Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine

Research projects involving human subjects

(Applicable to prospective studies)

Study Title:	Antithrombotic Therapy with Regulation of Blood Pressure in Non-Cardioembolic Progressive Stroke
Protocol Number:	2024213
Principal Investigator:	Longxuan Li
Department :	Neurology
Study Period :	August 1, 2024 - July 31, 2025

Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine

August 5, 2024

Version Number: 1.2

1. Research Summary

1.1 Summary

Study Title: Antithrombotic Therapy with Regulation of Blood Pressure in Non-Cardioembolic Progressive Stroke

Study Overview: Stroke has become the leading cause of death in China, with acute ischemic stroke still progressing within one week of onset, known as progressive ischemic stroke (PIS), which has a high rate of disability and mortality, accounting for 23-43% of the incidence of stroke. Non-cardioembolic PIS is one of the common types, and the current treatment mainly focuses on antithrombotic therapy, but the therapeutic effect is not satisfactory. More and more evidence suggests that hypotension is an unfavorable factor for PIS, so this study intends to explore the efficacy and safety of antithrombotic therapy with regulation of blood pressure in non-cardioembolic PIS.

Study Objective: To explore the efficacy and safety of antithrombotic therapy with regulation of blood pressure in non-cardioembolic PIS.

Study Population: The study plans to enroll 70 patients with PIS. All participants will be Han Chinese, aged 18 years or older. Gender and age will be statistical data after enrollment. Patients will participate in the study after informed consent.

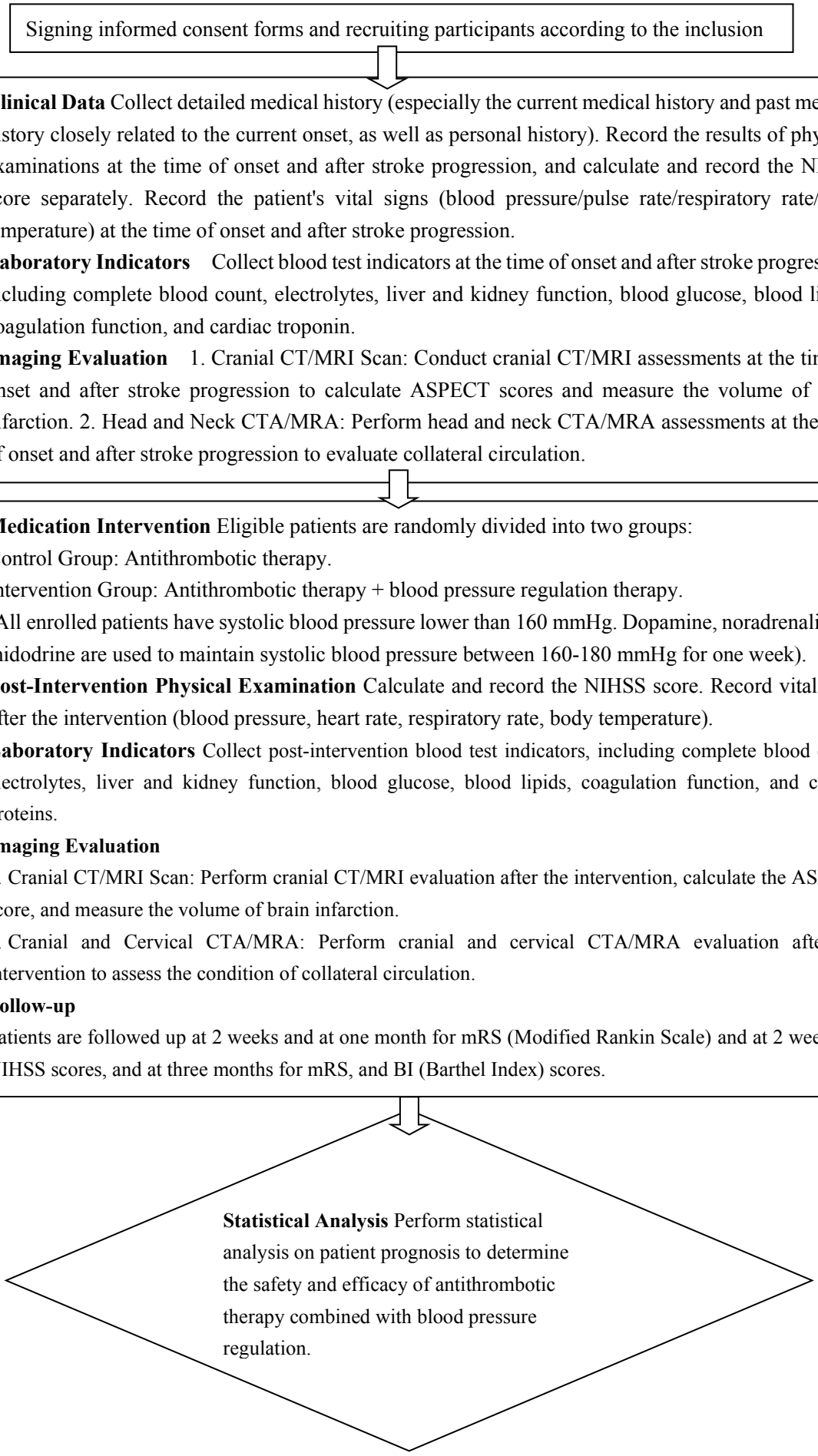
Study Site/Location: Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine

Study Interventions : Patients who meet the inclusion and exclusion criteria will be randomly assigned in a 1:1 ratio to the control group (antithrombotic therapy) or the intervention group (antithrombotic + blood pressure control therapy). In addition to antithrombotic therapy, the intervention group will use medications such as dopamine, metaraminol, or midodrine to control systolic blood pressure within the range of 160-180 mmHg and maintain it for one week.

