Protocol

Version 2.0

Normal Saline Infusion for Stroke after Intravenous Thrombolysis

A Randomized Clinical Trial

Principle Investigators

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Study Synopsis

Title	Normal Saline Infusion for Stroke after Intravenous Thrombolysis A Randomized Clinical Trial
Principle Centre	Tianjin Medical University General Hospital
Objective	To explore the safety and efficacy of immediate abundant normal saline infusion after intravenous thrombolysis for patients with stroke.
Efficacy Outcomes	Primary outcome 1. Proportion of the patients with mRS 0-2 at 90 days after randomization. Secondary outcomes 1. Proportion of the patients with early neurological deteiroration (END, △NIHSS≥2 and △NIHSS≥4) at 24 hours after randomization. 2. Proportion of the patients with cerebrovascular events within 1 days, 7 days and 90 days after randomization. 3. Peripheral blood inflammatory indices and laboratory examination (S100-β, brain derived neurotrophic factor and myeloperoxidase). 4. NIHSS scores at 7 days. 5. mRS scores at 30 days and 90 days. 6. Barthel index scores at 30 days and 90 days.
Safety Outcomes	1. Ejection fraction within 72 hours. 2. Mortality within 90 days 3. Proportion of intracranial hemorrhage (ICH) and symptomatic ICH within 7 days. 4. Infarction volume at 24 hours after randomization. 5. Blood pressure fluctuation within 24 hours.
Trial Design	NS-STAR is a prospective, multicentre, randomised, open-label, blinded outcome reading, Phase II trial (PROBE design). Subjects included are randomly assigned into the NS group and the control group. Follow-up is performed at baseline, 24 hours, 7 days, 30 days and 90 days after randomization. The outcome assessors are blinded to the allocation

	assignment.
Trial Population	Patients with acute ischemic stroke treated by intravenous thrombolysis
Sample Size	240
Inclusion Criteria	1. Acute ischemic stroke. 2. Age 18-80 years. 3. Pre-stroke mRS≤1. 4. NIHSS score 0-25 5. Onset-to-needle time≤4.5 h. 6. Sign the informed consent.
Exclusion Criteria	1. Thrombolysis contraindication. 2. Allergy to alteplase. 3. intention to undergo emergency endovascular treatment. 4. Any terminal illness such that patients would not be expected to survive>1 year. 5. Poor compliance. 6. Participating in other clinical trials within previous 3 months.
Withdrawal Criteria	1. Acute heart failure during interventions. 2. Hypertensive crisis during interventions. 3. Edema, such as pulmonary edema, limb edema and water intoxication. 4. Other severe complications that should terminate the interventions immediately. 5. Stroke mimics. 6. Decide to undergo emergency endovascular treatment during interventions. 7. Refuse subsequent treatment and follow-up.
Trial Cycle	All included patients are followed-up at baseline, 24 hours, 7 days, 30 days and 90 days after randomization, respectively.
Treatment Regimens	Eligible patients are randomly (a ratio of 1:1) assigned into the NS group: undergoing immediate 2000 mL intravenous infusion of NS after intravenous thrombolysis at a speed of 200 mL/h, or the control group: receiving

	200-400ml of NS after intravenous thrombolysis. A standard therapy according to Chinese guidelines for diagnosis and treatment of acute ischemic stroke 2023 will be performed during the whole treatment period.
Procedure	**Before intravenous thrombolysis:** The investigators will collect demographic characteristics, medical history, blood pressure, brain imaging (either computer tomography or magnetic resonance imaging), neurological measurements (NIHSS score and mRS score), haematological examination (blood routine, creatinine, urea, brain natriuretic peptide, etc.), laboratory examination (S100-β, brain derived neurotrophic factor and myeloperoxidase) and other pertinent information. **After intravenous thrombolysis:** The informed consent will obtained and patients will be randomly assigned into the NS group and the control group. The investigators will then collect blood pressure and neurological measurements (NIHSS score). **24-48 hours after randomization:** Brain imaging (either computer tomography or magnetic resonance imaging) will be performed at 24 hours. The 24-hour NIHSS score will be assessed and cerebrovascular events occurring within 24 hours will be recorded. Blood routine and laboratory examination (S100-β, brain derived neurotrophic factor and myeloperoxidase) will also be collected. **72 hours after randomization:** Cerebrovascular imaging (either computer tomography angiography or magnetic resonance angiography) and an ultrasonic cardiogram will be conducted within this period. **7 days after randomization:** Neurological measurements (NIHSS score and mRS score) will be evaluated. Cerebrovascular events during this 7-day period will also be recorded. **30 days after randomization:** The mRS score and Barthel index score will be assessed and cerebrovascular events or other adverse events will be recorded via a telephone interview.

