not statistically significant (Table 5).

4. Discussion

In acute ischemic stroke (AIS) treatment, recanalization of the occluded vessel is crucial for a good clinical outcome (27). Intravenous thrombolysis (IVT) with rt-PA is a conventional treatment for AIS. However, the low vascular recanalization rate led to many conflicting views (4, 28). When clinical symptoms of patients with LVO-AIS did not significantly improve after receiving IVT, endovascular therapy was required to achieve recanalization. For such patients, the role of IVT remains controversial. This study showed that IVT may reduce the core growth rate in patients with AIS, even if the vessel did not achieve recanalization. Moreover,

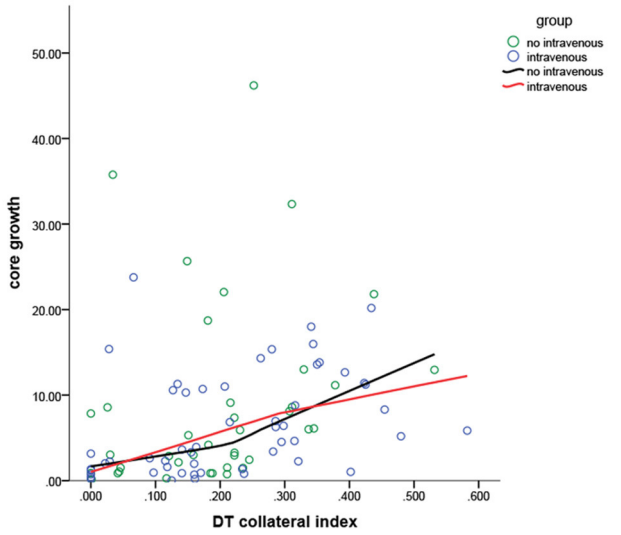

FIGURE 2
The core growth rate in different collateral indexes for the patients in the two groups. The IVT and non-IVT groups intersected two times, which suggested a significant interaction between the IVT and the collateral index, indicating that both of them interfere significantly with the core growth rate. The line of the non-IVT group showed a major increase when the DT collateral index was 0.250.

TABLE 3 Predicting core growth rate by IVT across DT collateral index.

	Adjusted p-value	Coefficient
DT collateral index < 0.1	0.616	−2.13
0.1 ≤ DT collateral index ≤ 0.25	0.426	−1.72
DT collateral index > 0.25	0.007	−15.38

Adjusted by DT collateral index, hypertension, and prior stroke.

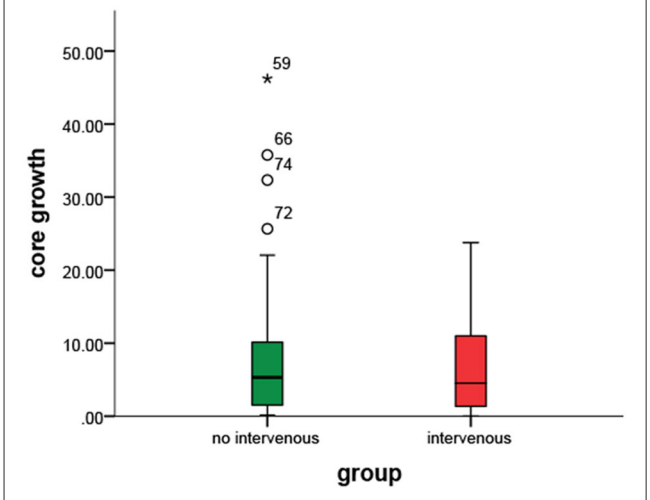

FIGURE 3
No clear difference was observed in the core growth rate between the two groups.

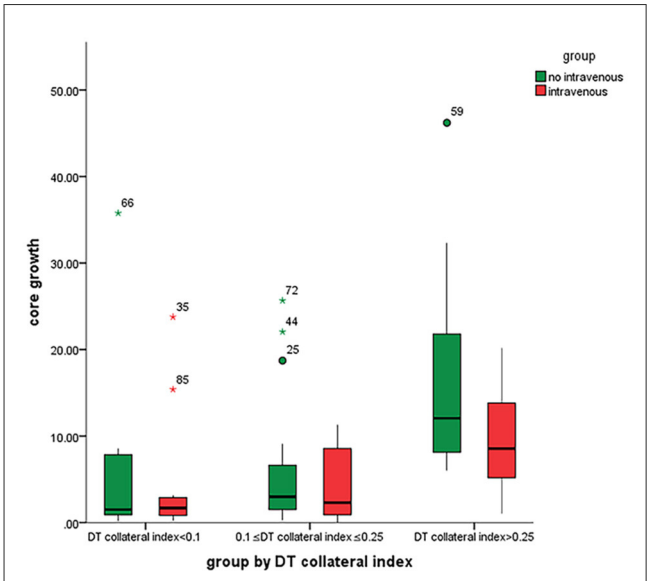

FIGURE 4
Differences in the core growth rate between within the IVT (or not) group and within a DT collateral index group. IVT significantly decreased the core growth rate in patients with poor collateral index.

this effect was influenced by collateral circulation and was clearer in patients with poor collateral circulation.

Whether patients with AIS can benefit from the "bridge" therapy (BT) is a research hotspot. In a meta-analysis of 38 eligible observational studies, BT was associated with a higher likelihood of 3-month functional independence compared to direct mechanical thrombectomy (29). Whether IVT will extend the total time of recanalization therapy remains controversial. In recent years, numerous studies showed that the average recanalization time was not statistically different between the "bridge" therapy and direct thrombectomy (14, 15, 29), indicating that IVT did not delay the time to recanalization. In addition, with the application of tenecteplase, the time difference between the two treatment modalities was further reduced (30). This study demonstrated that IVT may reduce the core growth rate without vessel recanalization. Some studies also found that, in time-window patients who were transferred to an EVT capable center, the outcomes were better in patients who had previously received IVT (31). Therefore, IVT is considered the first-line treatment for patients with AIS even with LVO and IVT is required in those patients as early as possible.

Infarct core volume is an independent predictor for the outcome of patients with AIS. The smaller the infarct core, the better the likelihood of clinical outcomes (32). In recent years, several studies demonstrated that the infarct core grows linearly during the first 6 h of AIS (17, 18). Wheeler suggested that the early stroke core growth curves exhibited a nearly linear growth during the first 8 h after symptom onset for patients with <10% reperfusion (23). Therefore, the core growth rate in the first 6 h from the onset can predict the infarct core to some extent. In this study, all patients successfully completed the CTP examination in 6 h from last known well time. IVT was validated to reduce the infarct core growth rate in this study, and one possible mechanism is probably due to the reduction in thrombus volume by IVT (33,

TABLE 4 Association between intravenous therapy and clinical outcomes.

	P value	Adjusted p-value	Adjusted OR (95%CI)
3-month good outcome	0.459	0.353	0.600 (0.205,1.760)
3-month poor outcome	0.620	0.673	0.832 (0.355,1.953)
3-month mortality	0.647	0.566	0.769 (0.314,1.886)
In-hospital mortality	0.979	0.603	0.768 (0.283,2.081)

Adjusted by hypertension, prior stroke, and DT collateral index.

TABLE 5 Association between intravenous therapy and tissue outcomes.

	Adjusted p-value	Coefficient
24 h-ASPECTS	0.224	0.451
ΔASPECTS	0.595	0.183

Adjusted by hypertension, prior stroke, and DT collateral index.

34). In addition, the rt-PA can act on the distal microvasculature and reduce microvenous thrombosis (35). Thus, the blood supply in infarct areas can be improved. A retrospective study showed that the rate of successful recanalization was significantly higher in patients who received IVT before mechanical thrombectomy (11), and it might be implicated in those mechanisms. In this study, we found that IVT may reduce the infarct core growth rate, and patients with AIS who had a lower collateral circulation and underwent IVT exhibited slower infarct growth rates. The impact of collateral circulation on infarct core growth is well established (19). Better collateral circulation indicates slower infarct growth, while the effect of IVT is not perfect. However, in patients who had poor collateral circulation, along with a decrease in the thrombosis volume and thrombus load in the local microcirculation, the blood flow might have improved more in the ischemic penumbra.

There was no statistical difference in a 3-month modified Rankin score between the two groups. The potential explanation offered might be that, although the rate of core growth was decreased in the IVT group, the clinical outcome was largely decided by the developed infarct core volume and the degree of recanalization. Several studies confirmed that collateral circulation is the factor that has the greatest impact on the growth of infarct core (36, 37). Moreover, the factors associated with collateral circulation were age, smoking, hypertension (38), and the use of statins (39, 40). All these factors had no statistical significance in this study, which may have resulted in no difference in collateral circulation between the two groups. All patients in this trial had LVO, and they accepted mechanical thrombectomy after the CTP examination, and the intraoperative recanalization levels have been shown to impact the prognosis of stroke (41). Thus, the follow-up treatments might heavily influence the clinical outcome. It is difficult to assess the precise relationship between IVT with patient prognosis. Further studies may be needed to elucidate this relationship in a future study.

Although the 24 h-ASPECT in the IVT group was slightly higher than that of the non-IVT group, there was no significant difference in the tissue outcomes. Two considerations may have contributed to this result. On the one hand, despite this study finding that IVT may reduce the core growth rate in patients with LVO, the final infarct core was largely decided by the collateral circulation and the efficacy of endovascular therapy. On the other hand, the sample size in our study is relatively small, which needs to be expanded for a more in-depth research in future, and the effects of intravenous thrombolysis on histological changes would have likely been observed.

This retrospective single-center study has several limitations. First, the lack of randomized treatment allocation in this study was the main limitation. Second, there were no clear differences in baseline core growth rates between the two groups, which might be affected by the collateral circulation. We further adjusted it by the multivariate regression models and removed the effects of the collateral index, which showed positive results. Third, this study was conducted with a small sample size, which might have led to a certain degree of bias. Fourth, some of the patients had unwitnessed onset and the time of stroke onset was uncertain. Hence, the estimation of time from onset to CTP may be extended.

Although the difference in the long-term prognosis of the patients was not observed, the present study provided important new information about the benefit of IVT. "Time is brain" in this study, and IVT successfully reduced the core growth rate, suggesting the underlying application in the treatment of acute cerebral infarction. For patients with AIS who arrive at the hospital within the time window, we suggest IVT in the absence of contraindications and expect more benefits from the "bridge" therapy. Further studies are required to confirm these conclusions.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Shanghai East Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YZ and ZH contributed to the conception and design of the present study. XW and HZ contributed to drafting a significant portion of the manuscript or figures. QW, HS, YX, and LX contributed to the acquisition and analysis of data. YL, CC, and GL helped perform the analysis with constructive discussions. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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References

1. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. (1995) 333:1581–7. doi: 10.1056/NEJM199512143332401

2. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D. et al. Thrombolysis with alteplase 3 to 45 hours after acute ischemic stroke. *N Engl J Med*. (2008) 359:1317–29. doi: 10.1056/NEJMoa0804656

3. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2019) 50:e344–418. doi: 10.1161/STR.0000000000000211

4. Seners P, Turc G, Maïer B, Mas JL, Oppenheim C, Baron JC. Incidence and predictors of early recanalization after intravenous thrombolysis: a systematic review and meta-analysis. *Stroke*. (2016) 47:2409–12. doi: 10.1161/STROKEAHA.116.014181

5. Campbell BC, Mitchell PJ. Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med*. (2015) 372:1009–18. doi: 10.1056/NEJMoa1414792

6. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. (2015) 372:11–20. doi: 10.1056/NEJMoa1411587

7. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med*. (2015) 372:1019–30. doi: 10.1056/NEJMoa1414905

8. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med*. (2015) 372:2296–306. doi: 10.1056/NEJMoa1503780

9. Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, et al. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. *N Engl J Med*. (2015) 372:2285–95. doi: 10.1056/NEJMoa1415061

10. Guedin P, Larcher A, Decroix JP, Labreuche J, Dreyfus JF, Evrard S, et al. Prior IV thrombolysis facilitates mechanical thrombectomy in acute ischemic stroke. *J Stroke Cerebrovasc Dis*. (2015) 24:952–7. doi: 10.1016/j.jstrokecerebrovasdis.2014.12.015

11. Behme D, Kabbasch C, Kowoll A, Dorn F, Liebig T, Weber W, et al. Intravenous thrombolysis facilitates successful recanalization with stent-retriever mechanical thrombectomy in middle cerebral artery occlusions. *J Stroke Cerebrovasc Dis*. (2016) 25:954–9. doi: 10.1016/j.jstrokecerebrovasdis.2016.01.007

12. Werner M, Lopez-Rueda A, Zarco F, Román LS, Blasco J, Amaro S, et al. Mechanical thrombectomy in acute basilar artery occlusion: a safety and efficacy single centre study. *Interv Neuroradiol*. (2016) 22:310–7. doi: 10.1177/15910199166 31145

13. Ohara T, Menon BK, Al-Ajlan FS, Horn M, Najm M, Al-Sultan A, et al. Thrombus migration and fragmentation after intravenous alteplase treatment: the INTERRSeCT study. *Stroke*. (2021) 52:203–12. doi: 10.1161/STROKEAHA.120.029292