Study duration : August 1, 2024 - July 31, 2025

Participant Duration: The duration of participation will depend on the patient's condition, with the typical treatment cycle being 2 weeks.

1.2 Technology Roadmap



2. Research background

2.1 Research significance

This study aims to explore the safety and efficacy of combined antithrombotic and blood pressure control therapy in treating non-cardioembolic progressive ischemic stroke（PIS）. By doing so, it seeks to improve the management of non-cardioembolic PIS, enhance patients' quality of life, reduce the medical burden on patients and their families, and promote related research and development. The study addresses critical issues related to the standardization and personalization of treatment for stroke, which are vital to public health.

2.2 Research Background

Acute stroke is one of the most common diseases in China, characterized by high disability and mortality rates. Ischemic stroke（IS）accounts for 60% to 80% of all strokes, with non-cardioembolic PIS being a common subtype and a major cause of disability and death in IS cases. The current treatment regimen for non-cardioembolic PIS includes oral antiplatelet agents (such as aspirin, clopidogrel), statins, and traditional Chinese medicines that promote blood circulation. However, these treatments have shown limited effectiveness. The ENCHANTED2/MT study indicated that for patients with acute large vessel occlusive IS, standard blood pressure management (140-180 mmHg) results in better clinical outcomes than intensive blood pressure management (<120 mmHg) (*Lancet, 2022 Nov 5;400(10363):1585-1596*). According to the "Chinese Guidelines for the Prevention and Treatment of Cerebrovascular Diseases 2005 Edition," when blood pressure exceeds 200/110 mmHg, cautious and stable blood pressure reduction is advised while lowering intracranial pressure, maintaining blood pressure slightly above pre-onset levels or at 180/105 mmHg. No antihypertensive treatment is needed for blood pressure ≤165/95 mmHg, and for excessively low blood pressure, an increase is recommended to ensure adequate cerebral perfusion. Since low perfusion is a key mechanism in IS, we propose that combined antithrombotic and blood pressure control (targeting 160-180 mmHg) may be more effective in treating non-cardioembolic PIS. This study aims to confirm the efficacy and safety of this combined treatment approach, thereby improving the treatment, and management of non-cardioembolic PIS and reducing the burden on patients and their families.

2.3 Expected outcomes of the study

The study aims to clarify the efficacy and safety of combined antithrombotic and blood pressure control therapy in treating non-cardioembolic PIS. The findings are expected to be published in 1-2 SCI-indexed journals.

2.4 Risk/Benefit Assessment

2.4.1 Known potential risks

Patients with non-cardioembolic PIS undergoing combined antithrombotic and blood pressure control therapy may face the following risks:

1）Bleeding Risks: Patients with large infarct areas may experience hemorrhagic transformation at the infarct site, new intracerebral hemorrhage, visceral bleeding, gum bleeding, or bleeding from the skin and mucous membranes during treatment with antithrombotic drugs combined with blood pressure control.

2）Hypertension-Related Complications: The requirement to maintain blood pressure around 160-180 mmHg for one week, along with antithrombotic therapy, may increase the risk of new intracerebral hemorrhage and other hypertension-related damages, such as hypertensive encephalopathy causing headaches and dizziness, cardiovascular damage leading to increased risks of heart disease, myocardial infarction, heart failure, and coronary artery disease, kidney damage, and an increased risk of aneurysm (blood vessel wall ballooning).

2.4.2 Known potential benefits

1) Antithrombotic Drugs: These drugs can benefit stroke patients by preventing clot formation.
2) Blood Pressure Control: The study's blood pressure control protocol aims to enhance cerebral perfusion in stroke patients, aiding in the recovery from cerebral infarction. Additionally, to mitigate the risk of new intracerebral hemorrhage from combined antithrombotic and elevated blood pressure therapy, insurance will be provided for participants before enrollment.
3) Educational Benefits: Participants will receive comprehensive education on stroke, which can improve treatment adherence and benefit long-term stroke prevention.

2.4.2 Potential Risk/Benefit Assessment

We will first determine the suitability of patients for enrollment based on their medical condition, carefully assessing clinical symptoms and various indicators to ensure that the benefits outweigh the risks. Additionally, we will prepare contingency plans in advance to address potential risks, ensuring patient safety during the implementation of the treatment protocol.