	90 days after randomization: The mRS score and Barthel index score will be again assessed and cerebrovascular events or other adverse events will be recorded via a telephone interview.
Concomitant Treatment	Guideline based treatment.
Statistical analysis	The primary outcome is performed in the intention-to-treat populations as well as per-protocol populations. Sensitivity analyses are performed as well in order to find out the influence factors. The $\chi 2$ test is used in the unadjusted analysis. Modified Poisson regression model with robust error estimation is used in analysis of the primary outcome and other dichotomous end points, with adjustment for prespecified covariates. The relative risk with their 95% confidence interval is calculated in both unadjusted and adjusted analysis. Generalized linear models are conducted for analyse continuous end points. The full range score on mRS is analyzed by an ordinal logistic-regression model, and odds ratios with 95% confidence interval are calculated. No interim analyses are performed in this study. A 2-sided *P* value <0.05 is considered statistical significance. Because multiple comparisons potentially cause type I error, findings for secondary outcome analyses should be interpreted as exploratory. SPSS software (version 26, IBM) and R software (version 4.1.0) are used for the statistical analyses.
Sites Number	3 centers
Collaborators	Affiliated Hospital of Jiujiang University Cangzhou Hospital of Integrated Traditional Chinese and Western Medicine
Duration	Enrollment about 1 year

Abbreviation

END, early neurological deteiroration

NIHSS, National Institutes of Health Stroke Scale

mRS, Modified Ranking Scale

BI, Barthel Index

ICH, intracranial hemorrhage

IVT, intravenous thrombolysis

AIS, acute ischemic stroke

CBF, cerebral blood flow

NS, normal saline

ESO, European Stroke Organisation

CT, computed tomography

MRI, magnetic resonance imaging

BNP, brain natriuretic peptide

MBP, myelin basic protein

GFAP, glial fibrillary acidic protein

MRA, magnetic resonance angiography

CTA, CT angiography

SBP, systolic blood pressure

DBP, diastolic blood pressure

NLR, neutrophil-to-lymphocyte ratio

PLR, platelet-to-lymphocyte

LMR, lymphocyte-to-monocyte

AEs, adverse events

SAEs, serious adverse events

RR, relative risk

GLMs, Generalized linear models

OR, odds ratio

ITT, Intention-To-Treat

PP, Per protocol

CRF, Case Report Forms

1. Background

It is well acknowledged that intravenous thrombolysis (IVT) is a safe and effective strategy for treating acute ischemic stroke (AIS) within 4.5 h in clinical settings.[1, 2] However, only approximately 30% of patients achieve long-term independence (modified Ranking scales 0-1).[3] A plethora of factors may influence the effectiveness of IVT, including age, comorbid diseases, stroke subtypes, infarct size and onset-to-needle time.[3, 4] Ultimately, all these factors can be attributed to reperfusion and the penumbra.[5] Therefore, the further elevation of the reperfusion rate and expansion of the ischemic penumbra after IVT are emerging challenges. A simple approach is to increase cerebral blood flow (CBF), which can reopen occluded vessels and rescue ischemic neurons after AIS. Intravenous administration of 0.9% normal saline (NS) is an easy-to-use strategy commonly used to expand volume.[6, 7] The benefit of NS infusion on stroke outcomes is not a concern, but for reperfusion therapies such as IVT, it seems to be practicable.

Investigations on volume expanders and hemodilution date back to the 1960s.[8] Since then, accumulating evidence has indicated that volume expansion can improve the collateral circulation status, reduce blood viscosity, and alleviate brain dysfunction.[9-12] Considering rescuing the ischemic penumbra, the early use of hemodilution, especially within 6 h after AIS onset, is inevitable to exert its powerful functions.[13] The no-reflow phenomenon and unexpected re-occlusion may be responsible for poor outcomes and early neurological deterioration (END) after IVT.[14] In theory, the early use of hemodilution after IVT can increase distal arterial perfusion and reduce the re-occlusion rate by increasing CBF. In addition, the prevalence of dehydration accounts for approximately 48%-53% of patients with stroke, in whom immediate fluid intake might improve their short- and long-term prognoses, especially in people undergoing thrombolysis.[15-19] Therefore, we hypothesized that immediate hemodilution after IVT may increase the reperfusion rate, reduce the re-occlusion rate, and improve prognosis.