14. Suzuki K, Matsumaru Y, Takeuchi M, Morimoto M, Kanazawa R, Takayama Y, et al. Effect of mechanical thrombectomy without vs with intravenous thrombolysis on functional outcome among patients with acute ischemic stroke: the SKIP randomized clinical trial. *JAMA*. (2021) 325:244–53. doi: 10.1001/jama.2020.23522

15. Zi W, Qiu Z, Li F, Sang H, Wu D, Luo W, et al. Effect of endovascular treatment alone vs intravenous alteplase plus endovascular treatment on functional independence in patients with acute ischemic stroke: the DEVT randomized clinical trial. *JAMA*. (2021) 325:234–43. doi: 10.1001/jama.2020.23523

16. Panni P, Gory B, Xie Y, Consoli A, Desilles JP, Mazighi M, et al. Acute stroke with large ischemic core treated by thrombectomy. *Stroke*. (2019) 50:1164–71. doi: 10.1161/STROKEAHA.118.024295

17. Markus R, Reutens DC, Kazui S, Read S, Wright P, Chambers BR, et al. Topography and temporal evolution of hypoxic viable tissue identified by 18F-fluoromisonidazole positron emission tomography in humans after ischemic stroke. *Stroke*. (2003) 34:2646–52. doi: 10.1161/01.STR.0000094422.74023.FF

18. Christoforidis GA, Vakil P, Ansari SA, Dehkordi FH, Carroll TJ. Impact of pial collaterals on infarct growth rate in experimental acute ischemic stroke. *AJNR Am J Neuroradiol*. (2017) 38:270–5. doi: 10.3174/ajnr.A5003

19. Lin L, Chen C, Tian H, Bivard A, Spratt N, Levi CR, et al. Perfusion computed tomography accurately quantifies collateral flow after acute ischemic stroke. *Stroke*. (2020) 51:1006–9. doi: 10.1161/STROKEAHA.119.028284

20. Lin L, Bivard A, Kleinig T, Spratt NJ, Levi CR, Yang Q, et al. Correction for delay and dispersion results in more accurate cerebral blood flow ischemic core measurement in acute stroke. *Stroke*. (2018) 49:924–30. doi: 10.1161/STROKEAHA.117.019562

21. Bivard A, Levi C, Krishnamurthy V, McElduff P, Miteff F, Spratt NJ, et al. Perfusion computed tomography to assist decision making for stroke thrombolysis. *Brain*. (2015) 138:1919–31. doi: 10.1093/brain/awv071

22. Lin L, Bivard A, Krishnamurthy V, Levi CR, Parsons MW. Whole-brain CT perfusion to quantify acute ischemic penumbra and core. *Radiology*. (2016) 279:876–87. doi: 10.1148/radiol.2015150319

23. Wheeler HM, Mlynash M, Inoue M, Tipirnini A, Liggins J, Bammer R, et al. The growth rate of early DWI lesions is highly variable and associated with penumbral salvage and clinical outcomes following endovascular reperfusion. *Int J Stroke*. (2015) 10:723–9. doi: 10.1111/ijs.12436

24. Sarraj A, Hassan AE, Grotta J, Blackburn S, Day A, Abraham M, et al. Early infarct growth rate correlation with endovascular thrombectomy clinical outcomes: analysis from the SELECT study. *Stroke*. (2021) 52:57–69. doi: 10.1161/STROKEAHA.120.030912

25. Barber PA, Demchuk AM, Zhang J, Buchan AM. Validity and reliability of a quantitative computed tomography score in predicting outcome of hyperacute stroke before thrombolytic therapy. ASPECTS study group Alberta stroke programme early CT score. *Lancet*. (2000) 355:1670–4. doi: 10.1016/S0140-6736(00)02237-6

26. Puetz V, Sylaja PN, Coutts SB, Hill MD, Dzialowski I, Mueller P, et al. Extent of hypoattenuation on CT angiography source images predicts functional outcome in patients with basilar artery occlusion. *Stroke*. (2008) 39:2485–90. doi: 10.1161/STROKEAHA.107.511162

27. Rha JH, Saver JL. The impact of recanalization on ischemic stroke outcome: a meta-analysis. *Stroke*. (2007) 38:967–73. doi: 10.1161/01.STR.0000258112.14918.24

28. Asadi H, Dowling R, Yan B, Wong S, Mitchell P. Advances in endovascular treatment of acute ischaemic stroke. *Intern Med J*. (2015) 45:798–805. doi: 10.1111/imj.12652

29. Katsanos AH, Malhotra K, Goyal N, Arthur A, Schellinger PD, Köhrmann M, et al. Intravenous thrombolysis prior to mechanical thrombectomy in large vessel occlusions. *Ann Neurol*. (2019) 86:395–406. doi: 10.1002/ana.25544

30. Huang X, Cheripelli BK, Lloyd SM, Kalladka D, Moreton FC, Siddiqui A, et al. Alteplase versus tenecteplase for thrombolysis after ischaemic stroke (ATTEST) : a phase 2, randomised, open-label, blinded endpoint study. *Lancet Neurol*. (2015) 14:368–76. doi: 10.1016/S1474-4422(15)70017-7

31. Sarraj A, Grotta J, Albers GW, Hassan AE, Blackburn S, Day A, et al. Clinical and neuroimaging outcomes of direct thrombectomy vs bridging therapy in large vessel occlusion: analysis of the SELECT cohort study. *Neurology*. (2021) 96:e2839–e53. doi: 10.1212/WNL.0000000000012063

32. Padroni M, Bernardoni A, Tamborino C, Roversi G, Borrelli M, Saletti A, et al. Cerebral blood volume ASPECTS is the best predictor of clinical outcome in

acute ischemic stroke: a retrospective, combined semi-quantitative and quantitative assessment. *PLoS ONE.* (2016) 11:e0147910. doi: 10.1371/journal.pone.0147910

33. Rossi R, Fitzgerald S, Molina S, Mereuta OM, Douglas A, Pandit A, et al. The administration of rtPA before mechanical thrombectomy in acute ischemic stroke patients is associated with a significant reduction of the retrieved clot area but it does not influence revascularization outcome. *J Thromb Thrombolysis.* (2021) 51:545–51. doi: 10.1007/s11239-020-02279-1

34. Rossi R, Molina S, Mereuta OM, Douglas A, Fitzgerald S, Tierney C, et al. Does prior administration of rtPA influence acute ischemic stroke clot composition? Findings from the analysis of clots retrieved with mechanical thrombectomy from the RESTORE registry. *J Neurol.* (2022) 269:1913–20. doi: 10.1007/s00415-021-10758-5

35. Desilles JP, Loyau S, Syvannarath V, Gonzalez-Valcarcel J, Cantier M, Louedec L, et al. Alteplase reduces downstream microvascular thrombosis and improves the benefit of large artery recanalization in stroke. *Stroke.* (2015) 46:3241–8. doi: 10.1161/STROKEAHA.115.010721

36. Lin L, Yang J, Chen C, Tian H, Bivard A, Spratt NJ, et al. Association of collateral status and ischemic core growth in patients with acute ischemic stroke. *Neurology.* (2021) 96:e161–e70. doi: 10.1212/WNL.0000000000011258

37. Puhr-Westerheide D, Tiedt S, Rotkopf LT, Herzberg M, Reidler P, Fabritius MP, et al. Clinical and imaging parameters associated with hyperacute infarction growth in large vessel occlusion stroke. *Stroke.* (2019) 50:2799–804. doi: 10.1161/STROKEAHA.119.025809

38. Wufuer A, Mijiti P, Abudusalamu R, Dengfeng H, Jian C, Jianhua M, et al. Blood pressure and collateral circulation in acute ischemic stroke. *Herz.* (2019) 44:455–9. doi: 10.1007/s00059-018-4691-5

39. Ovbiagele B, Saver JL, Starkman S, Kim D, Ali LK, Jahan R, et al. Statin enhancement of collateralization in acute stroke. *Neurology.* (2007) 68:2129–31. doi: 10.1212/01.wnl.0000264931.34941.f0

40. Lee MJ, Bang OY, Kim SJ, Kim GM, Chung CS, Lee KH, et al. Role of statin in atrial fibrillation-related stroke: an angiographic study for collateral flow. *Cerebrovasc Dis.* (2014) 37:77–84. doi: 10.1159/000356114

41. Dargazanli C, Fahed R, Blanc R, Gory B, Labreuche J, Duhamel A, et al. Modified thrombolysis in cerebral infarction 2C/thrombolysis in cerebral infarction 3 reperfusion should be the aim of mechanical thrombectomy: insights from the ASTER trial (contact aspiration vs. stent retriever for successful revascularization). *Stroke.* (2018) 49:1189–96. doi: 10.1161/STROKEAHA.118.020700

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Associations of plaque morphology and location with Intraplaque neovascularization in the carotid artery by contrast-enhanced ultrasound imaging

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Objective: Intraplaque neovascularization (IPN) is a known indicator of plaque vulnerability, and is thus considered a predictor of stroke. The morphology and location of the carotid plaque may be correlated with plaque vulnerability. Therefore, our study aimed to examine the associations of carotid plaque morphology and location with IPN.

Methods: A total of 141 patients with carotid atherosclerosis (mean age, 64.99±10.96years) who underwent carotid contrast-enhanced ultrasound (CEUS) between November 2021 and March 2022 were retrospectively analyzed. IPN was graded according to the presence and location of microbubbles within the plaque. The association of IPN grade with carotid plaque morphology and location was evaluated using ordered logistic regression.

Results: Of the 171 plaques, 89 (52%) were IPN Grade 0, 21 (12.2%) were Grade 1, and 61 (35.6%) were Grade 2. IPN grade significantly associated with both plaque morphology and location, with higher grades observed among Type III morphology and common carotid artery plaques. Significant negative association was further shown between IPN grade and serum high-density lipoprotein cholesterol (HDL-C) level. Plaque morphology and location, and HDL-C remained significantly associated with IPN grade after adjusting for confounding factors.

Conclusion: The location and morphology of carotid plaques were significantly associated with the IPN grade on CEUS, and therefore show potential as biomarkers for plaque vulnerability. Serum HDL-C was also identified as a protective factor against IPN, and may play a role in the management of carotid atherosclerosis. Our study provided a potential strategy for identification of vulnerable carotid plaques and elucidated the important imaging predictors of stroke.

KEYWORDS

carotid artery, plaque, atherosclerotic, contrast-enhanced ultrasound, neovascularization

1. Introduction

Stroke, with its high morbidity, mortality, and disability rates, represents one of the leading causes of death worldwide (1). Approximately 18–25% of all ischemic strokes are attributable to carotid plaque rupture (2, 3). Intraplaque neovascularization (IPN) represents an important feature of plaque vulnerability (4). IPN provides valuable insight to plaque activity, as it has been reported to associate with an increased risk of neovessel rupture, intraplaque hemorrhage, and inflammation (5).

The location and morphology of plaques have been shown to contribute to plaque vulnerability (6). The potential mechanism for this has been reported to relate to their influence on the shear stress generated on plaque surfaces (7), which is a known key player in the pathophysiology of atherosclerosis (8, 9). Indeed, areas of low shear stress are often accompanied by higher expressions of inflammatory mediators and greater degree of matrix metalloproteinase activity (10). Moreover, inflammation is known to initiate the process of neovascularization. However, the exact influence of plaque location and morphology on the degree of IPN remains unknown.

Contrast enhanced ultrasound (CEUS), a novel ultrasound technique, has been recognized as an effective imaging modality for detecting neovascularization (11, 12). Intensity of plaque enhancement on CEUS has been shown to significantly correlate with the degree of neovascularization (13–15). In addition, CEUS has allowed for clearer visualization of both the location and morphological features of carotid plaques as compared to standard duplex ultrasound.

As such, our study aimed to evaluate the association of plaque location and morphology with IPN grade on CEUS, to assess their role as potential biomarkers for plaque vulnerability.

2. Materials and methods

2.1. Study population

Consecutive patients diagnosed with carotid atherosclerotic plaques who underwent CEUS between November 2021 and March 2022 were retrospectively analyzed. The inclusion criteria involved carotid plaques of thickness ≥ 2.5 mm measured in the longitudinal axis at the point of greatest luminal narrowing. This was selected based on guideline reports that plaques of such size group are clinically significant, and can be accurately assessed on ultrasound (16). The exclusion criteria included: (1) maximum plaque thickness < 2.5 mm; (2) poor image quality such as severe plaque calcification; (3) allergy to CEUS contrast agent; (4) severe cardiopulmonary dysfunction or intolerance to CEUS; and (5) incomplete clinical data (Figure 1).

2.2. Clinical variables

The following variables were collected: (1) age, sex, and body mass index (BMI); (2) medical history, including hypertension, diabetes, and coronary artery disease; (3) smoking history; (4) statin use; and (5) blood test results, including low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and triglyceride (TG) levels.

2.3. Morphology and location of carotid plaques

The morphology and location of plaques were evaluated using combined B-mode ultrasound and CEUS.

Plaque morphology was assessed in terms of symmetric features in the longitudinal axis. Arc length was measured as the distance from each end of the plaque to point of maximum thickness. The morphology was classified as Type I (the greater arc-length of the carotid plaque was located in the downstream arterial wall above the site with maximum wall thickness), Type II (the arc-lengths of the carotid plaques in the downstream and upstream arterial walls from the site with maximum wall thickness were equal, and the tolerances were no less than 1 mm), or Type III (the greater arc-length of the carotid plaque was located in the upstream arterial wall below the site showing maximum wall thickness; Figure 2) (6).