3. Information of the principal investigator

3.1 Name, qualifications and contact information of the principal investigator

Longxuan Li , male, chief physician, professor, doctoral supervisor, Ruijin Hospital affiliated to Shanghai Jiaotong University, 13611649930

Serial number	Name	gender	age	job title	specialty	Whether accepted GCP training?	Roles in the research (eg. PI, sub-I, CRC)
1	Longxuan Li	male	52	Chief Physician	Neurology	yes	PI
2	Yi Zhang	male	33	Attending Physician	Neurology	yes	sub-I
3	Dou Yin	male	38	Deputy Chief Physician	Neurology	yes	sub-I
4	Pingchen Zhang	female	29	Resident Physician	Neurology	yes	CRC

4. Research objectives

The main purpose of this study is to explore the efficacy and safety of antithrombotic therapy combined with blood pressure regulation in the treatment of non-cardioembolic PIS.

5. Study Design

- Obtain informed consent and recruit subjects according to the inclusion and exclusion criteria.
- Clinical Data

(1) Collect detailed medical history, especially focusing on the current illness, past medical history, and personal history closely related to the onset of the disease.

(2) Record physical examination results at the time of onset and after stroke progression, and calculate and document the NIHSS scores.

(3) Document vital signs (blood pressure, pulse rate, respiratory rate, temperature) at onset and after stroke progression.

- Laboratory Indicators

Collect blood test indicators at onset and after stroke progression, including complete blood count, electrolytes, liver and kidney function, blood glucose, blood lipids, coagulation function, and cardiac proteins.

- Imaging Assessment

(1) Head CT/MRI: Perform head CT/MRI at onset and after stroke progression, and calculate ASPECTS scores and infarct volume.

(2) Head and Neck CTA/MRA: Conduct head and neck CTA/MRA at onset and after stroke progression to assess collateral circulation.

- Medication Intervention

Eligible patients are randomly divided into two groups:

Control Group: Antithrombotic therapy.

Intervention Group: Antithrombotic therapy + blood pressure regulation therapy.

(All enrolled patients have systolic blood pressure lower than 160 mmHg. Dopamine, noradrenaline, or midodrine are used to maintain systolic blood pressure between 160-180 mmHg for one week).

- Post-Intervention Physical Examination

Calculate and record the NIHSS score. Record vital signs after the intervention (blood pressure, heart rate, respiratory rate, body temperature).

- Laboratory Indicators

Collect post-intervention blood test indicators, including complete blood count, electrolytes, liver and kidney function, blood glucose, blood lipids, coagulation function, and cardiac proteins.

- Imaging Evaluation

Cranial CT/MRI Scan: Perform cranial CT/MRI evaluation after the intervention, calculate the ASPECT score, and measure the volume of brain infarction.

Cranial and Cervical CTA/MRA: Perform cranial and cervical CTA/MRA evaluation after the intervention to assess the condition of collateral circulation.

- Follow-up

Patients are followed up at 2 weeks and at one month for mRS scores, and at 2 weeks for NIHSS scores, and at three months for mRS, and BI (Barthel Index) scores.

- Statistical Analysis

Perform statistical analysis on patient prognosis to determine the safety and efficacy of antithrombotic therapy combined with blood pressure control.

5.2 Defining study endpoints

The study endpoint is defined as the completion of clinical data, specimen collection and follow-up for the research subjects.

6. Patient population

6.1 Inclusion criteria

(1) Adults (aged ≥18 years) with an AIS who have been able to complete usual activities in daily life without support before the stroke;

(2) One of the following PIS manifestations:

a. Within 7 days of onset, when symptom worsens and there are new lesions or infarct growth on DWI within 24 hours of aggravation, the National Institutes of Health Stroke Scale (NIHSS) score increases by ≥ 2 points;

b. Within 24 hours after IVT, when symptom worsens and there are new lesions or infarct growth on DWI within 24 hours of aggravation, the NIHSS score increases by ≥ 4 points compared to the baseline;

(3) Within 3h of stroke progression, ≥2 successive measurements of systolic blood pressure (SBP) < 160 mm Hg for >10 min.

(4) Computed tomographic angiography (CTA), magnetic resonance angiography (MRA), or digital subtraction angiography (DSA) confirms patients without visible large or medium-sized intracranial vessel occlusion.

6.2 Exclusion criteria

(1) After stroke progression, a head CT confirmed new cerebral hemorrhage or hemorrhagic transformation.

(2) Endovascualr treatment had been performed before stroke progression (thrombectomy, stent placement, balloon dilatation) or if surgery or interventional treatment had been scheduled;

(3) Current treatment with heparin therapy or oral anticoagulation (presumed cardiac source of embolus, such as atrial fibrillation, prosthetic cardiac valve, and known or suspected endocarditis);

(4) Previous diseases of the brain that include intracranial hemorrhage or amyloid angiopathy; brain surgery or hemorrhagic stroke; stroke within the last three months;

(5) Preexisting serious diseases: Cancer, AIDS, serious heart disease, dementia, liver diseases such as liver failure, cirrhosis, portal hypertension and active hepatitis, acute or chronic severe renal impairment (glomerular filtration rate < 30 ml/min/1̸73 m^2);

(6) Contraindication to aspirin or clopidogrel;

(7) Pregnant and lactating women.