To verify our hypothesis, we administered an intravenous NS infusion immediately after IVT termination. Generally, hemodilution-related drugs include colloids (such as hydroxyethyl and dextroside) and crystalloid (such as NS). An appropriate solution for hemodilution should be provided to optimize the perfusion of vital organs and avoid fluid overload. Although colloids can increase left ventricular end-diastolic volume, stroke volume, and cardiac output, they are also associated with high blood pressure, susceptibility to allergy, heart failure, and higher morbidity and mortality.[7] Compared

to colloids, crystalloids have an equivalent effect on stroke volume variability, which reflects fluid responsiveness.[7] Therefore, these two solutions are considered to have similar effects on fluid resuscitation, and crystalloids seem safer for patients with stroke undergoing reperfusion therapy. Therefore, NS may be a promising therapeutic strategy for volume expansion following IVT.

2. Objectives

This novel study explores the safety and efficacy of NS infusion for patients with stroke after IVT. This trial provides an innovative strategy for facilitating functional independence after the administration of IVT in patients with stroke.

2.1 Hypothesis tested

The study intends to demonstrate that immediate abundant normal saline infusion after IVT is safe and effective in improving long-term functional outcomes in AIS patients treated with IVT.

2.2 Primary objective

The proportion of favorable functional outcomes (mRS 0-2) at 90 days.

2.3 Secondary objective

1. Secondary efficacy including mRS score at 30 and 90 days, Barthel index at 30 and 90 days, cerebrovascular events within 24 hours, 7 days and 90 days, NIHSS score at 7 days, END within 24 hours and laboratory examination at 24-48 hours.

2. Secondary safety including ejection fraction within 72 hours, death rate with any reasons within 90 days, intracranial hemorrhage and symptomatic intracranial hemorrhage within 7 days, blood pressure fluctuation within 24 hours and infarction edema volume at 24 hours.

3. Study Design

3.1 Trial plan

This is an assessor-blinded, randomized, controlled, two-arm (1:1 ratio) clinical trial registered at Clinicaltrials.gov (http://www.clinicaltrials.gov; unique identifier: NCT05993078) and has been approved by the Institutional Ethics Committee.

All patients within 4.5 h from the definite onset time are screened by the criteria of IVT recommended by European Stroke Organisation (ESO) guidelines on IVT for AIS at admission.[20] A cranial CT scan is conducted to exclude the ICH and large brain infarctions. The participants are

allocated to two arms: NS and control groups. In the NS group, the patients undergo an NS 2000 mL intravenous infusion swiftly after IVT at a speed of 200 mL/h. In the control group, the patients receive an NS 200-400ml after IVT. All the participants are subjected to CT at 24-h after IVT; if no brain hemorrhage occurs, antiplatelet drugs are used. In the event of brain hemorrhage, antiplatelet drugs are not used until the hemorrhage diminishes. Subsequent therapy is based on Chinese guidelines for diagnosis and treatment of acute ischemic stroke 2023.[21] All of the subjects should be treated in the hospital at least 7 days according to the guidelines for early management of stroke.

3.2 Selection criteria

3.2.1 Inclusion criteria

(1) AIS;

(2) Age 18-80 years;

(3) Prestroke mRS≤1;

(4) NIHSS score 0-25;

(5) Onset-to-needle time≤4.5 h;

(6) Sign the informed consent.

3.2.2 Exclusion criteria

(1) Massive infarction, characterized by infarction area larger than 1/3 of the effected middle cerebral artery territory and/or the cerebellum territory presented in admitted computed tomography (CT) or magnetic resonance imaging (MRI);

(2) Intention to undergo endovascular treatment;

(3) History of heart failure or pre-IVT brain natriuretic peptide (BNP) ⩾ 500pg/ml or having presentations or signs indicating heart failure;

(4) Haemorrhage during IVT, including ICH, severe digestive haemorrhage and severe respiratory haemorrhage.

(5) Allergy to thrombolysis drugs;

(6) Intolerant to thrombolysis due to any reasons and had to terminate thrombolysis;

(7) Arterial puncture at a non-compressible site within previous 7 days, major surgery within previous 14 days, sever trauma, gastrointestinal or urinary tract bleeding within previous 21 days;

(8) Cerebral infarction or myocardial infarction within previous 3 months, previous ICH including parenchymal haemorrhage, intraventricular haemorrhage, subarachnoid haemorrhage, subdural/external haematoma, etc;

(9) Severe brain trauma, intracranial or intraspinal surgery within previous 3 months or known malignant intracranial neoplasm, giant intracranial aneurysm or arteriovenous malformation;

(10) Persistent systolic blood pressure≥180mmHg or diastolic blood pressure≥100mmHg;

(11) Admitted blood glucose<2.8mmol/L or >22.22mmol/L;

(12) Defect in coagulation, for example, current use of oral warfarin with an international normalised ratio>1.7, or prothrombin time>15s, or heparins during the last 48 hours, or use of direct thrombin inhibitors or direct factor Xa inhibitors during the last 48 hours or with an elevated activated partial thromboplastin time;

(13) Known defect of platelet or clotting function, platelet count<100×10^9/L;

(14) Stroke mimics, such as seizure and hysteria;

(15) Brain haemorrhage identified by CT or MRI;

(16) Any terminal illness such that patients would not be expected to survive>1 year;

(17) Pregnant women or nursing mother;

(18) Poor compliance, or inability to adhere to the trial protocol or follow-up

(19) Participating in other clinical trials within previous 3 months.