Plaque location was divided into the internal carotid artery (ICA), carotid bifurcation, and common carotid artery (CCA). In the case of

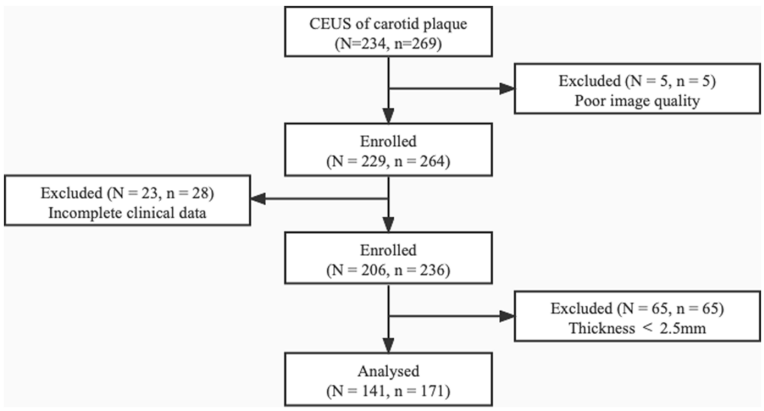

FIGURE 1
The patient selection process. Among the 234 patients diagnosed with carotid plaques, 93 (98 plaques) were excluded for poor image quality, incomplete clinical data, and plaque thickness<2.5 mm. *N*, number of patients; *n*, number of plaques.

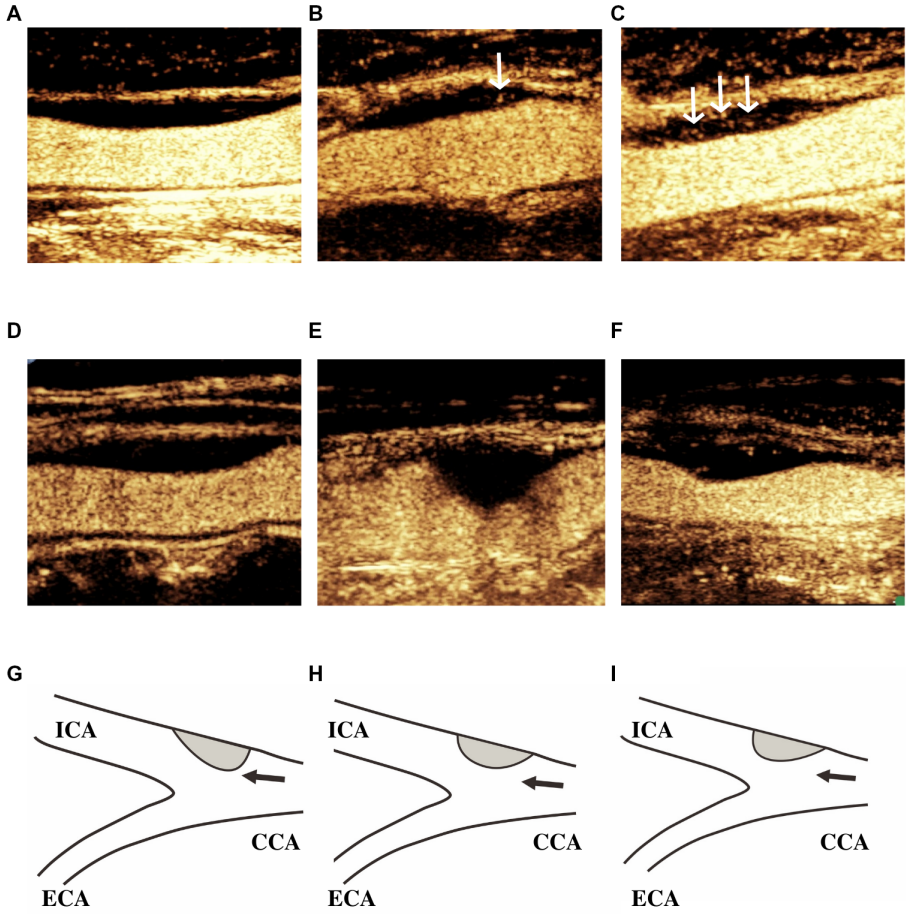

FIGURE 2
IPN grade and carotid plaque morphology. Classification of IPN grade: **(A)** Grade 0, no bubbles within the plaque, or bubbles confined to the adventitial side; **(B)** Grade 1, moderate intraplaque enhancement with moving bubbles at the adventitial side in the plaque shoulder; and **(C)** Grade 2, extensive intraplaque enhancement with clear appearance of bubbles moving into the plaque core. White arrows indicate intraplaque enhancement. Classification of plaque morphology: **(D,G)** Type I, the greater arc-length of the carotid plaques was located in the downstream arterial wall above the site showing maximum wall thickness; **(E,H)** Type II, the arc-lengths of the carotid plaques in the downstream and upstream arterial walls from the site showing maximum wall thickness were equal; and **(F,I)** Type III, the greater arc-length of carotid plaques was located in the upstream arterial wall below the site showing maximum wall thickness. Black arrows indicate the direction of blood flow. ICA, internal carotid artery; ECA, external carotid artery; and CCA, common carotid artery.

plaques spanning across two locations, the position of the point of maximum thickness was considered.

In addition, we recorded the presence of ulceration (cavities measuring at least 1 mm), which has been associated with the risk of plaque rupture (4, 17).

2.4. B-mode and contrast-enhanced ultrasound

All B-mode ultrasound and CEUS examinations were performed by an experienced radiologist using Philips EPIQ Elite (Philips, Netherlands) with a high frequency probe (eL18-4, MHz). After identification of the target plaque, the maximum longitudinal view of the plaque was determined for CEUS analysis.

CEUS was performed after bolus injection of 1.0 ml SonoVue solution (Bracco, Milan, Italy) followed by 5 ml saline flushing through a peripheral vein. The system settings were as follows: mechanical index, 0.06; gain, 60 ~ 70%; and depth, 2.5–3.5 cm. During the examination, the patients were encouraged to maintain calm breathing, and to avoid swallowing or coughing as best as possible.

Following contrast agent administration, IPN was graded using a semiquantitative visual approach according to the presence and location of microbubbles in the plaque. IPN grading was as follows: Grade 0 (no bubbles within the plaque, or bubbles confined to the adventitial side), Grade 1 (moderate intraplaque enhancement with moving bubbles at the adventitial side in the plaque shoulder), and Grade 2 (extensive intraplaque enhancement with clear appearance of bubbles moving into the plaque core; Figure 2) (18). All videos (of at least 2 min in duration) were stored digitally on magnetic optical disks for offline analysis.

Inter-observer consistency in IPN grading was analyzed by two independent radiologists (S-YG and L-NZ) who were blinded to each other's interpretation. To evaluate intra-observer consistency, the data was reanalyzed by the same radiologist (S-YG) after an interval of 1 month without reference to the initial results.

2.5. Statistical analysis

All statistical analyzes were performed using SPSS 25.0 (IBM, Armonk, NY, United States). Categorical and continuous variables were expressed as frequency (%) and mean ± standard deviation (SD), respectively. Analysis of variance was performed to compare the characteristics of both patient and plaque based on IPN grade. Ordered logistic regression analysis was used to analyze the relationship of IPN grade with selected factors after adjusting for confounding factors, with outcomes expressed as odds ratio (OR) and 95% confidence interval (CI). Intra- and inter-observer consistencies were analyzed using the intra-group correlation coefficient. Statistical significance was considered as $p < 0.05$.

3. Results

3.1. Baseline characteristics

Among the 234 patients who underwent carotid artery CEUS, 93 were excluded due to poor image quality (N = 5), incomplete clinical data ($N = 23$), and plaque thickness < 2.5 mm ($N = 65$; Figure 1). A total of 141 patients were eventually enrolled, of whom 107 (75.9%) were male. The average age was 64.99 ± 10.96 years. In terms of clinical characteristics, 52 (36.9%) had diabetes, 79 (56.0%) had hypertension, 20 (14.2%) had coronary heart disease, 42 (29.8%) had positive statin use history, and 74 (52.5%) had positive smoking history. All baseline characteristics of the included patients are presented in Table 1.

Bilateral carotid CEUS was performed in 30 (21.3%) patients. Among a total of 171 carotid plaques, 89 (51.4%) were Grade 0, 21 (12.1%) were Grade 1 and 63 (36.4%) were Grade 2. The comparison of patient and ultrasound characteristics based on IPN grade are shown in Tables 2, 3, respectively.

TABLE 1 Baseline clinical characteristics of the included patients.

Clinical characteristic	Patients (n=141)
Male sex, n (%)	107 (75.9)
Age (year)	64.99 ± 10.96
BMI (kg/m^2)	24.85 ± 4.81
Hypertension, n (%)	79 (56.0)
Diabetes, n (%)	52 (36.9)
Coronary heart disease, n (%)	20 (14.2)
LDL-C, mmol/L	2.81 ± 0.84
HDL-C, mmol/L	1.27 ± 0.37
TC, mmol/L	4.75 ± 1.17
TG, mmol/L	1.46 ± 0.97
Smoking history	74 (52.5)
Statin use history	42 (29.8)

BMI, body mass index; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; and TG, triglyceride.

3.2. Relationship between carotid plaque morphology and IPN grade

IPN grade was observed to significantly associate with plaque morphology (OR, 2.06; 95% CI, 1.47–2.90; $p < 0.01$; Table 3), with higher IPN grades observed among Type III plaques. Significant differences in IPN grade were observed between Type I and III plaques, as well as between Type II and III plaques ($p < 0.01$ and $p = 0.04$, respectively). However, no significant differences were demonstrated between Type I and II plaques ($p = 0.18$).

3.3. Relationship between carotid plaque location and IPN grade

Plaques located in the CCA demonstrated significantly higher IPN grades (OR, 1.30; 95% CI, 1.02–1.65; $p = 0.04$; Table 3). Significant differences in IPN grades were observed between plaques located in the CCA and the carotid bifurcation ($p = 0.03$), as well as between those in the CCA and the ICA ($p = 0.03$). However, no significant differences were shown between plaques located in the carotid bifurcation and the ICA ($p = 0.57$).

3.4. Relationship between serum HDL-C level and IPN grade

Lower serum HDL-C level was observed to significantly associate with higher IPN grade (OR, 0.33; 95% CI, 0.13–0.81; $p = 0.02$; Table 2).

3.5. Logistic regression analysis

After adjusting for confounding factors such as gender, BMI, hypertension, smoking history, and statin use, all 3 factors remained statistically significant. Significantly higher IPN grades were demonstrated among plaques of Type III morphology (OR, 2.09; 95%CI, 1.48–2.96; $p < 0.01$) and those located in the CCA (OR, 1.37; 95%CI, 1.04–1.77; $p = 0.02$). In contrast, a significant negative association was shown between serum HDL-C level and IPN grade (OR, 0.27; 95%CI, 0.10–0.76; $p = 0.01$; Table 4).

After adjusting for echo, maximum thickness, and ulceration, plaque morphology (OR, 2.25; 95%CI, 1.55–3.27; $p < 0.01$), plaque location (OR, 1.36; 95%CI, 1.04–1.77; $p = 0.02$), and serum HDL-C (OR, 0.27; 95%CI, 0.10–0.73; $p = 0.01$) remained statistically significant (Table 4).

3.6. Relationship of plaque morphology and location with ulceration

Ulceration was observed on 11 plaques, but did not demonstrate any significant correlation with morphology or location ($p = 0.25$ and $p = 0.13$, respectively; Supplementary Table S1). No significant correlation was demonstrated with IPN grade as well ($p = 0.62$; Table 3).

TABLE 2 Comparison of clinical characteristics based on IPN grade.

	Grade 0 (*n*=89)	Grade 1 (*n*=21)	Grade 2 (*n*=61)	OR	95% CI	*p*
Male, *n*	64	17	49	1.56	0.79–3.11	0.21
Age, year	65.30 ± 10.18	65.48 ± 10.61	65.61 ± 11.82	1.00	0.98–1.03	0.86
BMI, kg/m^2	24.19 ± 3.89	25.35 ± 6.47	25.12 ± 5.08	1.04	0.97–1.10	0.25
Hypertension, *n*	50	11	41	0.69	0.38–1.24	0.21
Diabetes, *n*	32	10	21	1.02	0.56–1.85	0.95
Coronary heart disease, *n*	12	4	8	0.99	0.43–2.27	0.99
LDL-C, mmol/L	2.94 ± 0.77	2.80 ± 1.01	2.85 ± 0.89	0.89	0.63–1.26	0.52
HDL-C, mmol/L	1.31 ± 0.40	1.17 ± 0.32	1.17 ± 0.29	0.33	0.13–0.81	0.02*
TC, mmol/L	4.78 ± 1.15	4.51 ± 1.30	4.62 ± 1.18	0.90	0.70–1.15	0.39
TG, mmol/L	1.39 ± 0.91	1.31 ± 0.43	1.55 ± 1.00	1.20	0.86–1.68	0.28
Statin use history	23	7	19	1.06	0.59–1.88	0.85
Smoking history	47	12	31	0.78	0.42–1.48	0.45

BMI, body mass index; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; and TG, triglyceride; CCA, common carotid artery; ICA, internal carotid artery.