6.3 Recruitment of research subjects

The study plans to recruit patients from the Department of Neurology at the Ruijin North Hospital. Doctors will conduct interviews with patients to identify those who meet the inclusion and exclusion criteria. The study aims to select 70 patients with non-cardioembolic PIS. All participants will be from the Han ethnic group. Gender and age will be recorded and analyzed after enrollment. The study targets middle-aged and elderly individuals, and does not include pregnant women or children. Patients will participate in the study after providing informed consent.

6.4 Method of allocating research subjects

Patients with non-cardioembolic PIS are randomly assigned to the control or intervention group in a 1:1 ratio. Control group: antithrombotic therapy; Intervention group: antithrombotic + blood pressure control therapy.

7. Study Intervention

7.1 Administration of the Study Intervention

7.1.1 Description of study intervention

- Control group : antithrombotic therapy

After stroke progression, all patients receive dual antiplatelet therapy (aspirin 100mg/day combined with clopidogrel 75mg/day) for the first 21 days, except in cases of cerebral hemorrhage. After this period, they continue to take aspirin 100mg/day orally for the long term.

- Intervention group : antithrombotic + blood pressure control therapy

Antithrombotic therapy is consistent with the control group. In terms of blood pressure control, medications such as dopamine, metaraminol, or midodrine are used to achieve a systolic blood pressure target range of 160-180 mmHg within 1 h of random assignment and to maintain this target for 7 days (or death, should this event occur earlier). BP measurements are routinely captured using automated devices fitted to the unaffected arm, following the protocol recommended by the standard guideline. The readings are taken at 15-minute intervals for the initial hour, hourly from the first to the sixth hour, every six hours from 6 to 24 hours, and then twice daily for 7 days (or death, if earlier). Subsequently, these data are uploaded into the research database.

7.1.2 Dosage and administration method

- Antithrombotic drugs:

  Aspirin: 100 mg orally once daily.

  Clopidogrel: 75 mg orally once daily.

- Blood pressure medications:

  Dopamine: Dopamine injection (200 mg) mixed with 30 ml of 0.9% sodium chloride injection. Administer intravenously using a micro-infusion pump at a rate of 1-8 μg/k g/min, adjusting the pump speed based on blood pressure dynamics.

  Metaraminol: Metaraminol injection (50 mg) mixed with 45 ml of 0.9% sodium chloride injection. Administer intravenously using a micro-infusion pump at a rate of 1-3 ml/h, adjusting the pump speed based on blood pressure dynamics.

  Midodrine: Take one tablet orally three times a day.

7.1.3 Establishment, storage, unblinding and emergency unblinding of trial drug codes

not applicable

7.1.4 Items and frequency of clinical and laboratory examinations to be conducted

Clinical data collection (3 times, at onset , after stroke progression , and after drug intervention)

at the onset of disease, after stroke progression , and after drug intervention were recorded, and the NHISS scores were calculated and recorded. The vital signs (blood pressure/pulse rate/respiratory rate/body temperature) of the patients at the onset of disease, after stroke progression , and after drug intervention were recorded.

Laboratory indicators (3 times, at onset , after stroke progression , and after drug intervention)

Blood test indicators were collected at the onset, after stroke progression , and after drug intervention , including routine blood tests, electrolytes, liver and kidney function, blood sugar, blood lipids, coagulation function, and myocardial protein.

Imaging evaluation (3 times, at onset , after stroke progression , and after drug intervention)

1. Plain scan of head CT/MRI: Complete head CT/MRI evaluation at the onset of the disease and after the progression of stroke, and calculate the ASPECT score and cerebral infarction volume.

2. Head and neck CTA/MRA: Complete head and neck CTA/MRA evaluation at the onset and after stroke progression to assess collateral circulation.

7.2 Preparation/Handling/Storage/Responsibility

7.2.1 Liability

not applicable

7.2.2 Composition, appearance, packaging and labelling

not applicable

7.2.3 Product Storage and Stability

not applicable

7.2.4 Preparation

not applicable

7.3 Measures to reduce bias: randomization and blinding

This study used random allocation to reduce bias.

7.4 Follow-up and Compliance

Patients were followed up by telephone, audio and video at 2 weeks and at 1 month respectively to assess mRS scores, at 2 weeks for NIHSS scores , and at 3 months respectively to follow up mRS, and BI scores. The following physicians were unaware of the type of treatment each patient received.

7.5 Research Intervention Commitment

Instruct participants to take medication according to the content of the research plan and obtain clinical data for subsequent results analysis.

