Protocol and Statistical Analysis Plans

Normobaric Hyperoxia for Intracerebral Hemorrhage

A Randomized Clinical Trial

NOTCH

Principle Investigators

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1. Protocol Title

Normobaric Hyperoxia for Intracerebral Hemorrhage

2. Trial Registration

Clinicaltrials.gov (http://www.clinicaltrials.gov; unique identifier: NCT04144868).

3. Funding

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4. Protocol Directors

Zhiying Chen, MD, PhD; Jiayue Ding, MD, PhD; Xiaoping Yin, MD, PhD; Ran Meng, MD, PhD.

5. Introduction

Perihematoma edema (PHE), as the major injury for intracerebral hemorrhage (ICH) involves more than the initial tissue damage induced directly by the hematoma.[1, 2] How to improve hypoxia in perihematoma seems to be a promising therapeutic candidate paradigm for ICH due to its pivotal role in the pathogenesis of perihematomas. Normobaric hyperoxia (NBO), supplied by a face mask (such as oxygen storage face mask) with atmosphere pressure (1ATA = 101.325 kPa, 100% O_2), has been considered a safe, convenient, and promising therapy for correcting various diseases and thus garnered great attention in recent years.[3] The previous study identified that early NBO could attenuate blood-brain barrier damage, rescue penumbra and finally improve the prognosis of ischemic stroke in patients with delayed rt-PA treatment.[4] Therefore, given the profound effectiveness in the ischemic penumbra, we hypothesized that NBO might yield additional benefits for the ischemic-hypoxic tissues

surrounding the hematoma in patients with ICH. Although many clinical trials have shown the effectiveness and safety of NBO in treating ischemic stroke, there is currently a lack of trials focusing on using NBO to treat ICH.[3, 5] Accordingly, we conducted a proof-of-concept, single-center, randomized controlled trial to evaluate the safety and efficacy of NBO in treating ICH patients so as to explore an innovative adjuvant therapy for ICH.

6. Objectives

This novel study explores the safety and efficacy of NBO administration for patients with ICH. This trial provides an innovative strategy for facilitating functional independence in patients with ICH.

7. Study Design

7.1 Trial plan

This is a single-center, prospective, assessor-blinded, randomized, controlled, two-arm (1:1 ratio) clinical trial registered at Clinicaltrials.gov (http://www.clinicaltrials.gov; unique identifier: NCT04144868) and has been approved by the Institutional Ethics Committee (Affiliated Hospital of Jiujiang University).

All patients within 24 hours from definite onset time are screened by the cranial computed tomography (CT) to confirm ICH at admission. And then the patients sign the consent and are allocated to two arms: the NBO group and the control group. In the NBO group, patients receive high-flow oxygen via oxygen storage face mask (100% O_2, flow rate 8 L/min, 1 hour, four times daily, and 2 L/min via nasal catheter during intermittent periods, for 7 days) immediately in the emergency room after randomization. In the control group, patients receive 2 L/min flow of 100% O_2 via nasal catheter in the emergency room after randomization, for 24 hours daily for 7 days. Meanwhile, the participants undergo routine medication treatment, including dehydration treatment, antihypertensive therapy and sedative treatment, and if necessary, hematoma aspiration or a decompressive craniectomy will be conducted.

7.2 Eligibility criteria

7.2.1 Inclusion criteria:

(1) supratentorial hematomas confirmed by admitted cranial CT, with the volume ranging from 10 to 30 mL;

(2) age 18–80 years;

(3) National Institute of Health Stroke Scale (NIHSS) ≥ 6 and Glasgow Coma Scale (GCS) > 8 at admission;

(4) onset-to-enrollment time ≤ 24 h;

(5) signed informed consent.

7.2.2 Exclusion criteria:

(1) a history of ICH, ischemic attack, brain tumor, brain trauma, and other intracranial injury or disorders;

(2) pre-stroke modified ranking scales (mRS) ≥ 1;

(3) life-threatening condition;

(4) pre-stroke complicated with austere diseases such as cancer, heart failure, and respiratory failures;

(5) severe liver and kidney disorders;

(6) a history of respiratory diseases;

(7) poor compliance;

(8) participation in other clinical trials within the previous three months.

7.2.3 Withdrawal criteria

(1) Acute respiratory diseases during interventions;

(2) Acid-base imbalances during interventions;

(3) Other severe complications that should terminate the interventions immediately;

(4) Decide to undergo hematoma aspiration or a decompressive craniectomy;

(5) Refuse subsequent treatment and follow-up.