TABLE 3 Comparison of ultrasound characteristics based on IPN grade.

	Grade 0 (*n*=89)	Grade 1 (*n*=21)	Grade 2 (*n*=61)	OR	95% CI	*p*
Echoes, hypoechoic	66	11	39	1.26	0.92–1.71	0.14
Ulceration	6 (4.5%)	1 (4.8%)	3 (6.6%)	1.38	0.39–4.87	0.62
Maximum thickness	3.22 ± 0.77	3.41 ± 1.08	3.35 ± 0.82	1.19	0.84–1.68	0.34
Carotid plaque morphology						
Type I	47	6	14	2.06	1.47–2.90	<0.01*
Type II	17	3	10			
Type III	25	12	37			
Carotid plaque location						
CCA	14	3	20	1.30	1.02–1.65	0.04*
Carotid bifurcation	59	15	34			
ICA	16	3	7			

TABLE 4 Logistic regression analysis.

	IPN grade								
	Univariate regression			Model 1			Model 2		
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Plaque morphology	2.06	1.47–2.90	<0.01*	2.09	1.48–2.96	<0.01*	2.25	1.55–3.27	<0.01*
Plaque location	1.30	1.02–1.65	0.04*	1.37	1.04–1.77	0.02*	1.36	1.04–1.77	0.02*
HDL-C	0.33	0.13–0.81	0.02*	0.29	0.11–0.83	0.02*	0.27	0.10–0.73	0.01*

Model 1, adjusted for gender, BMI. hypertension, smoking history, and statin use.
Model 2, adjusted for gender, BMI. hypertension, smoking history, statin use, echo, maximum thickness, and ulceration.

3.7. Intra- and inter-observer consistency analysis

Excellent agreement in the CEUS evaluation of IPN was demonstrated. The intra-observer consistency was 0.88 (95% CI, 2.32–2.45; $p < 0.01$), while the inter-observer consistency was 0.85 (95% CI, 2.23–2.43; $p < 0.01$).

4. Discussion

The morphology and location of carotid plaques, as well as serum HDL-C level, demonstrated a significant influence on IPN grade in our study.

Higher IPN grades were observed among plaques of Type III morphology. Plaques of such morphology are characterized by lower

upstream slopes, which may be subjected to lower shear stress (6). This is consistent with the notion that proatherogenic transcription factor upregulation and the resultant aggregation of inflammatory cells tend to occur in regions of low shear stress (19), ultimately resulting in a more fragile plaque phenotype (20, 21). Our findings of increased neovascularization in a low shear stress environment are further in correspondence to previous reports that macrophage infiltration often coexist with hypoxia and angiogenesis due to high metabolic demand (22, 23).

The bifurcation of the CCA has long been known as a common location for atherosclerotic plaque development due to the disturbance in flow (24, 25). However, we found that plaques located in the CCA were associated with a higher IPN grade instead. We postulate that this may be related to the greater length and area of such plaques (Supplementary Table S2; Supplementary Figure S1), which may have reflected greater plaque burden. In line with this, it has been reported that plaques of the CCA tend to grow along the longitudinal axis of the vessel wall and create greater lengths (26). Larger plaques may thereby associate with larger areas of anoxia, greater degrees of inflammation, and ultimately increased neovascularization (26, 27).

Serum HDL-C was found to be significantly protective against neovascularization. While low-density lipoprotein cholesterol (LDL-C) is widely accepted as an independent predictor of cardio- and cerebrovascular events, low HDL-C levels have been reported to be associated with an increased risk of cardiovascular diseases and stroke regardless of LDL-C levels (28–30). The HDL-C level was negatively associated with IPN grading in our study, and this association did not relate to statin use. Other lipid parameters, including the LDL-C level, were not significantly associated with IPN grading, highlighting the potential importance of HDL-C in plaque vulnerability. HDL-C is known to promote the reverse transport of cholesterol from atherosclerotic plaque (31). In addition, HDL-C portrays anti-atherosclerotic effects, which is mediated by its antioxidant, anti-inflammatory, and antithrombotic characteristics (32, 33). While Ying et al. (34) have found an association of carotid plaque neovascularization with total cholesterol and LDL-C levels. This was, however, not observed in our study, which may be attributable to the use of statins (Supplementary Table S3).

Ulceration is an important feature of ruptured plaques (35). However, we found no correlation of ulceration with IPN grade, plaque morphology, or plaque location. This may be due to the relatively few cases of ulcerated plaques among our patients, which hindered effective statistical analysis. However, a previous study showed that shear stress and local hemodynamics caused by anatomical differences of the carotid arteries did not influence the incidence of plaque ulceration (36). This suggests that morphology and location may not play any role in such feature. Nonetheless, further large-sample analyzes are warranted to elucidate the factors involved in the development of ulcerated plaques.

There were several limitations in our study. First, this was a single-centered study with a relatively small sample size. Large-sample studies involving the assessment of other potential associating factors such as clinical symptoms are thereby warranted. Second, plaque morphology was assessed based on only the longitudinal views of B-mode ultrasound and CEUS. However, the spatial morphology of plaques is complicated and may be correlated with plaque vulnerability. In future studies, we aim to evaluate the spatial diversity of plaque morphology through three-dimensional ultrasound imaging. Third, the geometry of calcification, which may affect plaque stability, was overlooked in our study. This was due to their acoustic attenuation effects on CEUS, which would hinder our assessment of neovascularization. Further studies on the effects of calcification on plaque vulnerability are thus required. Finally, plaques with thickness < 2.5 mm were excluded due to the difficulties in performing CEUS for plaques with a thinner wall, which could have negatively affected the accuracy of our results. Some studies on the IPN grade excluded plaques with thickness < 2.5 mm for similar reasons (37). To obtain more accurate results, we excluded these data. In future studies, we will attempt to include other imaging modalities to study the neovascularization of plaques with thickness < 2.5 mm. In addition, we included plaques with thickness ≥ 2.5 mm, which were considered high-risk plaques in a previous study. In future, we hope to obtain more information regarding plaques in high-risk groups and provide better management strategies.

5. Conclusion

The location and morphology of carotid plaques significantly associated with IPN grade on CEUS, and thereby carry the potential as biomarkers for plaque vulnerability. Serum HDL-C was further found as a protective factor against IPN, and may play a role in the management of carotid atherosclerosis. Our study not only provided a potential strategy for the identification of vulnerable carotid plaques, but also elucidated the important imaging predictors of stroke.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Shanghai General hospital institutional review board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

S-YG: conceptualization, writing–original draft, writing–review and editing, and investigation. L-NZ: investigation and writing–review and editing. FL: data curation. JC: writing–review and editing. M-HY: formal analysis and writing–review and editing. C-XJ: funding acquisition and writing–review and editing. RW: resources, funding acquisition, and writing–review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1097070/full#supplementary-material

References

1. Lou M, Ding J, Hu B, Zhang Y, Li H, Tan Z, et al. Chinese Stroke Association guidelines for clinical management of cerebrovascular disorders: executive summary and 2019 update on organizational stroke management. *Stroke Vasc Neurol*. (2020) 5:260–9. doi: 10.1136/svn-2020-000355

2. Ooi YC, Gonzalez NR. Management of extracranial carotid artery disease. *Cardiol Clin*. (2015) 33:1–35. doi: 10.1016/j.ccl.2014.09.001

3. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation*. (2015) 131:e29–e322. doi: 10.1161/CIR.0000000000000152

4. Saba L, Saam T, Jäger HR, Yuan C, Hatsukami TS, Saloner D, et al. Imaging biomarkers of vulnerable carotid plaques for stroke risk prediction and their potential clinical implications. *Lancet Neurol*. (2019) 18:559–72. doi: 10.1016/S1474-4422(19)30035-3

5. Horie N, Morofuji Y, Morikawa M, Tateishi Y, Izumo T, Hayashi K, et al. Communication of inwardly projecting neovessels with the lumen contributes to symptomatic intraplaque hemorrhage in carotid artery stenosis. *J Neurosurg*. (2015) 123:1125–32. doi: 10.3171/2014.12.JNS142371

6. Lu M, Cui Y, Peng P, Qiao H, Cai J, Zhao X. Shape and location of carotid atherosclerotic plaque and Intraplaque hemorrhage: A high-resolution magnetic resonance imaging study. *J Atheroscler Thromb*. (2019) 26:720–7. doi: 10.1016/j.jcmg.2016.04.012

7. Lee JM, Choi G, Hwang D, Park J, Kim HJ, Doh JH, et al. Impact of longitudinal lesion geometry on location of plaque rupture and clinical presentations. *JACC Cardiovasc Imaging*. (2017) 10:677–88. doi: 10.1016/j.jcmg.2016.04.012

8. Woo HG, Kim HG, Lee KM, Ha SH, Jo H, Heo SH, et al. Wall shear stress associated with stroke occurrence and mechanisms in middle cerebral artery atherosclerosis. *J Stroke*. (2023) 25:132–40. doi: 10.5853/jos.2022.02754

9. Russo G, Pedicino D, Chiastra C, Vinci R, Lodi Rizzini M, Genuardi L, et al. Coronary artery plaque rupture and erosion: role of wall shear stress profiling and biological patterns in acute coronary syndromes. *Int J Cardiol*. (2023) 370:356–65. doi: 10.1016/j.ijcard.2022.10.139

10. Malik J, Novakova L, Valerianova A, Chytilova E, Lejsek V, Buryskova Salajova K, et al. Wall shear stress alteration: a local risk factor of atherosclerosis. *Curr Atheroscler Rep*. (2022) 24:143–51. doi: 10.1007/s11883-022-00993-0

11. Fedak A, Chrzan R, Chukwu O, Urbanik A. Ultrasound methods of imaging atherosclerotic plaque in carotid arteries: examinations using contrast agents. *J Ultrason*. (2020) 20:191–e200. doi: 10.15557/JoU.2020.0032

12. Huang R, Abdelmoneim SS, Ball CA, Nhola LF, Farrell AM, Feinstein S, et al. Detection of carotid atherosclerotic plaque neovascularization using contrast enhanced ultrasound: A systematic review and Meta-analysis of diagnostic accuracy studies. *J Am Soc Echocardiogr*. (2016) 29:491–502. doi: 10.1016/j.echo.2016.02.012

13. Zhang Y, Cao J, Zhou J, Zhang C, Li Q, Chen S, et al. Plaque elasticity and Intraplaque neovascularisation on carotid artery ultrasound: A comparative histological study. *Eur J Vasc Endovasc Surg*. (2021) 62:358–66. doi: 10.1016/j.ejvs.2021.05.026

14. Zhang Q, Li C, Zhou M, Liao Y, Huang C, Shi J, et al. Quantification of carotid plaque elasticity and intraplaque neovascularization using contrast-enhanced ultrasound and image registration-based elastography. *Ultrasonics*. (2015) 62:253–62. doi: 10.1016/j.ultras.2015.05.025

15. Lyu Q, Tian X, Ding Y, Yan Y, Huang Y, Zhou P, et al. Evaluation of carotid plaque rupture and neovascularization by contrast-enhanced ultrasound imaging: an exploratory study based on histopathology. *Transl Stroke Res*. (2021) 12:49–56. doi: 10.1007/s12975-020-00825-w

16. Johri AM, Nambi V, Naqvi TZ, Feinstein SB, Kim ESH, Park MM, et al. Recommendations for the assessment of carotid arterial plaque by ultrasound for the characterization of atherosclerosis and evaluation of cardiovascular risk: from the American Society of Echocardiography. *J Am Soc Echocardiogr*. (2020) 33:917–33. doi: 10.1016/j.echo.2020.04.021

17. Dilba K, van Dam-Nolen DHK, van Dijk AC, Kassem M, van der Steen AFW, Koudstaal PJ, et al. Plaque composition as a predictor of plaque ulceration in carotid artery atherosclerosis: the plaque at RISK study. *AJNR Am J Neuroradiol*. (2021) 42:144–51. doi: 10.3174/ajnr.A6868

18. Coli S, Magnoni M, Sangiorgi G, Marrocco-Trischitta MM, Melisurgo G, Mauriello A, et al. Contrast-enhanced ultrasound imaging of intraplaque neovascularization in carotid arteries: correlation with histology and plaque echogenicity. *J Am Coll Cardiol*. (2008) 52:223–30. doi: 10.1016/j.jacc.2008.02.082

19. Helderman F, Segers D, de Crom R, Hierck BP, Poelmann RE, Evans PC, et al. Effect of shear stress on vascular inflammation and plaque development. *Curr Opin Lipidol*. (2007) 18:527–33. doi: 10.1097/MOL.0b013e3282ef7716

20. Zhao Y, Ren P, Li Q, Umar SA, Yang T, Dong Y, et al. Low shear stress upregulates CX3CR1 expression by inducing VCAM-1 via the NF-κB pathway in vascular endothelial cells. *Cell Biochem Biophys*. (2020) 78:383–9. doi: 10.1007/s12013-020-00931-4

21. Ding Z, Liu S, Wang X, Deng X, Fan Y, Sun C, et al. Hemodynamic shear stress via ROS modulates PCSK9 expression in human vascular endothelial and smooth muscle cells and along the mouse aorta. *Antioxid Redox Signal*. (2015) 22:760–71. doi: 10.1089/ars.2014.6054