7.6 Research Plan

efficacy and safety of antithrombotic therapy with regulation of blood pressure in non-cardioembolic progressive ischemic stroke

Visit	**Screening period**	**Treatment period**		**Follow-up after termination of treatment**	**Follow-up period**	
	1	2	3		Follow-up 1	Follow-up 2
Treatment cycle (days)	1	7	7	1	3 0	9 0
Informed consent	√					
Population and Baseline	√					
Inclusion/Exclusion	√					
Various inspections ...	Blood tests, imaging examinations, NHISS score	Blood test, Imaging examination, NHISS score	Blood test, Imaging examination, NHISS score	NHISS、mRS score	mRS score	mRS、BI score

8. Discontinuation of study intervention and discontinuation/withdrawal of study subjects

8.1 Discontinuation of Study Intervention

The following adverse drug reactions occurred and the study intervention was discontinued:

1. Increased risk of bleeding: The use of antithrombotic drugs may lead to an increased risk of bleeding, including nasal bleeding, gingival bleeding, skin ecchymosis, etc.;

2. Allergic reaction: Some patients may experience allergic reaction after using antithrombotic drugs, manifested by symptoms such as rash, itching, and difficulty breathing;

3. Symptoms of hypertensive encephalopathy:

- ✓ Increased intracranial pressure: patients experience severe headache, projectile vomiting, optic disc edema, amaurosis, retinal artery spasm with flame-like hemorrhages and arterial spasm, and villous exudates ;
- ✓ Disturbance of consciousness: In severe cases, irritability, drowsiness, or even coma may occur, and mental confusion may also occur ;
- ✓ Epileptic seizures: may be generalized or localized, and some may present with status epilepticus ;
- ✓ Paroxysmal dyspnea: caused by vasospasm, ischemia and acidosis of the respiratory center ;
- ✓ Other symptoms of brain dysfunction: Focal signs of the nervous system such as aphasia and hemiplegia are rare ;
- ✓ Headache: It is often an early symptom of hypertensive encephalopathy. Most cases are full-headed headache or obvious forehead pain. The headache is obvious when coughing or exerting force, accompanied by nausea and vomiting. The headache can be relieved when blood pressure drops ;
- ✓ The main symptoms of brain edema are headache, convulsion and impaired consciousness, which is called the triad of hypertensive encephalopathy ;

8.2 Discontinuation/Withdrawal of Study Subjects

The researcher may terminate or withdraw the research subject if the research subject :

- significant nonadherence to study intervention;
- The occurrence of severe clinical adverse reactions or other clinical conditions makes continued participation in the study no longer in the best interests of the subject;

8.3 Loss to follow-up

not applicable

9. Outcome Measures

9.1 Efficacy end points

The primary efficacy end point is the percentage of patients with an excellent outcome, defined as a score of 0 or 1 on the modified Rankin scale (mRS) at 90 days after randomization. Secondary efficacy outcomes at 90 days are measured by the Barthel index (BI) and the mRS. Specifically, we measure how many patients achieved ≥ 95 for the BI, and 0-2 for the mRS. Other efficacy end points include the proportion of patients with an excellent outcome or functional independence (0-2 for the mRS) at 2 weeks

and at day 30. In addition, we measure how many patients achieved a score of 0 or 1 for the NIHSS at 2 weeks after randomization.

9.2 Safety end points

The primary safety outcome is severe or moderate bleeding as defined by the Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) criteria at 90 days. Secondary safety outcomes included any bleeding, death, adverse events, and severe adverse events through 90 days of follow-up.

9.3 Adverse events and serious adverse events

9.3.1 Definition of Adverse Event (AE)

If patients experience drug side effects and unexpected events during the study, they are defined as adverse events. Details are as follows:

1. Increased risk of bleeding: The use of antithrombotic drugs may lead to an increased risk of bleeding, including nasal bleeding, gingival bleeding, skin ecchymosis, etc.;

2. Allergic reaction: Some patients may experience allergic reaction after using antithrombotic drugs, manifested by symptoms such as rash, itching, and difficulty breathing;

3. Symptoms of hypertensive encephalopathy:

- ✓ Increased intracranial pressure: patients experience severe headache, projectile vomiting, optic disc edema, amaurosis, retinal artery spasm with flame-like hemorrhages and arterial spasm, and villous exudates ;
- ✓ Disturbance of consciousness: in severe cases, irritability, drowsiness, or even coma may occur, and mental confusion may also occur ;
- ✓ Epileptic seizures: may be generalized or localized, and some may present with status epilepticus ;
- ✓ Paroxysmal dyspnea: caused by vasospasm, ischemia and acidosis of the respiratory center ;

9.3.2 Definition of Serious Adverse Event (SAE)

If patients experience serious drug side effects and serious unexpected events during the study, they are defined as serious adverse events.

9.3.3 Classification of adverse events

9.3.3.1 Incident severity

Patients rated the severity of events based on their health status during their participation in the study.

9.3.3.2 Relevance to the study intervention

Adverse events were classified as relevant or irrelevant based on whether they were related to the study intervention;

9.3.3.3 Anticipation

After the patient is admitted to the hospital, the patient's basic health condition will be assessed in advance and safety precautions will be informed.

9.3.4 Timing, frequency , follow-up and outcomes of adverse event assessment

After the patient is admitted to the hospital, the patient's basic health condition is assessed and safety precautions are informed. If any adverse events occur during hospitalization, they are evaluated, recorded and reported in a timely manner.

9.3.5 Adverse Event Reporting

Any adverse events experienced by patients should be reported promptly to the project leader, department head, and the Ruijin Hospital Ethics Committee.

9.3.6 Serious Adverse Event Reporting

If a patient experiences serious adverse events, they should be reported promptly to the project leader, department head, and Ruijin Hospital Ethics Committee.