7.3 Follow-up time point

All included patients are followed-up at baseline, 3 days, 7 days, 14 days and 90 days after randomization, respectively.

7.4 Randomization and blinding

All patients are enrolled consecutively and randomized in 1:1 ratio to receive 8 L/min flow of 100% O_2 via oxygen storage face mask or 2 L/min flow of 100% O_2 via nasal catheter using a computer-generated randomization code. This is an open label trial, so we adopt a partial blinding strategy where the clinical assessors are blinded to the treatment allocation but the operators are not. That is, the neurological functional scores and imaging tests are assessed by investigators blinded to the

intervention and grouping. The operators responsible for oxygen interventions to the patients are unblinded.

8. Trial Flow Chart

	STUDY PROTOCOL					
	Enrollment	**Allocation**	**Follow-up**			
TIMEPOINT	0 day	0 day	3 days	7 days	14 days	90 days
ENROLLMENT						
Eligibility screen	X					
Informed consent	X					
Allocation		X				
INTERVENTIONS						
NBO		←	—	→		
Control		←	—	→		
ASSESSMENTS						
Personal data		X				
Safety evaluation				X		
NIHSS	X		X	X	X	
Glasgow Coma Scale	X		X	X	X	
Barthel index						X
mRS						X
Cranial CT	X		X	X	X	
CT perfusion	X			X		

NBO, normobaric hyperoxia; CT, computed tomography; NIHSS, National Institute of Health Stroke Scale; mRS, modified Rankin Scale.

9. Interventions

All patients within 24 hours from definite onset time are screened by the cranial CT to confirm ICH at admission. After diagnosis of ICH, the patient or his certigiers will decide whether to participate in this

trial. If they agree, an informed consent will be signed, and allocation will proceed.

Participants will be allocated into two arms:

The NBO group, undergoing high-flow oxygen via oxygen storage face masks (100% O_2, flow rate 8 L/min, 1 hour, four times daily, and 2 L/min via nasal catheter during intermittent periods, for 7 days) immediately in the emergency room after randomization.

The control group, undergoing 2 L/min flow of 100% O_2 via nasal catheter in the emergency room after randomization, for 24 hours daily for 7 days.

Secondary stroke prevention follows the 2022 AHA/ASA guidelines for the diagnosis and treatment of acute intracerebral hemorrhage. All participants are hospitalized for at least 14 days, and if the hematoma expands and causes cerebral hernia, hematoma aspiration or a decompressive craniectomy will be conducted.

10. Measurements

10.1 Imaging test

10.1.1 Non-contrast cranial CT image

Non-contrast head CT scans are performed to assess the severity of hematoma and perihematomal edema (PHE). A 256-slice CT scanner is used following standard local protocols. Next, 5-mm-thick CT images are measured for hematoma and PHE volumes using the General Electric Company workstation built-in image software. All volume measurements are performed using a semi-automated process involving tracking the perimeter of appropriately high- and low-attenuation areas and calculating the lesion area for each slice multiplied by the slice thickness to obtain the lesion volume. Independent analysis of all images is performed by two doctors blinded to the treatment assignment, and any discrepancies are resolved consensually.

Cranial CT scans are performed at admission, day 3, 7, and 14. Software from United Imaging (United Imaging Healthcare Co., Ltd., Shanghai, China) is used for the automated calculation of hematoma and absolute PHE volumes. The relative PHE is calculated by dividing the absolute PHE volume by the baseline hematoma volume to obtain a dimensionless ratio. The reduction volumes of the hematoma, absolute PHE, and relative PHE follow the defined formula: baseline volume − follow-up volume. Besides, the clearance rate of the hematoma, absolute PHE and relative PHE are calculated as a percentage using the defined formula: 100% × (baseline volume − follow-up

volume)/baseline volume.

10.1.2 CT perfusion (CTP) images

Using a high-pressure injector, 50 mL of iodixanol (Visipaque, 370 mg/mL) is rapidly injected through the cubital vein at 8 mL/s. The injection is completed simultaneously with the continuous scanning of a selected single layer for 50 s. A head-fixing strap is used to secure the patient during scanning. Cerebral perfusion parameters are transmitted to specialized perfusion CT software (Revolution, GE Healthcare, USA) for manual post-processing. The perfusion parameters, including cerebral blood volume (CBV), CBF, time to peak (TTP), and mean transit time (MTT), are measured by post-processing software (Advantage Workstation 4.7, Revolution, GE Healthcare, USA).