3.2.3 Withdrawal criteria

(1) Acute heart failure during interventions.

(2) Hypertensive crisis during interventions.

(3) Edema, such as severe cerebral edema, pulmonary edema, limb edema and water intoxication.

(4) Other severe complications that should terminate the interventions immediately.

(5) Stroke mimics.

(6) Decide to undergo emergency endovascular treatment during interventions.

(7) Refuse subsequent treatment and follow-up.

3.3 Follow-up time point

All included patients are followed-up at baseline, 24 hours, 7 days, 30 days and 90 days after randomization, respectively.

3.4 Randomization and masking

All patients are enrolled consecutively and randomized in 1:1 ratio to receive NS 2000ml or 200-400ml using a computer-generated randomization code. Sealed envelopes containing the allocation information (NS or control) will be labeled with sequential numbers prepared by an investigator who does not know screening of subjects. The researchers will enroll potential patients with a sequential number of opaque sealed envelopes, which will be opened only after obtaining informed consent. It is ensured that investigators are masked to the treatment allocation.

This is an open label trial, so we adopt a partial blinding strategy where the clinical assessors are blinded but the operators are not. That is, the neurological functional scores, imaging tests and laboratory assessments are assessed by investigators blinded to the intervention and grouping. The operators responsible for NS infusion to the patients are unblinded.

4. Trial Flow Chart

	STUDY PROTOCOL								
	Enrollment	**IVT ending**	**Interventions**	**Follow-up**					
TIMEPOINTS	-1 h	0 h	0-10 h	24 h	48 h	Day 3	Day 7	Day 30	Day 90
ENROLLMENT									
Eligible Screen	**X**								
Informed Consent		**X**							
Allocation		**X**							
Termination Screen			**X**						
INTERVENTIONS									
NS 2000 mL			→	→					
NS 200-400 mL			→	→					
ASSESSMENT									
Baseline Data	**X**								
NIHSS score	**X**	**X**		**X**			**X**		
Cranial CT	**X**			**X**					
Blood Indices	**X**			—	—				
Laboratory examination	**X**			—	—				
Ultrasonic Cardiogram				—	—	—			
Cranial MRA/CTA				—	—	—	—	—	
Blood pressure	—	—	—	—					
mRS							**X**	**X**	**X**

Barthel index		**X**	**X**

IVT: intravenous thrombolysis; NS: 0.9% normal saline; NIHSS: Institute of Health Stroke Scale; CT: computed tomography; MRA: magnetic resonance angiography; CTA: computed tomography angiography; mRS: modified Ranking scales.

5. Interventions

All patients within 4.5 hours from the definite onset time will be screened by the criteria of IVT recommended by European Stroke Organisation (ESO) guidelines.[20]

During thrombolysis, the patient or his certigiers will decide whether to participate in this trial. If they agree, an informed consent for NS-STAR trial will be signed, and allocation will proceed.

Participants will be allocated into two arms:

the NS group, undergoing immediate 2000 mL intravenous infusion of NS after IVT at a speed of 200 mL/h.

the control group, receiving 200-400ml of NS after IVT.

Subsequent therapy will follow the Chinese guidelines for the diagnosis and treatment of acute ischemic stroke 2023.[21] All subjects will be hospitalized for at least 7 days according to the guidelines for early stroke management.

6. Measurement

6.1 Haematological examination and laboratory assessment

Baseline blood samples are obtained before IVT administration. The blood indices comprise blood routine, creatinine, urea, BNP, electrolyte and coagulation function. The blood routine is reassessed at 24-48 hours after randomization. Peripheral blood biomarkers that can reflect brain injury, including S100-β, brain derived neurotrophic factor (BDNF) and myeloperoxidase (MPO) are assessed before IVT administration and at 24-48 hours after randomization.

6.2 Imaging tests

Cranial CT scans are performed before IVT and 24 hours after randomization. The infarction area is confirmed using CT maps and the infarction volume is calculated by 3D-Slicer (Version 4.6.2, https://www.slicer.org/). Magnetic resonance angiography (MRA), CT angiography (CTA), or carotid artery ultrasound are used to evaluate cranial vessels.