10. Statistical Analysis

Intention-to-treat (ITT) analysis will be used to analyze the therapeutic effects of the two groups and all the data will be analyzed with SPSS 21.0 Software. Categorical variables are described as frequencies and percentages; they are compared using a Chi-squared test or Fisher’s exact test. Continuous variables are described as the mean ±standard deviation (SD) or the median and quartile spacing (IQR); they are compared using an independent Student’s t-test or ANCOVA (analysis of covariance). Difference of the efficacy endpoints and safety endpoints will be compared using binary logistic regression. Distribution of mRS at 90 days between two groups will be compared using the

ordinal logistic regression. Change in NIHSS score between two groups will be compared using general linear model. There is statistical significance if p-value<0.05.

10.1 General Methods

SPSS 21.0 software was used for statistical analysis. All data results are presented in the form of mean ± standard deviation. For quantitative data, the t test was used to compare the two groups of samples that conformed to the normal distribution, and the Mann–Whitney U test was used for the comparison of data samples that did not conform to the normal distribution and data samples with uneven variance. $P < 0.05$ was considered statistically significant.

10.2 Analysis of primary and secondary study endpoints

- Primary outcome analysis:

The proportions of patients with excellent outcome 90 days after treatment in the two groups were compared using the chi-square test or Fisher's precision probability test.

- Secondary outcome analysis:

The proportions of patients with secondary outcomes 2 weeks, 30 days and 90 days after treatment in the two groups were compared using the chi-square test or Fisher's precision probability test.

10.3 Security Analysis

Severe or moderate bleeding as defined by the Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) criteria at 90 days, any bleeding, death, adverse events, and severe adverse events through 90 days of follow-up are recorded.

10.4 Baseline Descriptive Analysis

Descriptive statistics were used to compare the demographic characteristics and laboratory parameters at baseline among the groups.

10.5 Subgroup Analysis

The primary and secondary outcomes were analyzed according to subgroups such as large vessel disease group vs small vessel disease group.

11. Supporting Documents and Notes

11.1 Informed Consent Process

First, we explain to patients and their families the necessity of our research and the benefits it will bring to them. Our research is exploratory and can provide feedback, with traceable data sources, and the results are credible. At the same time, we will also conduct detailed physical examinations, blood tests, and imaging assessments on patients to fully understand their disease conditions. We can treat patients' current conditions and treatment complications accordingly, achieve high-quality disease management, and significantly improve patients' prognosis and quality of life.

After that, the patient was explained the necessity of informed consent for participating in the study. Informed consent is based on respect for the patient, informing the patient of the relevant content of the study and giving the patient autonomy, which has legal and medical significance. Therefore, the patient or his/her authorized representative or legal representative will be asked to sign to express consent. In view of the fact that cerebrovascular disease is a disease seen in middle-aged and elderly people, and according to the experimental principles, all participants are adults, and no minors will participate.

A sample of the informed consent form is attached.

11.2 Privacy Protection

We will choose a relatively private environment to ask questions to patients. The acquired data will be sealed, and a unified label will be used when entering data to weaken the possibility of leakage of individual patient information. The corresponding documents will be encrypted, and participants will be trained. Data collection is carried out by clinical researchers under the supervision of the person in charge, who will be responsible for the accuracy, completeness and timeliness of the reported data. All data should be clear to ensure accurate interpretation and ensure traceability. Clinical data will be stored in a database, which is password protected.

11.3 Collection and use of specimens and data

This study does not involve the collection and use of samples.

11.4 Quality Control and Quality Assurance

Two researchers will conduct the study on site, one of whom will conduct the evaluation and the other will conduct on-site monitoring and record the safety status of the subjects. After each data collection is completed, the results and the safety status of this study will be reported to the principal investigator verbally, and reported again in writing within 48 hours and filed. If an adverse event or adverse safety event occurs, the principal investigator will be notified immediately and actively handled.

After the end of each day's study, the principal investigator will review the paper records to ensure data integrity and seal them to ensure that patient information is not leaked. Within 72 hours after the participating researchers enter the data, the principal investigator and another participating researcher will review the data results.

11.5 Data Processing and Record Keeping

11.5.1 Data Collection and Management

The acquired data will be sealed, and a unified label will be used when entering data to reduce the possibility of leakage of individual patient information. The corresponding documents will be encrypted, and participants will be trained. Data collection will be carried out by clinical researchers under the supervision of the person in charge, who will be responsible for the accuracy, completeness and timeliness of the reported data. All data should be clear to ensure accurate interpretation and traceability. Clinical data will be stored in a database, which will be password protected.

11.5.2 Research Data Retention

All research data and original documents will be retained until the paper is published.

11.6 Publication and Data Sharing Agreement

not applicable

11.7 Conflict of Interest Statement

There are no conflicts of interest related to this study.