22. Moerman AM, Korteland S, Dilba K, van Gaalen K, Poot DHJ, van Der Lugt A, et al. The correlation between wall shear stress and plaque composition in advanced human carotid atherosclerosis. *Front Bioeng Biotechnol*. (2022) 9:828577. doi: 10.3389/fbioe.2021.828577

23. Moreno PR, Purushothaman M, Purushothaman KR. Plaque neovascularization: defense mechanisms, betrayal, or a war in progress. *Ann N Y Acad Sci*. (2012) 1254:7–17. doi: 10.1111/j.1749-6632.2012.06497.x

24. Gallo D, Steinman DA, Morbiducci U. Insights into the co-localization of magnitude-based versus direction-based indicators of disturbed shear at the carotid bifurcation. *J Biomech*. (2016) 49:2413–9. doi: 10.1016/j.jbiomech.2016.02.010

25. Ahmadpour BM, Nooraeen A, Tafazzoli-Shadpour M, Taghizadeh H. Contribution of atherosclerotic plaque location and severity to the near-wall hemodynamics of the carotid bifurcation: an experimental study and FSI modeling. *Biomech Model Mechanobiol*. (2021) 20:1069–85. doi: 10.1007/s10237-021-01431-x

26. Paritala PK, Yarlagadda T, Mendieta JB, Wang J, McGahan T, Lloyd T, et al. Plaque longitudinal heterogeneity in morphology, property, and Mechanobiology. *Cerebrovasc Dis*. (2021) 50:510–9. doi: 10.1159/000515690

27. Boswell-Patterson CA, Hétu MF, Kearney A, Pang SC, Tse MY, Herr JE, et al. Vascularized carotid atherosclerotic plaque models for the validation of novel methods of quantifying Intraplaque neovascularization. *J Am Soc Echocardiogr*. (2021) 34:1184–94. doi: 10.1016/j.echo.2021.06.003

28. Honda S, Sidharta SL, Shishikura D, Takata K, Di Giovanni GA, Nguyen T, et al. High-density lipoprotein cholesterol associated with change in coronary plaque lipid

burden assessed by near infrared spectroscopy. *Atherosclerosis*. (2017) 265:110–6. doi: 10.1016/j.atherosclerosis.2017.08.016

29. Touboul PJ, Labreuche J, Bruckert E, Schargrodsky H, Prati P, Tosetto A, et al. HDL-C, triglycerides and carotid IMT: a meta-analysis of 21,000 patients with automated edge detection IMT measurement. *Atherosclerosis*. (2014) 232:65–71. doi: 10.1016/j.atherosclerosis.2013.10.011

30. Sacco RL, Benson RT, Kargman DE, Boden-Albala B, Tuck C, Lin IF, et al. High-density lipoprotein cholesterol and ischemic stroke in the elderly: the northern Manhattan stroke study. *JAMA*. (2001) 285:2729–35. doi: 10.1001/jama.285.21.2729

31. Rosenson RS, Brewer HB Jr, Davidson WS, Fayad ZA, Fuster V, Goldstein J, et al. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation*. (2012) 125:1905–19. doi: 10.1161/CIRCULATIONAHA.111.066589

32. Wu Y, Xu Y, Chen J, Zhao M, Rye KA. HDL and endothelial function. *Adv Exp Med Biol*. (2022) 1377:27–47. doi: 10.1007/978-981-19-1592-5_3

33. Fotakis P, Kothari V, Thomas DG, Westerterp M, Molusky MM, Altin E, et al. Anti-inflammatory effects of HDL (high-density lipoprotein) in macrophages predominate over Proinflammatory effects in atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*. (2019) 39:e253–72. doi: 10.1161/ATVBAHA.119.313253

34. Wang Y, Yao M, Zou M, Ge Z, Cai S, Hong Y, et al. Relationship between serum lipid profiles and carotid Intraplaque neovascularization in a high-stroke-risk population: A cross-sectional study in China. *J Am Heart Assoc*. (2021) 10:e021545. doi: 10.1161/JAHA.121.021545

35. Bos D, van Dam-Nolen DHK, Gupta A, Saba L, Saloner D, Wasserman BA, et al. Advances in multimodality carotid plaque imaging: AJR expert panel narrative review. *AJR Am J Roentgenol*. (2021) 217:16–26. doi: 10.2214/AJR.20.24869

36. Schulz UG, Rothwell PM. Association between arterial bifurcation anatomy and angiographic plaque ulceration among 4,627 carotid stenoses. *Cerebrovasc Dis*. (2003) 15:244–51. doi: 10.1159/000069491

37. Meng Q, Xie X, Li L, Jiang C, Zhao K, Bai Z, et al. Assessment of neovascularization of carotid artery atherosclerotic plaques using superb microvascular imaging: a comparison with contrast-enhanced ultrasound imaging and histology. *Quant Imaging Med Surg*. (2021) 11:1958–69. doi: 10.21037/qims-20-933

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Texture analysis of apparent diffusion coefficient maps in predicting the clinical functional outcomes of acute ischemic stroke

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Purpose: To investigate texture analysis (TA) based on apparent diffusion coefficient (ADC) map in predicting acute ischemic stroke (AIS) prognosis and discriminating TA features in stroke subtypes.

Methods: This retrospective study included patients with AIS between January 2018 and April 2021. The patients were assigned to the favorable [modified Rankin Scale (mRS) score≤2] and unfavorable (mRS score>2) outcome groups. All patients underwent stroke subtyping according to the Trial of Org 10,172 in Acute Stroke Treatment (TOAST) classification. The TA features were extracted from infarction lesions on the ADC map. The demographic characteristics, clinical characteristics, and texture features were used to construct prediction models with recurrent neural network (RNN). The receiver operating characteristic (ROC) curves were implemented to evaluate the performance of the predictive models.

Results: A total of 1,003 patients (682 male; mean age 65.90±12.44) with AIS having documented the 90-day mRS score were identified, including 840 with favorable outcomes. In the validation set, the area under the curve (AUC) of the predictive model using only clinical characteristics achieved an AUC of 0.56, texture model 0.77, the model combining both clinical and texture features showed better with an AUC of 0.78. The texture feature profiles differed between large artery atherosclerosis (LAA) and small artery occlusion (SAO) subtypes (all $p<0.05$). The AUC of combined prediction models for LAA and SAO subtypes was 0.80 and 0.81.

Conclusion: Texture analysis based on ADC map could be useful as an adjunctive tool for predicting ischemic stroke prognosis.

KEYWORDS

ischemic stroke, radiology, diffusion magnetic resonance imaging, texture analysis, prognosis

Introduction

Stroke remains the second-leading cause of death and the third-leading cause of death and disability combined globally (1). The burden of stroke has increased substantially over the past few decades due to an increasing and aging population as well as the increased prevalence of modifiable stroke risk factors, particularly in low-and middle-income countries (2). More than 12

million people worldwide suffer a stroke each year, and approximately 70–80% of stroke cases are attributed to ischemic stroke (3, 4). The distribution of ischemic stroke subtypes varies among different racial or ethnic groups (5). Since accurate clinical strategies can improve the outcomes in patients with acute ischemic stroke (AIS), the obtainability of robust and validated prognostic biomarkers is essential to optimize early individualized therapy and rehabilitation strategies.

Texture analysis (TA) is an effective quantitative image analysis tool to explore the microstructural changes that cannot be explored by humans visually. TA defines the measure of voxel intensities, voxel inter-relationships, and the gray-level patterns in the image. TA has been successfully used for characterizing multiple sclerosis (6–8), small vessel disease (9), and dementia with Lewy bodies (10). It has been demonstrated that TA is an effective tool for image analysis. In the field of ischemic stroke, TA based on magnetic resonance imaging (MRI) has been reported to be applied to the early identification of ischemic lesions (11), stroke severity classification (12), post-stroke cognitive impairment (13) and detecting the effects of stroke therapy (14).

Diffusion-weighted imaging (DWI) is the most important and commonly used part of routine clinical stroke neuroimaging protocols as it facilitates the identification of stroke lesions. The apparent diffusion coefficient (ADC) yielded by DWI is sensitive to the initial cell swelling of a cytotoxic edema. ADC maps have been found to reveal the early indications and progression of cerebral ischemic infarction (15). Each voxel intensity of ADC maps is determined partly by intracellular water and partly by extracellular water. The changes in the distribution of extracellular water entering cells after infarction may possibly be reflected in the texture features. Previous studies demonstrated that the texture features based on DWI and ADC maps could evaluate ischemic stroke severity (12, 16). ADC changes in motor structures have also been shown to be predictors of acute stroke outcomes (17). A recent study reported that radiomics features based on DWI and ADC could predict stroke outcomes (18). However, the information on whether the texture features of infarct lesions correlate with the prognosis of AIS is limited.

We hypothesized that the ADC-based texture features might differ between patients with AIS having favorable and unfavorable clinical outcomes. Thus, this study aimed to explore the role of texture features in predicting AIS prognosis. We further investigated the characteristics difference of texture features in different stroke subtypes.

Materials and methods

Study population

Between January 2018 and April 2021, all participants, aged >18 years, presenting to our stroke center with signs and symptoms of AIS were enrolled in this retrospective study. The study had approval from the institutional ethics committee of our hospital (approval number: 2022–013-01 K). Patients with confirmed acute DWI lesions on brain MRI scans performed within 72 h of symptom onset were included in this analysis. Of 1,580 participants, we excluded participants with cerebral hemorrhage ($n = 21$), traumatic brain injury ($n = 7$), previous neurological or psychiatric disorder ($n = 163$), severe MRI artifacts ($n = 17$), contradiction to MR examination ($n = 9$), feature extraction failure ($n = 80$), or loss to follow-up ($n = 280$). The flowchart is shown in Figure 1. The requirement for informed consent was waived because of the retrospective nature of the study.

Clinical variables

Age, sex, National Institutes of Health Stroke Scale (NIHSS) score, antecedent hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation, tobacco or alcohol use, low-density lipoprotein cholesterol (LDL-C), and discharge medications were abstracted from the medical record. The AIS subtypes were assigned according to the Trial of Org 10,172 in Acute Stroke Treatment (TOAST) classification (19).

Clinical outcome assessment

Patients or their caregivers were interviewed in person or by telephone at 90 days after stroke to assess the functional outcomes using the modified Rankin Scale (mRS) score. The patients were assigned to the favorable (mRS score ≤ 2) and unfavorable (mRS score > 2) outcome groups (20).

MRI examination

All MRI examinations were performed on a 1.5-T MRI scanner (GE Healthcare, WI, United States) or a 3.0-T MRI scanner (United Imaging Healthcare, Shanghai, China). The scan parameters for the 1.5-T scanner were axial DWI based on a single-shot echo planar imaging (SSEPI) sequence, with repetition time (TR)/echo time (TE) = 3,203 ms/83.9 ms, slice thickness/gap = 5 mm/1.5 mm, FOV = 240 × 240 mm^2, *b* values = 0 and 1,000 s/mm^2, and matrix = 96 × 96. The scan parameters for the 3.0-T scanner were axial DWI based on the SSEPI sequence, with TR/TE = 2,800 ms/75.4 ms, slice thickness/gap = 5 mm/1.5 mm, FOV = 230 × 220 mm^2, *b* values = 0 and 1,000 s/mm^2, and matrix = 128 × 128.

Segmentation of infarction lesions

A total of 100 patients were randomly selected for manual segmentation by ITK-SNAP 3.8.[1] The volume of interest (VOI) was sketched by slice-by-slice stacking on DWI. The 100 VOIs were brought into a 2D U-Net network for automatic segmentation (21). Then, the trained segmentation network model was used to segment the remaining cases. For automatic segmentation assessment, 100 random cases from the testing set were chosen to calculated the DICE coefficient. Image intensity parameters were normalized to 0–1 using window width and window level before the process of automatic image segmentation. The region of VOI on DWI was copied to the corresponding ADC maps. The mask matrix based on DWI and ADC maps was used for further texture extraction. The framework of the proposed method is given in Figure 2.