6.3 Clinical observation

National Institutes of Health Stroke Scale (NIHSS) is commonly used to evaluate neurological deficits in patients with AIS and includes 15 items in 11 fields of different neurological statuses. The NIHSS is scored at admission, at the end of IVT, 24-h and 7-day after randomization during hospitalization.

END indicates an increase of $\geqslant 2$ or $\geqslant 4$ in the NIHSS score within 24 h after IVT, and the deterioration is not caused by ICH, which is confirmed by cranial CT.

Modified Rankin Scale (mRS) reflects disability at follow-up time, which consists of 7 disability levels, ranging from 0 (no symptoms) to 5 (severe disability), and 6 (death). We intend to assess the mRS on days 7, 30, and 90 after randomization.

Barthel index (BI) is a standardized tool used to evaluate a person's ability to perform basic activities of daily living independently at follow-up time, and consists of 10 items, ranging from 0 (total dependence) to 100 (complete independence). We intend to assess the BI on days 30 and 90 after randomization.

The ejection fraction is assessed by ultrasonic cardiogram within 72 hours after randomization.

Blood pressure, including systolic blood pressure (SBP) and diastolic blood pressure (DBP), is monitored from IVT initiation every 5 min for 2 h and then continuously assessed every 30 min for 6 h and every hour for 16 h. The 24-hour blood pressures are recorded (before IVT, after IVT, 10 hours, 12 hours and 24 hours after randomization).

7. Outcomes

7.1 Efficacy outcomes

7.1.1 Primary outcomes

Primary efficacy is independence at 90 days, as scored using the mRS, and dichotomized as a favorable (score 0-2) or an unfavorable outcome (score 2-6), following the Cochrane analysis of thrombolysis in stroke. The proportion of patients in each arm with favorable outcomes is compared at 90 days.

7.1.2 Secondary outcomes

(1) Proportion of patients with END (△NIHSS≥2 and △NIHSS≥4) at 24 hours after randomization. △NIHSS indicates 24-hour NIHSS score minus post-IVT NIHSS score.

(2) Proportion of patients with cerebrovascular events within 1 day, 7 days and 90 days after randomization. Cerebrovascular events included progressive stroke (NIHSS deterioration≥2 scores),

transient ischemic attack or new ischemic lesions confirmed by imaging.

(3) NIHSS scores at 7 days.

(4) Proportion of patients with mRS 0-2 at 30 days.

(5) mRS distribution at 30 days and 90 days.

(6) BI scores at 30 days and 90 days.

(7) Proportion of the patients with BI 60-100 at 30 days and 90 days.

(8) Peripheral blood inflammatory indices, including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte (PLR), lymphocyte-to-monocyte (LMR) and (platelet × neurophil)/lymphocyte (SII) at 24-48 hours after randomization.

(9) Laboratory examinations (S100-β, BDNF, and MPO) at 24-48 hours after randomization.

7.2 Safety outcomes

(1) The ejection fraction evaluated by ultrasonic cardiogram within 72 hours.

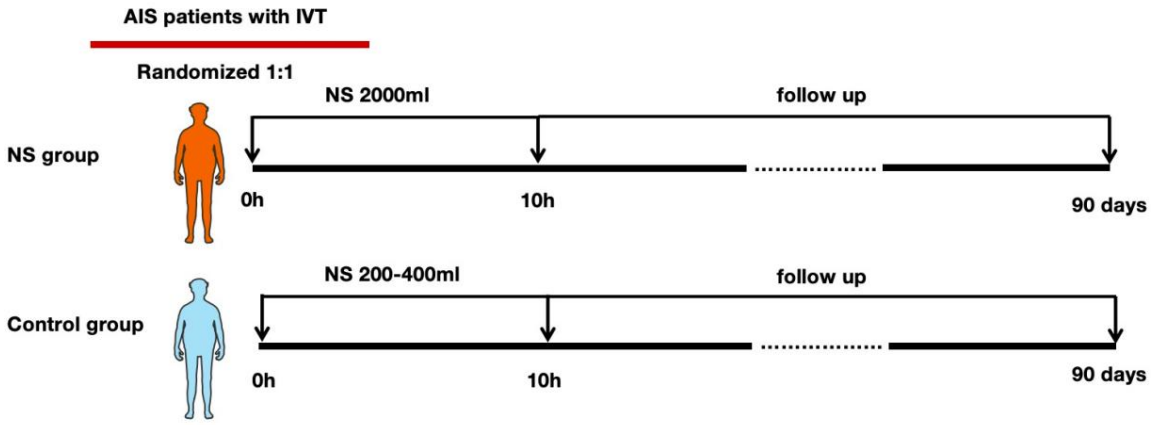
(2) Death rate within 90 days.