Attached research scale

mRS score

Classification Description

0 No symptoms at all

1 Despite symptoms, no significant disability; able to perform all usual duties and activities

2 Mild disability; cannot perform all activities previously performed, but can manage personal affairs without assistance

3 Moderate disability; requires some assistance but does not need assistance walking

4 Severe disability; unable to walk without assistance from others and unable to take care of own physical needs

5 Severe disability; bedridden, incontinent, requiring constant care and attention

6 Death

ASPECTS score

On the CT images, 10 regions at two levels of the middle cerebral artery (MCA) blood supply area were selected:

① The nuclear level (i.e., thalamus and striatum plane) is divided into seven areas: M1, M2, M3, insula, lentiform nucleus, caudate nucleus, and posterior limb of the internal capsule;

② Levels above the nucleus (2 cm above the level of the nucleus), including M4, M5 and M6. The boundary between the two is the head of the caudate nucleus. In the cross-sectional CT images, any ischemic changes located in the caudate nucleus and below are defined as the level of the nucleus, and ischemic changes above the level of the head of the caudate nucleus are defined as the level above the nucleus. These 10 areas have the same weight, all 1 point. When scoring, the number of areas with EIC is subtracted from 10 points. 10 points represent normal CT scans, and 0 points represent extensive ischemia in the MCA blood supply area.

pc-ASPECTS divides the posterior circulation into 10 points. Each EIC of the left and right thalamus, cerebellum, and posterior cerebral artery is subtracted by 1 point, and any EIC in the midbrain or pons is defined as a low-density lesion or gray-white blur on NCCT.

ASPECTS reduction area			
Insula		Caudate nucleus	
Lentiform nucleus		Posterior limb of internal capsule	
M1		M4	
M2		M5	

M3		M6	
Total score			

pc-ASPECTS reduction points			
Left thalamus 1		Right thalamus 1	
Left cerebellum 1		Right cerebellum 1	
Left posterior cerebral artery supply Zone 1		Left brain posterior movement Arterial blood supply area 1	
Midbrain 2		Pons 2	
Total score			

NHISS score

1a. Level of Consciousness:

Even if a full evaluation is not possible (due to tracheal intubation, language disorder, tracheal trauma, bandages, etc.), the examiner must choose one response. Only score 3 points if the patient shows no response to harmful stimuli (not reflexive).

- Awake and alert (0 points)
- Somnolent, can be awakened with mild stimulation, able to answer questions, follow commands (1 point)
- Sleepy or lethargic, requires repeated or strong stimuli to respond, non-stereotyped response (2 points)
- Comatose, only reflexive activity or spontaneous response, or no response, flaccid, no reflexes (3 points)

1b. Level of Consciousness Questions: Month, Age

Ask the patient about the current month and their age. Only the first response is scored. If the patient cannot understand the question due to aphasia or coma, score 2 points. If the patient cannot complete the response due to tracheal intubation, tracheal trauma, severe dysarthria, language disorder, or any other reason (excluding aphasia), score 1 point. The patient may respond in writing.

- Both answers correct (0 points)
- One answer correct (1 point)
- Both answers incorrect (2 points)

1c. Level of Consciousness Commands: Open/close eyes; make a fist/release on non-paralyzed side
Only the initial response is scored, even if there is clear effort but incomplete execution. If there is no response to commands, use gestures and then record the score. Appropriate commands should be given for those with trauma, amputation, or other physical defects.

- Both actions correct (0 points)
- One action correct (1 point)
- Both actions incorrect (2 points)

2. Gaze:
Only test horizontal eye movement. Score voluntary or reflexive eye movements. If skewed eye position can be corrected by voluntary or reflexive movement, score 1 point. Score 1 point for isolated peripheral eye muscle paralysis. Gaze can be tested in aphasic patients. For those with eye trauma, bandages, blindness, or other vision impairments, the examiner should choose a reflex movement to test, ensuring eye linkage, and then move the eyes from side to side, occasionally detecting partial gaze paralysis.
- Normal (0 points)
- Partial gaze palsy (abnormal gaze in one or both eyes, but no forced deviation or total gaze palsy) (1 point)
- Forced deviation or total gaze palsy (cannot be overcome by head-eye reflex) (2 points)

3. Visual Field:
Record normal if the patient can see fingers in the peripheral vision. If one eye is blind or enucleated, test the other eye. Score 1 point for definite asymmetrical blindness (including quadrantanopia). Score 3 points for total blindness (for any reason). For patients near death, score 1 point; use the result to answer question 11.

- No visual field loss (0 points)
- Partial hemianopia (1 point)
- Complete hemianopia (2 points)
- Bilateral hemianopia (including cortical blindness) (3 points)

4. Facial Palsy:

- Normal (0 points)
- Minor (flattening of the nasolabial fold, asymmetry on smiling) (1 point)
- Partial (total or near-total paralysis of the lower face) (2 points)
- Complete (paralysis of the upper and lower face on one or both sides, no movement) (3 points)

5 & 6. Motor Function of Upper and Lower Limbs:

Place the limb in the appropriate position: for the upper limb, raise to 90° when sitting or 45° when lying; for the lower limb, raise to 30° when lying. If the limb falls within 10 seconds for the upper limb or 5 seconds for the lower limb, score 1-4 points. Encourage aphasic patients with language or gestures, without using harmful stimuli. Check each limb in sequence, starting with the non-paralyzed upper limb.