Cranial CTP images are conducted at admission and day 7. The area around the marginal hematoma is selected as the region of interest (ROI), and the symmetrical area in the opposite hemisphere is regarded as the contralateral mirror ROI. The CBF, CBV, MTT, and TTP are assessed in both the area around the hematoma and the contralateral mirror area.

10.2 Clinical scale

10.2.1 Modified Rankin Scale (mRS)

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

10.2.2 Barthel index (BI)

The BI represents functional status at follow-up time, the scores of which range from 0 (complete dependence) to 100 (complete independence) measured by several items, including feeding, bathing, grooming, dressing, bowels, bladder, toilet use, transfers, and stairs. We intend to assess the BI on days 90 after randomization.

10.2.3 National Institute of Health Stroke Scale (NIHSS)

The NIHSS is commonly used to evaluate neurological deficits in stroke and comprises five items in 11 fields of different neurological statuses (scores range from 0–42, representing normal to severe neurological deficits). The NIHSS is scored at admission, 3-day, 7-day and 14-day after randomization during hospitalization.

10.2.4 Glasgow Coma Scale (GCS)

GCS is a practical method for the evaluation of impairment of conscious level in response to defined

stimuli, which contains three parts, including eye-opening, verbal response, and motor response (scores range from 3–15, representing deep coma to normal). The GCS is scored at admission, 3-day, 7-day and 14-day after randomization during hospitalization.

11. Outcomes

11.1 Efficacy outcomes

11.1.1 Primary outcome

Primary efficacy is independence at 90 days, as scored using the mRS, and dichotomized as a good (score 0-3) or a poor outcome (score 4-6), following the Cochrane analysis of thrombolysis in stroke. The proportion of patients in each arm with good outcomes is compared at 90 days.

11.1.2 Secondary outcomes

(1) mRS distribution at 90 days.

(2) BI scores at 90 days.

(3) NIHSS scores at 3, 7 and 14 days.

(4) GCS scores at 3, 7 and 14 days.

(5) Hematoma volumes, clearance rate and reduction volume at 3, 7, 14 day.

(6) Absolute PHE volumes, clearance rate and reduction volume at 3, 7, 14 day.

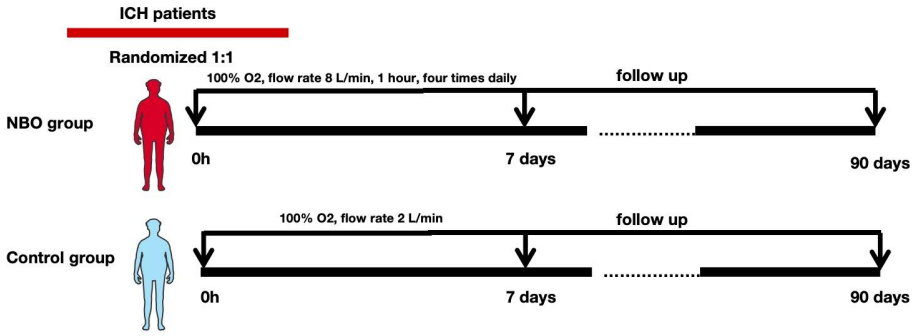
(7) Relative PHE volumes, clearance rate and reduction volume at 3, 7, 14 day.

(8) CBF, CBV, MTT, and TTP at 7 days.

11.2 Safety outcomes

The 7-day oxygen pressure (PO_2), the 7-day carbon dioxide pressure (PCO_2) and the 7-day PH value, as well as the death rate and the complication rate (including hematoma expansion, pulmonary infections, acid-base imbalances and so forth) during hospitalization.

12. Study Flow Diagram



13. Study Periods

13.1 Screening periods

Within 24 hours of symptom onset, participants who are diagnosed as ICH are assigned to either the NBO group or control group. The demographic features, medical history, onset-to-needle time, cranial CT imaging, blood pressure, blood samples and neurological measurements (NIHSS score, mRS score and GCS score) are collected.

13.2 In-hospital periods

The patients who are assigned to the NBO group receive 100% O_2 via oxygen storage face masks (flow rate 8 L/min, 1 hour, four times daily, and 2 L/min via nasal catheter during intermittent periods, for 7 days), otherwise, the patients receive 2 L/min flow of 100% O_2 via nasal catheter for 24 hours daily for 7 days. Secondary stroke prevention follows the 2015 AHA/ASA guidelines for the diagnosis and treatment of acute intracerebral hemorrhage.[6] Blood samples are collected at 7 days. Cranial CT is performed at 3 days, 7 days and 14 days, and cranial CTP is performed at 7 days after randomization. NIHSS score and GCS score are assessed at 7 days and 14 days after randomization.