(3) Intracranial hemorrhage and symptomatic intracranial hemorrhage within 7 days.

(4) Blood pressure fluctuation within 24 hours.

(5) Infarction edema volume evaluated by cranial CT at 24 hours.

8. Study flow diagram



AIS: acute ischemic stroke; IVT: intravenous thrombolysis; NS: normal saline.

9. Study periods

9.1 Screening periods

Before IVT administration, enrollment screening and collection of demographic features, medical history, onset-to-needle time, cranial CT imaging, blood pressure, neurological measurements (NIHSS score and mRS score), haematological examinations (blood routine, creatine, urea, BNP, electrolyte, coagulation function and laboratory biomarkers including S100-β, BDNF and MPO), and other information are necessary.

9.2 Treatment periods

After IVT administration, patients are randomized into two groups: receiving intravenous NS 2000ml and NS 200-400ml respectively. Therapy follows the Chinese guidelines for diagnosis and treatment of acute ischemic stroke 2023[21]. Blood pressures are recorded at 0 hour, 10 hours, 12 hours and 24 hours after randomization. NIHSS scores are assessed immediately after IVT administration and 24 hours after randomization. Cranial CT scan is performed at 24 hours. Haematological examinations including blood routine and laboratory biomarkers (S100-β, MBP and GFAP), and routine urine test are conducted at 24-48 hours after randomization.

9.3 In-hospital periods

Ultrasonic cardiogram is performed at 72 hours after randomization. NIHSS score and mRS score are assessed at 7 days after randomization.

9.4 Follow-up periods

Neurological measurements including mRS score and Barthel index are assessed via a telephone interview at 30 and 90 days. Cerebrovascular events and death events are recorded carefully at this time. All the adverse events should be recorded and tracked until properly resolved.

10. Adverse events monitoring

10.1 Adverse events (AEs)

All AEs information should be carefully recorded and tracked until properly resolved. AEs in this trial include acute heart failure, hypertensive crisis, edema (such as severe cerebral edema, pulmonary edema, limb edema and water intoxication) and other severe complications during and after interventions that are considered to be associated with the interventions.

10.2 Serious adverse events (SAEs)

SAEs are any untoward medical occurrence (whether related to interventions or not) meeting any of the following criteria: (1) fatal (AEs causes death); (2) life-threatening; (3) causing or prolonging hospitalization; (4) disability or organ function impairment.

10.3 Safety assessment

For AEs and SAEs occurring in this study, the following factors will be evaluated for safety: (1) severity criteria for AEs; (2) causal relationship between the event and intervention; (3) anticipation of events.

10.4 Severity categorization and relationship definitions of AEs

10.4.1 Severity categorization

(1) Grade I, mild

A transient symptoms or the event that requires only minimal therapeutic interventions. The event does not affect usual activities of daily living.

(2) Grade II, moderate

A significant symptoms or the event that requires additional specific therapeutic interventions. The event affect usual activities of daily living, causing discomfort or disability but with no profound or permanent risk of harm to the participant.

(3) Grade III, severe

A profoundly severe symptoms or the event that requires intensive therapeutic interventions. The event profoundly affects or interrupts usual activities of daily living, causing disability and even posing permanent risk of harm to the participant.

(4) Grade IV, life-threatening

The event leads to death at any time.

(5) Grade V, death

Death.

10.4.2 Causal relationship between the event and the intervention

(1) Definitely related

AEs occur during the intervention and/or the AEs disappear when the intervention discontinues. The occurrence of AEs cannot be explained by factors other than the intervention. Similar AEs re-occur when re-use of the intervention.

(2) Probably related

AEs occur during the intervention and/or the AEs relieve or not when the intervention discontinues. The occurrence of AEs can or cannot be explained by factors other than the intervention. It is not sure similar AEs re-occur when re-use of the intervention.

(3) Probably unrelated

AEs do not occur during the intervention and/or the AEs do not relieve when the intervention discontinues. The occurrence of AEs can or cannot be explained by factors other than the intervention. It is not sure similar AEs re-occur when re-use of the intervention.

(4) Definitely unrelated

AEs do not occur during the intervention and/or the AEs do not relieve when the intervention discontinues. The occurrence of AEs can be explained by factors other than the intervention. Similar AEs do not re-occur when re-use of the intervention.

(5) Unjudged

The judgment cannot be made due to incomplete information.