Texture features extraction and analysis

Texture features extraction were performed on the MATLAB 2019a (The MathWorks Inc., Natick, MA, USA). Based on the

1 www.itk-snap.org

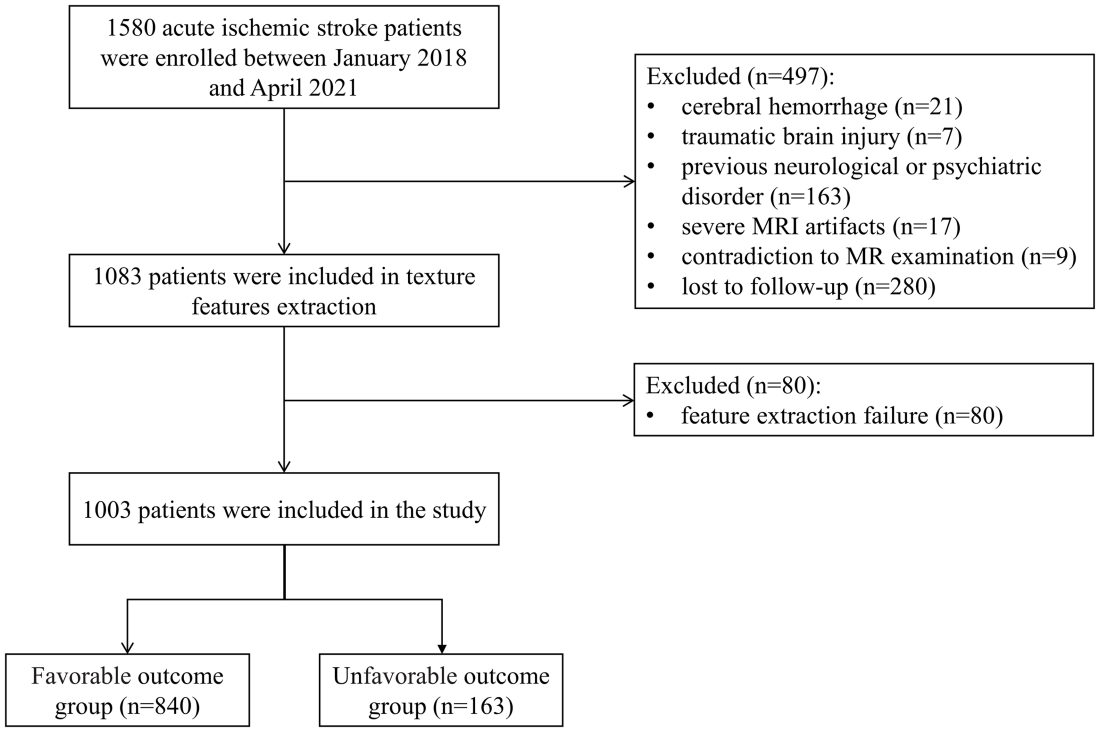

FIGURE 1
Flowchart of case selection for analysis.

extraction results of texture features and clinical characteristics, we use sparse representation for feature selection to reduce the redundant information in features and improve classification model accuracy. Specifically, we use sample features for sparse representation in sample labeling, so the highest label correlated feature subsets are selected. Meanwhile, the sparsity constraints on the model effectively removed the correlation and redundancy in the features of the feature subsets. Following is the model: $\hat{\boldsymbol{w}} = \arg\min_{\boldsymbol{w}} \| \boldsymbol{l} - \boldsymbol{F}\boldsymbol{w} \|_2^2 + \eta \| \boldsymbol{w} \|_0$ (22). $\boldsymbol{l} \in \boldsymbol{R}^m$ being the training sample label, m being the size of the training sample, $\boldsymbol{F} = [\boldsymbol{f}_1, \boldsymbol{f}_2 \cdots \boldsymbol{f}_m]^T \in \boldsymbol{R}^{m \times 2K}$ being feature set of training sample, ?being the sparse representation parameter, the absolute value of factors in sparse representation coefficient, $\boldsymbol{w}$ is the importance of the features. When $\boldsymbol{w}$ is computed, key features can be selected by simply comparing the thresholds.

The following features were extracted: eight gray-level co-occurrence matrix (GLCM) features, thirteen gray-level run-length matrix (GLRLM) features, thirteen gray-level size zone matrix (GLSZM) features, and five neighborhood gray-tone difference matrix (NGTDM) features. Additional information on TA features is provided in Supplementary Table S1.

Model classification and data distribution

Based on the selected feature subsets, we established our classification prediction models based on recurrent neural network (RNN) (23). Weighted cross-entropy loss function was used to optimize the network. In network training, we used an Adam optimizer with a learning rate of 0.0001 and a batch size of 10.

The patients were randomly divided into the training and validation sets by stratified sampling. Due to the imbalance of sample number in our study, we used under-sampling method to build the dataset. The unfavorable-outcome group was randomly divided into training and validation sets at the ratio of 2:1. Then, the patients in the favorable-outcome group were randomly assorted into the training set 1.5 times the number in the training set with an unfavorable outcome. The remaining patients were included in the validation set to validate the reliability and robustness of the models.

Statistical analysis

The continuous variables with normal distribution were reported as mean ± standard deviation, non-normally distributed variables as median (interquartile range), and classification variables as frequency (%). We used the independent-samples *t*-test or Mann–Whitney *U* test for continuous variables and the chi-square for categorical dependent variables between favorable-outcome and unfavorable-outcome groups, as appropriate. The receiver operating characteristic (ROC) curve was generated to evaluate the performances of the predictive models. All statistical analyses were carried out using SPSS (version 26.0, SPSS Inc., Chicago, IL, United States). All the reported *p* values were based on the two-tailed tests, and *p* values less than 0.05 indicated a statistically significant difference.

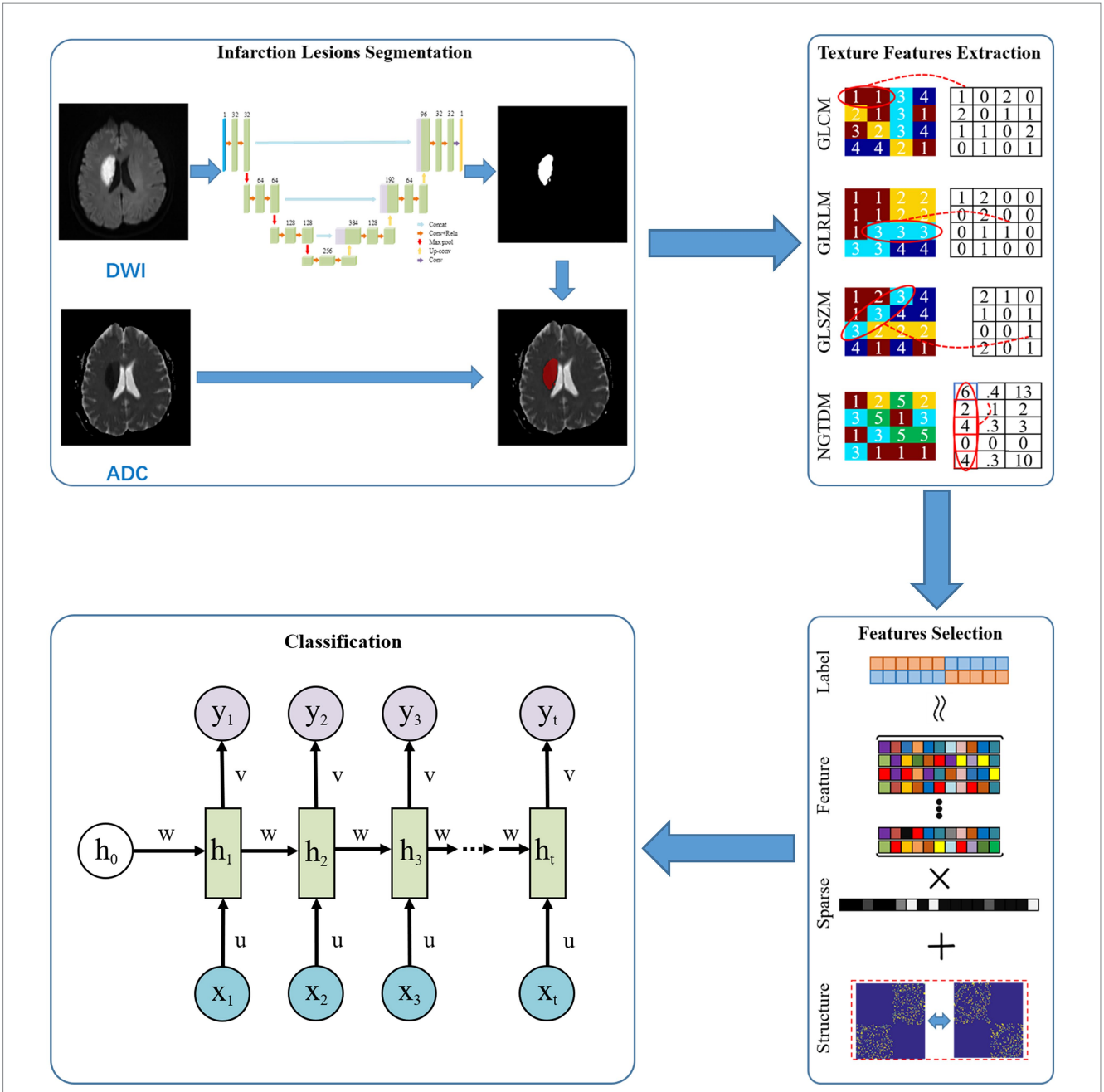

FIGURE 2
Pipeline of texture analysis for clinical outcome prediction.

Results

Demographic and clinical characteristics

We included 1,003 patients with AIS having a 90-day mRS score available for this analysis. Of these, 682 (68.0%) were men, and the mean age was 65.90 years (SD 12.44 years). Table 1 summarizes the demographic and clinical features of the study population and comparisons between the groups. In reproducibility analysis, the manually drawn results and the network's automatic segmentation exhibited excellent agreement (DICE coefficient = 0.886).

Functional outcomes in the entire cohort

Of 1,003 patients, 840 (83.7%) showed functional independence (mRS ≤ 2) at 90 days. Further, 163 participants (16.3%) had unfavorable outcomes (74 patients with mRS = 3, 47 with mRS = 4, 28 with mRS = 5, and 14 with mRS = 6). Strokes with favorable outcomes had a lower admission NIHSS score and smaller stroke volume (both $p < 0.001$). The stroke subtype showed a significant difference between the two groups ($p = 0.012$). The patients with unfavorable outcomes were less likely to receive antiplatelet therapy after discharge ($p = 0.030$) (Table 1).

Eleven texture features, including five GLRLM features, five GLSZM features, and one NGTDM feature demonstrated statistically

TABLE 1 Demographic and clinical characteristics of AIS patients with favorable and unfavorable outcome.

Characteristics	Functional outcome		*p*-value
	Favorable outcome (*n*=840)	Unfavorable outcome (*n*=163)	
Age, y	66.02 ± 12.21	65.33 ± 13.57	0.516
Men, *n* (%)	578 (68.8%)	104 (63.8%)	0.210
Smoking, *n* (%)	318 (37.9%)	60 (36.8%)	0.801
Drinking, *n* (%)	114 (13.6%)	20 (12.3%)	0.655
Hypertension, *n* (%)	563 (67.0%)	113 (69.3%)	0.566
Hyperlipidemia, *n* (%)	229 (27.3%)	48 (29.4%)	0.568
Diabetes Mellitus, *n* (%)	292 (34.8%)	58 (35.6%)	0.841
Atrial fibrillation, *n* (%)	92 (11.0%)	21 (12.9%)	0.475
Stroke subtype (TOAST), *n* (%)			**0.012**
LAA	445 (53.0%)	99 (60.7%)	
CE	66 (7.9%)	14 (8.6%)	
SAO	262 (31.2%)	36 (22.1%)	
Other	7 (0.8%)	6 (3.7%)	
Undetermined	60 (7.1%)	8 (4.9%)	
Discharge statin, *n* (%)	526 (62.6%)	108 (66.3%)	0.378
Discharge antiplatelet, *n* (%)	759 (90.4%)	138 (84.7%)	**0.030**
Discharge anticoagulant, *n* (%)	39 (4.6%)	6 (3.7%)	0.587
LDL-C mmol/L	3.01 ± 0.93	3.09 ± 1.13	0.314
Admission NIHSS score	3 (1–4)	4 (2–7)	**<0.001**
Stroke volume, ml	1.68 (0.70–7.12)	2.35 (0.78–13.41)	**<0.001**

LAA, large artery atherosclerosis; CE, cardioembolism; SAO, small artery occlusion; HbA1c, glycosylated hemoglobin; LDL-C, low-density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale. The bold values indicate the value of p less than 0.05.

TABLE 2 Texture features analysis ($p<0.05$) in AIS patients with and without favorable outcome.

Method	Texture features	*p*-value
GLRLM (gray-level run-length matrix)	Short run emphasis (SRE)	0.008
	Long run emphasis (LRE)	0.006
	Gray-level nonuniformity (GLN)	<0.001
	Run-length nonuniformity (RLN)	<0.001
	Run percentage (RP)	0.007
GLSZM (gray-level size zone matrix)	Gray-level nonuniformity (GLN)	<0.001
	Zone-size nonuniformity (ZSN)	<0.001
	Zone percentage (ZP)	0.003
	High gray-level zone emphasis (HGZE)	0.043
	Large zone low gray-level emphasis (LZLGE)	0.004
NGTDM (neighborhood gray-tone difference matrix)	Busyness	<0.001

significant differences. The information of the 11 features is provided in Table 2.

Based on RNN, all the clinical models and texture models were constructed, the clinical characteristics and texture feature selection was shown in the Supplementary Table S2. According to the above clinical characteristics and texture features, the combined model was constructed. In the validation set, the area under the ROC curve (AUC) of the combined prediction model was 0.78, and the accuracy, sensitivity, and specificity were 0.81, 0.74, and 0.82, respectively (Table 3). The AUC of the texture model was 0.77. The model using only the clinical characteristics achieved a low AUC of 0.56 in the validation cohort (Figure 3A).

Functional outcomes in the large artery atherosclerosis type

A summary of the demographic and clinical features of the patients with large artery atherosclerosis (LAA) having different clinical outcomes is shown in Table 4. The patients resulting in favorable 90-day outcomes had a lower NIHSS score at admission and smaller stroke volume than those with unfavorable outcomes (both $p < 0.001$). The proportion of statin after discharge in the unfavorable-outcome group was higher than that in the favorable group ($p = 0.045$).

Thirteen texture features, including five GLRLM features, seven GLSZM features, and one NGTDM feature demonstrated statistically significant differences. The information of the 13 features is provided in Table 5.