- Upper Limb:
  - No drift, holds position at 90° (or 45°) for 10 seconds (0 points)
  - Can lift but not hold for 10 seconds, does not hit the bed or other support when falling (1 point)
  - Attempts to resist gravity, but cannot maintain 90° sitting or 45° supine (2 points)
  - Cannot resist gravity, limb falls quickly (3 points)
  - No movement (4 points)
  - Amputation or joint fusion, specify: 5a left upper limb; 5b right upper limb (9 points)
- Lower Limb:
  - No drift, holds position for 5 seconds (0 points)
  - Falls within 5 seconds without hitting the bed (1 point)
  - Falls to the bed within 5 seconds, can partially resist gravity (2 points)
  - Falls immediately to the bed, cannot resist gravity (3 points)
  - No movement (4 points)

- Amputation or joint fusion, specify: 6a left lower limb; 6b right lower limb (9 points)

7. Limb Ataxia:

The goal is to detect unilateral cerebellar lesions. Check with eyes open; if there is a visual deficit, ensure testing occurs in a vision-free area. Perform bilateral finger-to-nose and heel-to-shin tests, scoring when ataxia is disproportionate to weakness. Do not score if the patient cannot understand or has limb paralysis. Blind patients use extended arms to touch their nose. Amputation or joint fusion scores 9 points, with explanation.

- No ataxia (0 points)
- Ataxia in one limb (1 point)
- Ataxia in two limbs, specify: right upper limb 1=yes, 2=no (2 points)
- Amputation or joint fusion, specify: left upper limb 1=yes, 2=no (9 points)
- Amputation or joint fusion, specify: right lower limb 1=yes, 2=no (9 points)
- Amputation or joint fusion, specify: left lower limb 1=yes, 2=no (9 points)

8. Sensation:

Check for response to pinprick and expression, or withdrawal from painful stimuli in patients with consciousness impairment or aphasia. Only score sensory loss related to stroke. For hemibody sensory loss, perform a precise examination, testing multiple areas (upper limb excluding hand, lower limb, trunk, face) to confirm hemibody sensory loss. Score 2 points for severe or total sensory loss. Score 1 or 0 points for drowsy or aphasic patients. Score 2 points for bilateral sensory loss in brainstem strokes. Score 2 points for unresponsive or quadriplegic patients. Comatose patients (1a=3) score 2 points.

- Normal (0 points)
- Mild to moderate sensory loss (patient feels pinprick as less sharp or dull, or has diminished but present sensation) (1 point)
- Severe to total sensory loss (no sensation in face, upper limb, lower limb) (2 points)

9. Language:

Tests for naming and reading. If visual impairment interferes, have the patient identify objects placed in their hand, repeat, and pronounce. Intubated patients write responses. Score 3 points for comatose patients. Choose a score for confused or uncooperative patients, but only score 3 points for patients who cannot speak or follow any commands.

- Normal (0 points)
- Mild to moderate aphasia: fluency and comprehension somewhat reduced, but expression not markedly limited (1 point)
- Severe aphasia: communication via broken language (2 points)
- Mute or global aphasia: complete inability to speak or understand (3 points)

10. Dysarthria:

Test for clarity of articulation during spontaneous speech. Score 9 points for tracheal intubation or other physical barriers, with explanation. Do not tell the patient why the test is being conducted.

- Normal (0 points)
- Mild to moderate dysarthria: some slurred speech, but understandable (1 point)
- Severe dysarthria: speech unclear, not understandable, but without aphasia or disproportionate to aphasia (2 points)
- Tracheal intubation or other physical barriers, explain: (9 points)

11. Neglect:

If severe visual loss affects bilateral visual field testing, score as normal. Score as normal if the patient shows bilateral attention despite aphasia. Score as abnormal if there is evidence of visuospatial neglect or anosognosia.

- Normal (0 points)
- Neglect in vision, touch, hearing, spatial awareness, or personal space; or neglect in one sensory modality (1 point)
- Severe neglect in more than one sensory modality; unawareness of personal space; or only orienting to one side (2 points)

Eating: A score of 10 indicates the ability to eat independently, while a score of 0 indicates complete dependence on others.

Bathing: A score of 5 indicates the ability to complete the bathing process independently, while a score of 0 indicates the need for assistance.

Grooming: A score of 5 indicates the ability to independently maintain personal hygiene, while a score of 0 indicates the need for help.

Dressing: A score of 10 indicates the ability to dress independently, while a score of 0 indicates complete dependence on others.

Bowel and Bladder Control: A score of 10 indicates the ability to control independently, while a score of 0 indicates complete incontinence or dependence on a catheter.

Toileting: A score of 10 indicates the ability to complete independently, while a score of 0 indicates complete dependence on others.

Bed to Chair Transfer: A score of 15 indicates the ability to complete the transfer independently, while a score of 0 indicates complete dependence on others.

Walking on Flat Surface: A score of 15 indicates the ability to walk independently for 45 meters, while a score of 0 indicates complete dependence on others.

Stair Climbing: A score of 10 indicates the ability to complete independently, while a score of 0 indicates complete dependence on others.