13.3 Follow-up periods

Neurological measurements including mRS score and Barthel index are assessed via a telephone interview at 90 days.

14. Adverse events monitoring

14.1 Adverse events (AEs)

All AEs information should be carefully recorded and tracked until properly resolved. AEs in this trial

include pulmonary infections, acid-base imbalances, hematoma expansion, and other severe complications during and after interventions that are considered to be associated with the interventions.

14.2 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.

14.3 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.

14.4 Severity categorization and relationship definitions of AEs

14.4.1 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.

14.4.2 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.

15. Statistics

15.1 Sample size calculation

The sample size was estimated according to previous trials, with an expected between-group difference of 18 percentage points in favorable outcomes (90-day mRS 0-3, 81% in the NBO group, and 61% in the control group). We set the effective size to 0.25, α error probability to 0.05, power (1-β error probability) to 0.8, and the loss rate to 10%, the final result is that the total sample size is 154. However, the interim analysis showed the profound efficacy of NBO on ICH patients demonstrated by both imaging presentations and clinical evaluations. So, we terminated the trial ahead of schedule, and enrolled 96 participants (NBO group, 48 cases, and control group, 48 cases) at last.

15.2 Statistical methods

The modified intention-to-treat analysis of efficacy outcomes and safety outcomes were performed on the full analysis set, which was composed of all patients who received randomization independent of treatment and at least 1 efficacy evaluation after enrollment. The χ^2 test was used in the unadjusted

analysis. A modified Poisson regression model with robust error estimation was used to analyze the primary outcome and other dichotomous endpoints, with adjustment for prespecified covariates (age, male and onset-to-needle time). Relative risk (RR) with a 95% confidence interval (CI) was calculated in both unadjusted and adjusted analyses. Generalized linear models (GLMs) were used to analyze continuous endpoints, including NIHSS scores, Barthel scale scores, and hematoma and edema volumes. The distribution of mRS was analyzed using an ordinal logistic regression model, and odds ratios (OR) with 95% CI were calculated. Post hoc subgroup analysis was performed for the primary outcome according to age, sex, and baseline NIHSS scores, with an OR of 95% CI. No interim analyses were performed.

A 2-sided *P* value <.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.

16. Data Management

The enrolled participants were entered in the case report form (CRF) by the evaluator within 24 hours and entered in the electronic form within 3 days. All of the entered personnel signed on the CRF. The assessors were blinded to allocation information. A data supervisor who was not involved in clinical research is set up for data verification. Two investigators who blinded to allocation information rechecked the original data, and if the data was found to be wrong, the data should be corrected in time. The data of modification and the person who modified it should be recorded to ensure that the data is correct.

17. Data and Security Monitoring Board (DSMB)

A Data and Security Monitoring Board (DSMB) was established in this study in order to ensure the integrity of the trial and protect the rights and health of subjects. During the study period, regular monitoring visits will be made to the clinical site to ensure that all procedures were complied with the approved protocol/amendment. The safety and efficacy of the interventions were assessed at interim time-point and DSMB had the authority to terminate the trial.

18. Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee (EC) of the Affiliated Hospital of Jiujiang University (Jiujiang, China, 2020001) in accordance with the guidelines of the 1964 Declaration of Helsinki. All participants or their legally authorized representative provided written informed consent.

Modify the protocol: The research agreement must be strictly followed. The revised research agreement must be approved by the ethics committee, and the revised research agreement can be implemented after approval.

Protocol adherence: Prior to beginning the study, the Investigator must sign the Investigator Agreement and Signature page documenting his/her agreement to conduct the study in accordance with the protocol. An Investigator must not make any changes or deviate from this protocol, except to protect the life and physical well-being of a subject in an emergency. Each deviation from the protocol must be documented with the date and reason for the deviation and reported to EC as soon as possible.

Written informed consent form: The informed consent Form (ICF) document should be translated into a language that the subject can understand. The informed consent must be approved by the ethics committee before use. ICF must agree with the current Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki. Before participating in a clinical trial, each subject or its legally authorized representative (LAR) must understand the content of the informed consent form and sign it. The informed consent should clearly indicate the rights and obligations of the subjects. The written informed consent must be properly recorded with the signature of the person or LAR. The consent process must be recorded in the subject's medical record.

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