The clinical model, including stroke volume, NIHSS score, discharge statin, discharge anticoagulant and LDL-C, exhibited an AUC of 0.58 with accuracy, sensitivity and specificity of 0.61, 0.55 and 0.61, respectively, in the validation cohort. The effectiveness of clinical model was lower than the texture model (AUC: 0.80) and the combined model (AUC: 0.80) (Table 3 and Figure 3B).

Functional outcomes in the small artery occlusion type

A total of 298 patients, including 262 with favorable outcomes and 36 with unfavorable outcomes, were classified into the small artery occlusion (SAO) type. No significant differences were found in demographic and clinical data (Table 4).

Three GLCM texture features were significantly different in these two outcome groups. GLCM dissimilarity ($p = 0.022$) and contrast ($p = 0.022$) were higher in the unfavorable-outcome group, whereas homogeneity was higher in the favorable-outcome group ($p = 0.048$) (Figure 4).

In the validation set, the AUC of clinical-texture model was 0.81, and the accuracy, sensitivity and specificity were 0.84, 0.75, and 0.84, respectively. The texture model showed an AUC of 0.74 with accuracy, sensitivity and specificity of 0.84, 0.67, and 0.85, while the AUC of clinical model was 0.64, and the accuracy, sensitivity and specificity were 0.79, 0.50, and 0.81 (Table 3 and Figure 3C).

TABLE 3 The performance of the prediction models.

	Total AIS			LAA subtype			SAO subtype		
	Clinical	Texture	Combined	Clinical	Texture	Combined	Clinical	Texture	Combined
AUC	0.56	0.77	0.78	0.58	0.80	0.80	0.64	0.74	0.81
Accuracy	0.69	0.78	0.81	0.61	0.82	0.83	0.79	0.84	0.84
Sensitivity	0.41	0.61	0.74	0.55	0.70	0.79	0.50	0.67	0.75
Specificity	0.71	0.79	0.82	0.61	0.83	0.83	0.81	0.85	0.84

AUC, area under curve; AIS, acute ischemic stroke; LAA, large artery atherosclerosis; CE, cardioembolism; SAO, small artery occlusion.

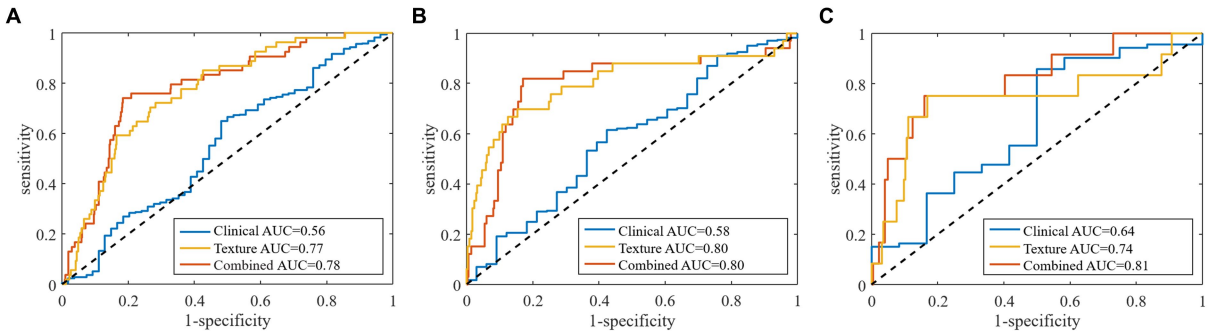

FIGURE 3
Receiver operator characteristic curves for **(A)** total stroke, **(B)** large artery atherosclerosis type, and **(C)** small artery occlusion type by clinical characteristics, texture features and combined models in predicting of stroke outcomes.

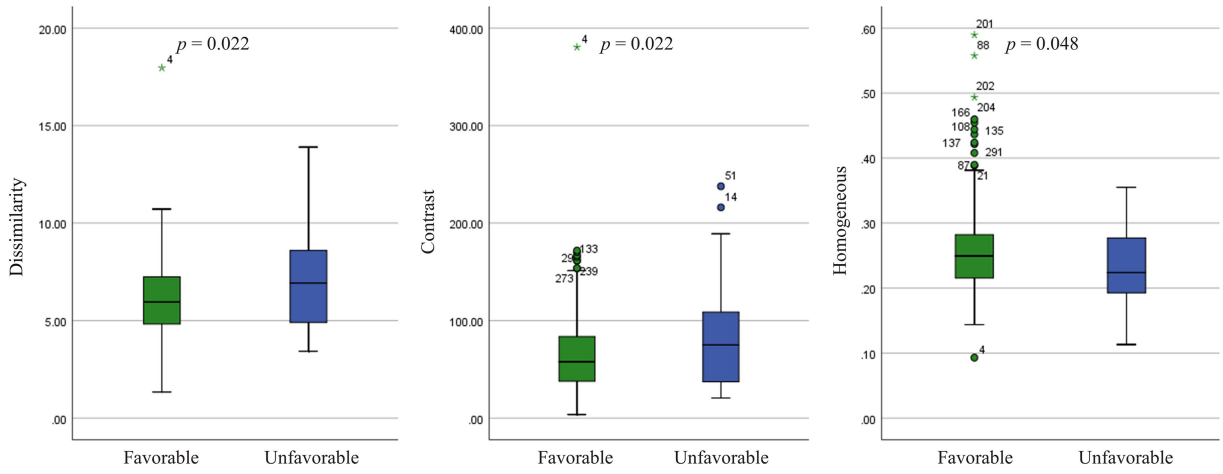

FIGURE 4
Texture features analysis (p<0.05) in the small artery occlusion type between favorable-outcome and unfavorable-outcome groups.

Discussion

In this study, we developed new models that could predict 90-day functional outcomes in patients with AIS. Our findings indicated that the second-order texture characteristics reflected the heterogeneity of stroke lesions. The predictive models of LAA and SAO stroke outcomes had moderate sensitivity and specificity. We demonstrated that the texture feature profiles differed between LAA and SAO subtypes.

TA features proved to be efficient in describing the voxel inter-relationships and the gray-level distributions within images, allowing the quantification of the intrinsic heterogeneity invisible to the naked eye. We extracted the second-order features of texture features, which consisted of GLCM, GLRLM, GLSZM, and NGTDM. The GLCM features quantified the relationship between gray levels by counting the pairs with predefined distance and direction that had the same distribution of gray-level values (24). The GLRLM features quantified the length and number of consecutive voxels that had the same gray-level value (25). The GLSZM features quantified the number of connected voxels that shared the same gray-level intensity (26). The NGTDM features quantified the difference between a gray value and the average gray value of its neighbors within the predefined distance (27). The significant differences in second-order

TABLE 4 Demographic and clinical characteristics in LAA and SAO strokes with favorable and unfavorable outcome.

Characteristics	LAA		*p*-value	SAO		*p*-value
	Favorable outcome (*n*=445)	Unfavorable outcome (*n*=99)		Favorable outcome (*n*=262)	Unfavorable outcome (*n*=36)	
Age, y	66.17 ± 12.19	65.21 ± 12.59	0.483	64.15 ± 12.03	66.06 ± 13.88	0.383
Men, *n* (%)	295 (66.3%)	68 (68.7%)	0.647	194 (74.0%)	22 (61.1%)	0.103
Smoking, *n* (%)	154 (34.6%)	39 (39.4%)	0.368	111 (42.4%)	13 (36.1%)	0.475
Drinking, *n* (%)	59 (13.3%)	15 (15.2%)	0.619	44 (16.8%)	4 (11.1%)	0.553
Hypertension, *n* (%)	292 (65.6%)	74 (74.7%)	0.080	180 (68.7%)	20 (55.6%)	0.115
Hyperlipidemia, *n* (%)	125 (28.1%)	34 (34.3%)	0.216	65 (24.8%)	8 (22.2%)	0.735
Diabetes Mellitus, *n* (%)	140 (31.5%)	35 (35.4%)	0.453	99 (37.8%)	14 (38.9%)	0.898
Atrial fibrillation, *n* (%)	36 (8.1%)	11 (11.1%)	0.333	5 (1.9%)	1 (2.8%)	0.740
Discharge statin, *n* (%)	276 (62.0%)	72 (72.7%)	**0.045**	164 (62.6%)	21 (58.3%)	0.621
Discharge antiplatelet, *n* (%)	411 (92.4%)	86 (86.9%)	0.79	248 (94.7%)	33 (91.7%)	0.732
Discharge anticoagulant, *n* (%)	17 (3.8%)	3 (3.0%)	0.934	2 (0.8%)	0 (0.0%)	1.000
LDL-C, mmol/L	3.05 ± 0.96	3.15 ± 1.24	0.337	2.99 ± 0.86	3.01 ± 0.90	0.895
Admission NIHSS score	3 (2–5)	4 (3–8)	**<0.001**	2 (1–3)	2 (1–3)	0.343
Stroke volume, ml	3.68 (1.41–13.40)	6.19 (1.59–15.02)	**<0.001**	0.56 (0.30–0.93)	0.41 (0.27–0.64)	0.198

LAA, large artery atherosclerosis; SAO, small artery occlusion; HbA1c, glycosylated hemoglobin; LDL-C, low-density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale. The bold values indicate the value of p less than 0.05.

TABLE 5 Texture features analysis ($p<0.05$) in the large artery atherosclerosis type between favorable-outcome and unfavorable-outcome groups.

Method	Texture features	*p*-value
GLRLM (gray-level run-length matrix)	Short run emphasis (SRE)	0.041
	Long run emphasis (LRE)	0.049
	Gray-level nonuniformity (GLN)	<0.001
	Run-length nonuniformity (RLN)	<0.001
	Run percentage (RP)	0.040
GLSZM (gray-level size zone matrix)	Large zone emphasis (LZE)	<0.001
	Gray-level nonuniformity (GLN)	<0.001
	Zone-size nonuniformity (ZSN)	<0.001
	Zone percentage (ZP)	0.005
	High gray-level zone emphasis (HGZE)	0.022
	Large zone low gray-level emphasis (LZLGE)	0.008
	Large zone high gray-level emphasis (LZHGE)	<0.001
NGTDM (neighborhood gray-tone difference matrix)	Busyness	<0.001

texture features might suggest that the spatial inter-relationship between adjacent voxels in patients with AIS having functional independence was different from that in patients with disability.

The pathophysiological mechanisms underlying ischemic stroke termed the ischemic cascade, which consisted of the formation of reactive oxygen species, release of glutamate, accumulation of intracellular calcium, and induction of inflammatory processes. A previous study showed that the texture features based on DWI were closely related to edema after cerebral infarction (28). Electrico-physiological phenomena such as cortical spreading depolarisations with associated energy failure and altered intracellular calcium concentration particularly from cells of the neuromuscular unit resulting into further neuronal cell injury, blood–brain barrier (BBB) break-down and related changes of the microstructures and thereby of the ADC maps and other radionics (29, 30). TA has been useful in examining subtle BBB leakage and inflammatory process after brain ischemia (14, 31). However, the relationship between TA features and pathological changes of stroke is unclear. We speculated that the complex pathophysiological processes of stroke might lead to the microstructural changes, which could be reflected in texture features.

A recent study found that the radiomics signatures based on ADC maps were associated with unfavorable outcomes and served as a risk factor (32). The radiomics features of computed tomography reflected the heterogeneity of stroke infarction and had good performance in predicting patient prognosis (33–35). One recent study also confirmed that a clinical-radiomics nomogram from DWI was a good predictor for ischemic stroke prognosis (36). Our study also demonstrated a positive signal, indicating the ADC-based texture analysis could be a useful tool in predicting stroke prognosis.

In a previous study, several texture features, principally the GLRLM features, differed between patients with AIS undergoing mechanical thrombectomy with good versus bad outcomes (37). Although the findings were partially similar, our study highlighted the differences in texture feature categories by the stroke subtype. We found that adjacent voxel relationships of images had higher dissimilarity and contrast and less homogeneity in unfavorable-outcome patients with SAO than those in favorable-outcome patients, which were diametrically contradictory to previous results (37). These implied that the brain tissue textures on infarction lesions with bad outcomes might be more complex and heterogeneous than those on lesions with good outcomes. Similar results

were obtained in previous studies of chronic ischemic stroke (38). Furthermore, we showed that five GLRLM features, seven GLSZM features, and one NGTDM feature were associated with stroke outcomes in LAA, whereas only three GLCM features were associated with stroke outcomes in SAO. One possible explanation could be ascribed to the different pathogenic mechanisms of ischemic stroke. TA has been used to automatically differentiate lacunar syndrome and partial or total anterior circulation stroke based on MRI images (12). The occluded arteries in lacunar infarcts were end arteries, which was in contrast to the large cerebral artery disease; no collaterals were formed with the adjacent vascular territories. The findings of such specific subtypes might support the concept of a different underlying etiologic disease process.

As expected, a larger volume of infarct lesions and increasing stroke severity in terms of the admission NIHSS score were associated with unfavorable outcomes in both the entire cohort and the LAA subtype. This finding was in accordance with the previous literature (39). The reason why no difference was found in age and sex between two groups was probably that the majority of enrolled patients had a good prognosis. The clinical characteristics in SAO did not correlate with the clinical outcomes was likely because the patients with SAO tended to have smaller infarct volumes and mild clinical symptoms.