11. Statistics

11.1 Sample size

The sample-size is estimated according to previous trials with an expected between-group difference of 18 percentage points in favorable outcome (90-day mRS 0-2, 67.3% in the NS group and 49.3% in the control group).[4] Based on 0.8 power to detect a significant difference (2-sided P=0.05) and to compensate for nonadherence or dropout rate of 5%, we intend to enroll 120 patients per group (calculated by PASS, version 15.0, https://passapp.io/).

11.2 Statistical analysis

The modified intention-to-treat analysis of efficacy outcomes and safety outcomes are performed on the full analysis set, which is composed of all patients who receive randomization independent of treatment and at least 1 efficacy evaluation after enrollment. The primary outcome is also performed with sensitivity analyses, including per-protocol analysis; imputation of missing primary outcome data under the scenarios of worst possible outcome, and best possible outcome. The $\chi 2$ test is used in the unadjusted analysis. Modified Poisson regression model with robust error estimation is used in analysis of the primary outcome and other dichotomous end points, with adjustment for prespecified covariates

(age, sex, post-IVT NIHSS scores and onset-to-needle time). The relative risk (RR) with their 95% confidence interval (95%CI) is calculated in both unadjusted and adjusted analysis. Generalized linear models (GLMs) are conducted for analyse continuous end points, including NIHSS scores, Barthel scale, blood pressure, infarction volumes, ejection fraction at each follow-up time point. The full range score on mRS is analyzed by an ordinal logistic-regression model, and odds ratio (OR) with 95%CI are calculated. Cox regression model are used for analyzing the follow-up cerebrovascular events, presented with cumulative probability of events in Kaplan Meier cures. No interim analyses are performed in this study. The missing values of baseline variables in the covariate-adjusted analyses are replaced by mean values (continous variates) or mode (dichotomous variates) only if the number of them account for less than 10% in total.

A 2-sided *P* value <0.05 is considered statistical significance. Because multiple comparisons potentially cause type I error, findings for secondary outcome analyses should be interpreted as exploratory. SPSS software (version 26, IBM) and R software (version 4.1.0) are used for the statistical analyses.

11.3 Analysis population

11.3.1 Intention-To-Treat (ITT)

The ITT population involves all participants in the groups to which they are originally assigned, regardless of whether they complete the treatment or adhere to the protocol. It can reflect the real-world effectiveness of the treatment and remain unbiased by the participants' adherence to the treatment plan.

11.3.2 Per protocol (PP)

The PP population involves the participants who fully complete the treatment and adhere to the protocol. It can be used to assess the efficacy of the treatment on those who followed the prescribed procedures.

11.3.3 Safety population

The safety population included all participants who receive the protocol treatment and have at least one time of safety evaluation.

12. Data management and monitoring

12.1 Case Report Forms (CRF)

A CRF should be completed and signed by the investigators for each participant randomized. The

reasons for subject withdrawing from the study must be noted on the CRF. Adverse events and its follow-up interviewing should be recorded carefully on the CRF. All forms should be completed within 1 week after subject visit. The investigators should ensure the accuracy, completeness, legibility and timeliness of the data submitted to the sponsor in the CRF and in all required reports.

12.2 Monitoring

Sponsor will keep close touch with each study center and will provide any study information whenever the investigators need. Regular visits includes as follows:

(1) Confirm the adherence of the protocol and the timeliness of the data in the CRF.

(2) Verify the completeness, accuracy and legibility of the data in the CRF, including the baseline and the follow-up measurements and neurological scores.

13. References

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Appendix

Appendix 1: National Institutes of Health Stroke Scale (NIHSS)

Instructions	Scale Definition	Score
1a. Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	0 = **Alert;** keenly responsive. 1 = **Not alert**; but arousable by minor stimulation to obey, answer, or respond. 2 = **Not alert**; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped). 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and flexic.	
1b. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = **Answers** both questions correctly. 1 = **Answers** one question correctly. 2 = **Answers** neither question correctly.	
1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but	0 = **Performs** both tasks correctly. 1 = **Performs** one task correctly. 2 = **Performs** neither task correctly.	____

not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.		
2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.	0 = **Normal.** 1 = **Partial gaze palsy;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = **Forced deviation,** or total gaze paresis not overcome by the oculocephalic maneuver.	____
3. Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If	**0 = No visual loss.** **1 = Partial hemianopia.** **2 = Complete hemianopia.** **3 = Bilateral hemianopia** (blind including cortical blindness).	

patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.		
4. Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.	**0 = Normal** symmetrical movements. **1 = Minor paralysis** (flattened nasolabial fold, asymmetry on smiling). **2 = Partial paralysis** (total or near-total paralysis of lower face). **3 = Complete paralysis** of one or both sides (absence of facial movement in the upper and lower face).	_____
5. Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	**0 = No drift**; limb holds 90 (or 45) degrees for full 10 seconds. **1 = Drift;** limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support. **2 = Some effort against gravity**; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. **3 = No effort against gravity**; limb falls. **4 = No movement.** **UN** = Amputation or joint fusion, explain: _____ **5a. Left Arm** **5b. Right Arm**	_____

6. Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	**0 = No drift;** leg holds 30-degree position for full 5 seconds. **1 = Drift;** leg falls by the end of the 5-second period but does not hit bed. **2 = Some effort against gravity;** leg falls to bed by 5 seconds, but has some effort against gravity. _____ **3 = No effort against gravity;** leg falls to bed immediately. **4 = No movement.** **UN** = Amputation or joint fusion, explain: _____ **6a. Left Leg** **6b. Right Leg**	
7. Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	**0 = Absent.** **1 = Present in one limb.** **2 = Present in two limbs.** **UN = Amputation or joint fusion**, explain: _____ _____	

8. Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma (item 1a=3) are automatically given a 2 on this item.	**0 = Normal;** no sensory loss. **1 = Mild-to-moderate sensory loss**; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched. **2 = Severe to total sensory loss;** patient is not aware of being touched in the face, arm, and leg.	
9. Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor	**0 = No aphasia;** normal. **1 = Mild-to-moderate aphasia;** some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response. **2 = Severe aphasia**; all communication is through	

or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.	fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response. **3 = Mute**, global aphasia; no usable speech or auditory comprehension.	
10. Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	**0 = Normal.** **1 = Mild-to-moderate dysarthria**; patient slurs at least some words and, at worst, can be understood with some difficulty. **2 = Severe dysarthria;** patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric. **UN = Intubated** or other physical barrier, explain:_____	
11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of	**0 = No abnormality.** **1 = Visual, tactile, auditory, spatial, or personal inattention** or extinction to bilateral simultaneous stimulation in one of the sensory modalities. **2 = Profound hemi-inattention or extinction to more than one modality;** does not recognize own hand or orients to only one side of space.	

abnormality. Since the abnormality is scored only if present, the item is never untestable.		
	Total NIHSS:	

Appendix 2. Modified Ranking Scale (mRS)

Grade	Description
0	No symptoms
1	Symptoms without any incapacity (able to perform all usual activities)
2	Mild incapacity (unable to perform all usual activities but able to look after his/her affairs alone)
3	Moderate incapacity (requires assistance but walks alone)
4	Severe incapacity (requires assistance for walking and physical body needs)
5	Severe incapacity (bedbound, incontinent, permanent surveillance required)
6	Death

Appendix 3. Barthel Index (BI)

Items	Criteria	Score
Bowel Control	Incontinent Occasional incontinence or requires assistance with devices Able to control, and can use enemas or suppositories if needed	0 5 10
Bladder Control	Incontinent Occasional incontinence or requires assistance with devices Able to control, and can use urine collectors if needed	0 5 10
Grooming	Needs assistance Able to wash face, comb hair, brush teeth, and shave independently	0 5
Bathing	Depends on others Can complete independently	0 5
Toileting	Depends on others Needs partial help, such as with dressing/undressing or using toilet paper Can use the toilet or bedpan independently, including dressing/undressing and flushing or cleaning the bedpan	0 5 10
Feeding	Depends on others Needs partial help (e.g., cutting food, stirring food) Can use any necessary devices to eat independently within a reasonable time	0 5 10
Dressing	Depends on others Needs help but can complete at least half of the work within a reasonable time Can dress and undress independently (including buttoning, zipping, and putting on/taking off braces)	0 5 10
Transfer	Completely depends on others, unable to sit Can sit, but needs a lot of help (2 people) to transfer Needs little help (1 person) or guidance Can transfer independently from bed to wheelchair and back, including sitting up in bed, braking the wheelchair, and lifting	0 5 10 15

Mobility	Unable to move Can move independently in a wheelchair for 45 meters Needs help from 1 person (physical or verbal guidance) to walk 45 meters Can walk 45 meters on a flat surface, can use assistive devices (excluding wheeled walkers)	0 5 10 15
Stairs	Unable to Needs help and supervision Can do it independently, can use assistive devices	0 5 10

Total score interpretation:

- 0 - 20 points: Extremely severe functional impairment, completely dependent on others for daily life.
- 20 - 40 points: Great need for help in daily life.
- 40 - 60 points: Needs help in daily life.
- Above 60 points: Basically able to take care of oneself in daily life.