This study had several limitations. First, the retrospective data might lead to selection bias. Second, the clinical symptoms of patients in this cohort were relatively mild, and a large proportion of patients had a good prognosis. We used deep learning algorithms to tackle the imbalance of data distribution and hence improve the performance of our predictive models. Third, considering our small study population, we did not analyze the textures of the other three subtypes of TOAST. Fourth, we included patients with AIS, however, we did not analyze whether different therapies vary different texture features. Further research is still needed in the future. Fifth, other relevant factors were not accounted for, such as hemorrhagic transformation or white matter hyperintensities, which were previously linked to texture features (40, 41). Future work should contemplate enlarging the sample size, finding TA features relevant to each stroke subtype, and demonstrating the robustness of these results in a prospective randomized multicenter study.

In conclusion, the TA base on ADC maps showed potential value in predicting the prognosis of patients with AIS. TA features differed in LAA and SAO stroke subtypes. Combined with clinical characteristics, TA could be used to improve the efficacy for predicting the functional outcomes in AIS.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the institutional ethics committee of Minhang Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

HW: conceptualization. YS: methodology and writing – original draft preparation. BS: formal analysis. JZ: investigation. YZ: project administration. HW: writing – review and editing. YS and HW: funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1132318/full#supplementary-material

References

1. Collaborators GBDS. Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the global burden of disease study 2019. *Lancet Neurol.* (2021) 20:795–820. doi: 10.1016/S1474-4422(21)00252-0

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

3. Feigin VL, Brainin M, Norrving B, Martins S, Sacco RL, Hacke W, et al. World stroke organization (WSO): global stroke fact sheet 2022. *Int J Stroke.* (2022) 17:18–29. doi: 10.1177/17474930211065917

4. Meschia JF, Brott T. Ischaemic stroke. *Eur J Neurol.* (2018) 25:35–40. doi: 10.1111/ene.13409

5. Ornello R, Degan D, Tiseo C, Di Carmine C, Perciballi L, Pistoia F, et al. Distribution and temporal trends from 1993 to 2015 of ischemic stroke subtypes: a systematic review and Meta-analysis. *Stroke.* (2018) 49:814–9. doi: 10.1161/STROKEAHA.117.020031

6. Caruana G, Pessini LM, Cannella R, Salvaggio G, de Barros A, Salerno A, et al. Texture analysis in susceptibility-weighted imaging may be useful to differentiate acute

from chronic multiple sclerosis lesions. *Eur Radiol*. (2020) 30:6348–56. doi: 10.1007/s00330-020-06995-3

7. Harrison LC, Raunio M, Holli KK, Luukkaala T, Savio S, Elovaara I, et al. MRI texture analysis in multiple sclerosis: toward a clinical analysis protocol. *Acad Radiol*. (2010) 17:696–707. doi: 10.1016/j.acra.2010.01.005

8. Zhang Y, Moore GR, Laule C, Bjarnason TA, Kozlowski P, Traboulsee A, et al. Pathological correlates of magnetic resonance imaging texture heterogeneity in multiple sclerosis. *Ann Neurol*. (2013) 74:91–9. doi: 10.1002/ana.23867

9. Tozer DJ, Zeestraten E, Lawrence AJ, Barrick TR, Markus HS. Texture analysis of T1-weighted and fluid-attenuated inversion recovery images detects abnormalities that correlate with cognitive decline in small vessel disease. *Stroke*. (2018) 49:1656–61. doi: 10.1161/STROKEAHA.117.019970

10. Tak K, Lee S, Choi E, Suh SW, Oh DJ, Moon W, et al. Magnetic resonance imaging texture of medial Pulvinar in dementia with Lewy bodies. *Dement Geriatr Cogn Disord*. (2020) 49:8–15. doi: 10.1159/000506798

11. Ortiz-Ramon R, Valdes Hernandez MDC, Gonzalez-Castro V, Makin S, Armitage PA, Aribisala BS, et al. Identification of the presence of ischaemic stroke lesions by means of texture analysis on brain magnetic resonance images. *Comput Med Imaging Graph*. (2019) 74:12–24. doi: 10.1016/j.compmedimag.2019.02.006

12. Faust O, En Wei Koh J, Jahmunah V, Sabut S, Ciaccio EJ, Majid A, et al. Fusion of higher order spectra and texture extraction methods for automated stroke severity classification with MRI images. *Int J Environ Res Public Health*. (2021) 18:8059. doi: 10.3390/ijerph18158059

13. Betrouni N, Yasmina M, Bombois S, Petrault M, Dondaine T, Lachaud C, et al. Texture features of magnetic resonance images: an early marker of post-stroke cognitive impairment. *Transl Stroke Res*. (2020) 11:643–52. doi: 10.1007/s12975-019-00746-3

14. Chen G, Strzelecki M, Pang Q, Kim H, Stodkilde-Jorgensen H. Textures in magnetic resonance images of the ischemic rat brain treated with an anti-inflammatory agent. *Clin Imaging*. (2010) 34:7–13. doi: 10.1016/j.clinimag.2009.02.004

15. Hjort N, Christensen S, Solling C, Ashkanian M, Wu O, Rohl L, et al. Ischemic injury detected by diffusion imaging 11 minutes after stroke. *Ann Neurol*. (2005) 58:462–5. doi: 10.1002/ana.20595

16. Wang H, Lin J, Zheng L, Zhao J, Song B, Dai Y. Texture analysis based on ADC maps and T2-FLAIR images for the assessment of the severity and prognosis of ischaemic stroke. *Clin Imaging*. (2020) 67:152–9. doi: 10.1016/j.clinimag.2020.06.013

17. Rosso C, Colliot O, Pires C, Delmaire C, Valabregue R, Crozier S, et al. Early ADC changes in motor structures predict outcome of acute stroke better than lesion volume. *J Neuroradiol*. (2011) 38:105–12. doi: 10.1016/j.neurad.2010.05.001

18. Zhou Y, Wu D, Yan S, Xie Y, Zhang S, Lv W, et al. Feasibility of a clinical-Radiomics model to predict the outcomes of acute ischemic stroke. *Korean J Radiol*. (2022) 23:811–20. doi: 10.3348/kjr.2022.0160

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

20. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Correction to: 2018 guidelines for the early Management of Patients with Acute Ischemic Stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. (2018) 49:233–4. doi: 10.1161/STR.0000000000000163

21. Norman B, Pedoia V, Majumdar S. Use of 2D U-net convolutional neural networks for automated cartilage and Meniscus segmentation of knee MR imaging data to determine relaxometry and morphometry. *Radiology*. (2018) 288:177–85. doi: 10.1148/radiol.2018172322

22. Wu G, Chen Y, Wang Y, Yu J, Lv X, Ju X, et al. Sparse representation-based Radiomics for the diagnosis of brain tumors. *IEEE Trans Med Imaging*. (2018) 37:893–905. doi: 10.1109/TMI.2017.2776967

23. Sherstinsky A. Fundamentals of recurrent neural network (RNN) and long short-term memory (LSTM) network. *Physica D Nonlinear Phenomena*. (2020) 404:132306. doi: 10.1016/j.physd.2019.132306

24. Haralick RM, Shanmugam K, Dinstein IH. Textural features for image classification. *IEEE Trans Syst Man Cybern*. (1973) SMC-3:610–21. doi: 10.1109/TSMC.1973.4309314

25. Galloway MM. Texture analysis using gray level run lengths. *Comput Graph Image Process*. (1975) 4:172–9. doi: 10.1016/S0146-664X(75)80008-6

26. Thibault G, Angulo J, Meyer F. Advanced statistical matrices for texture characterization: application to cell classification. *IEEE Trans Biomed Eng*. (2014) 61:630–7. doi: 10.1109/TBME.2013.2284600

27. Amadasun M, King R. Textural features corresponding to textural properties. *IEEE Trans Syst Man Cybern*. (1989) 19:1264–74. doi: 10.1109/21.44046

28. Jiang L, Zhang C, Wang S, Ai Z, Shen T, Zhang H, et al. MRI Radiomics features from infarction and cerebrospinal fluid for prediction of cerebral edema after acute ischemic stroke. *Front Aging Neurosci*. (2022) 14:782036. doi: 10.3389/fnagi.2022.782036

29. Dohmen C, Sakowitz OW, Fabricius M, Bosche B, Reithmeier T, Ernestus RI, et al. Spreading depolarizations occur in human ischemic stroke with high incidence. *Ann Neurol*. (2008) 63:720–8. doi: 10.1002/ana.21390

30. Lauritzen M, Dreier JP, Fabricius M, Hartings JA, Graf R, Strong AJ. Clinical relevance of cortical spreading depression in neurological disorders: migraine, malignant stroke, subarachnoid and intracranial hemorrhage, and traumatic brain injury. *J Cereb Blood Flow Metab*. (2011) 31:17–35. doi: 10.1038/jcbfm.2010.191

31. Valdes Hernandez MDC, Gonzalez-Castro V, Chappell FM, Sakka E, Makin S, Armitage PA, et al. Application of texture analysis to study small vessel disease and blood-brain barrier integrity. *Front Neurol*. (2017) 8:327. doi: 10.3389/fneur.2017.00327

32. Quan G, Ban R, Ren JL, Liu Y, Wang W, Dai S, et al. FLAIR and ADC image-based Radiomics features as predictive biomarkers of unfavorable outcome in patients with acute ischemic stroke. *Front Neurosci*. (2021) 15:730879. doi: 10.3389/fnins.2021.730879

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

34. Ramos LA, van Os H, Hilbert A, Olabarriaga SD, van der Lugt A, Roos Y, et al. Combination of radiological and clinical baseline data for outcome prediction of patients with an acute ischemic stroke. *Front Neurol*. (2022) 13:809343. doi: 10.3389/fneur.2022.809343

35. Wen X, Li Y, He X, Xu Y, Shu Z, Hu X, et al. Prediction of malignant acute middle cerebral artery infarction via computed tomography Radiomics. *Front Neurosci*. (2020) 14:708. doi: 10.3389/fnins.2020.00708

36. Wang H, Sun Y, Ge Y, Wu PY, Lin J, Zhao J, et al. A clinical-Radiomics nomogram for functional outcome predictions in ischemic stroke. *Neurol Ther*. (2021) 10:819–32. doi: 10.1007/s40120-021-00263-2

37. Sarioglu O, Sarioglu FC, Capar AE, Sokmez DFB, Topkaya P, Belet U. The role of CT texture analysis in predicting the clinical outcomes of acute ischemic stroke patients undergoing mechanical thrombectomy. *Eur Radiol*. (2021) 31:6105–15. doi: 10.1007/s00330-021-07720-4

38. Sikio M, Kolhi P, Ryymin P, Eskola HJ, Dastidar P. MRI texture analysis and diffusion tensor imaging in chronic right hemisphere ischemic stroke. *J Neuroimaging*. (2015) 25:614–9. doi: 10.1111/jon.12185

39. Griessenauer CJ, McPherson D, Berger A, Cuiper P, Sofoluke N, Adams MD, et al. Effects of white matter Hyperintensities on 90-day functional outcome after large vessel and non-large vessel stroke. *Cerebrovasc Dis*. (2020) 49:419–26. doi: 10.1159/000509071

40. Kassner A, Liu F, Thornhill RE, Tomlinson G, Mikulis DJ. Prediction of hemorrhagic transformation in acute ischemic stroke using texture analysis of postcontrast T1-weighted MR images. *J Magn Reson Imaging*. (2009) 30:933–41. doi: 10.1002/jmri.21940

41. Bretzner M, Bonkhoff AK, Schirmer MD, Hong S, Dalca AV, Donahue KL, et al. MRI Radiomic signature of white matter Hyperintensities is associated with clinical phenotypes. *Front Neurosci*. (2021) 15:691244. doi: 10.3389/fnins.2021.691244

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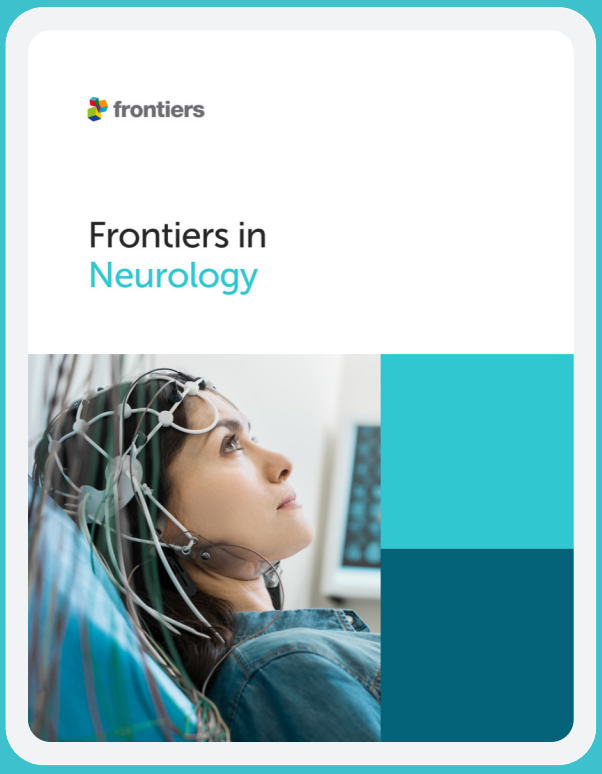

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