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Transcutaneous auricular vagus nerve stimulation in poststroke cognitive impairment: protocol for a randomised controlled trial

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ABSTRACT

Background As one of the most common stroke sequelae, poststroke cognitive impairment significantly impacts 17.6%–83% of survivors, affecting their rehabilitation, daily living and quality of life. Improving cognitive abilities among patients in stroke recovery is therefore critical and urgent. Transcutaneous auricular vagus nerve stimulation (TAVNS) is a non-invasive, safe, cost-effective treatment with great potential for improving the cognitive function of poststroke patients. This clinical research will evaluate the effectiveness, and help elucidate the possible underlying mechanisms, of TAVNS for improving poststroke cognitive function.

Methods and analysis A single-centre, parallel-group, allocation concealment, assessor-blinded randomised controlled clinical trial. We will allocate 88 recruited participants to the TAVNS or sham group for an intervention that will run for 8 weeks, 5 days per week with twice daily sessions lasting 30 min each. Blood tests will be performed and questionnaires issued at baseline and 8-week and 12 week follow-ups. Primary outcomes will be changes in cognitive function scores. Secondary outcomes will be changes in activities of daily living, quality of life and serum oxidative stress indicators.

Ethics and dissemination The Ethics Committee of the First Affiliated Hospital of Hunan University of Chinese Medicine has approved the protocol (No. HN-LL-YJSLW-2022200). Findings will be published in peer-reviewed academic journals and presented at scientific conferences.

Trial registration number ChiCTR2200057808.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The first single-centre, parallel-group, assessor-blinded, randomised controlled clinical trial to test the effects of transcutaneous auricular vagus nerve stimulation (TAVNS) on poststroke cognitive impairment.
⇒ Integrates diverse outcomes to examine the effects and underlying mechanisms of TAVNS in poststroke cognitive improvement.
⇒ Adherence to out-of-hospital self-administered TAVNS treatments may influence study results.
⇒ Because stimulation site differences are obvious, incomplete patient and accessor blinding may limit study results.

INTRODUCTION

Among stroke sequelae, poststroke cognitive impairment (PSCI) is common, affecting 17.6%–83% of stroke survivors, depending on assessment timing, research setting, demographics and cognitive test/cut-off.[1] PSCI is a clinical syndrome characterised by cognitive deterioration after a clinical cerebrovascular incident, which persists for up to 6 months; it includes both PSCI no dementia and the more severe form, post-stroke dementia.[2 3] Core impaired domains include executive function, memory, attention, language and visuospatial function, which not only lead to disability, dependence and low quality of life,[4] but are closely associated with high risk of recurrent ischaemic stroke[5] and reduced 5-year survival.[2] As the tremendous burdens of stroke continue to rise,[6 7] PSCI has become a mounting public healthcare challenge that urgently needs to be addressed.

Currently, the most relevant PSCI-prevention measures are acute therapy and recurrence prevention.[1] However, there are no unambiguously successful therapeutic techniques, and prospective pharmaceuticals have yet to show efficacy in decreasing or preventing cognitive deterioration following stroke. Acetylcholinesterase inhibitors (AChEIs) and memantine, which are approved for use in Alzheimer's disease (AD), have shown some clinical benefits in vascular dementia.[8 9] However, a recent study revealed little evidence that pharmaceutical therapies, including AChEIs and memantine, help reduce symptoms or slow dementia progression in patients with PSCI.[10] Pharmaceutical therapies also cause side effects like

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gastrointestinal issues (diarrhoea, constipation), headaches and dizziness.[11] Thus, it is critical to develop viable treatments that slow or stop PSCI progression.

Vagus nerve stimulation (VNS) is approved by the United States Food and Drug Administration as an adjunct to medication for treating partial epilepsy, depression and primary headache disorders[12]; it was also recently recognised to have the potential for improving cognitive impairment.[13 14] The vagus nerve connects to the nucleus of the solitary tract (NTS), which activates noradrenergic neurons in the locus coeruleus (LC). This causes norepinephrine (NE) release in memory-forming areas like the hippocampus, basolateral amygdala and medial prefrontal cortex.[15–18] Because noradrenergic stimulation has been demonstrated to improve memory performance, the NTS-LC-NE pathway is thought to play a key role in memory enhancement, which may be activated with VNS.[19–21] In clinical practice, there are two VNS types: invasive (iVNS) and non-invasive (nVNS). Interest in nVNS is greater, because iVNS necessitates a surgical procedure under general anaesthesia and is associated with potential treatment-related complications like cardiac arrhythmias, peritracheal haematomas and vocal cord dysfunction.[22]

Transcutaneous auricular VNS (TAVNS) is a safe, well-tolerated, non-invasive alternative[23] that activates the vagal projections and vagally mediated pathways similar to iVNS.[24] The auricular branch of the vagus nerve (ABVN) is the only branch of the vagus to reach the body surface, at the cymba and cavum conchae.[25] Its afferent fibres pass through the cervical ganglion into the vagal trunk and project to the NTS.[26] Functional brain imaging has shown that TAVNS and iVNS have similar activation patterns, suggesting that the former effectively activates vagal trajectories and has the potential to induce similar effects.[27 28] Clinical studies have also shown that TAVNS can effectively improve cognitive function in those with epilepsy[29] and depression,[30] and in healthy individuals.[31 32] However, whether TAVNS has the same effects in those with PSCI is unclear.

PSCI has a complex set aetiologies, including dysfunctions of the neurovascular unit,[33] neuroinflammation response[34 35] and glymphatic pathway,[36] and in relation to infarcts with AD pathology.[37] Recent studies have shown that low serum superoxide dismutase (SOD) is associated with a high risk of cognitive impairment after acute ischaemic stroke (AIS), suggesting that SOD is a potentially modifiable factor in PSCI.[38] Consistent with this, two animal model studies have shown that iVNS increases serum SOD, attenuating myocardial ischaemic injury and renal ischaemic injury.[39 40] However, it remains to be determined whether TAVNS has the same effects, and is effective, in PSCI.

In this single-centre randomised clinical trial, we aim to assess the efficacy of TAVNS vs sham in PSCI. We hypothesise that compared with sham, TAVNS improves cognitive function, activities of daily living (ADL), quality of life and serum oxidative stress indicators.

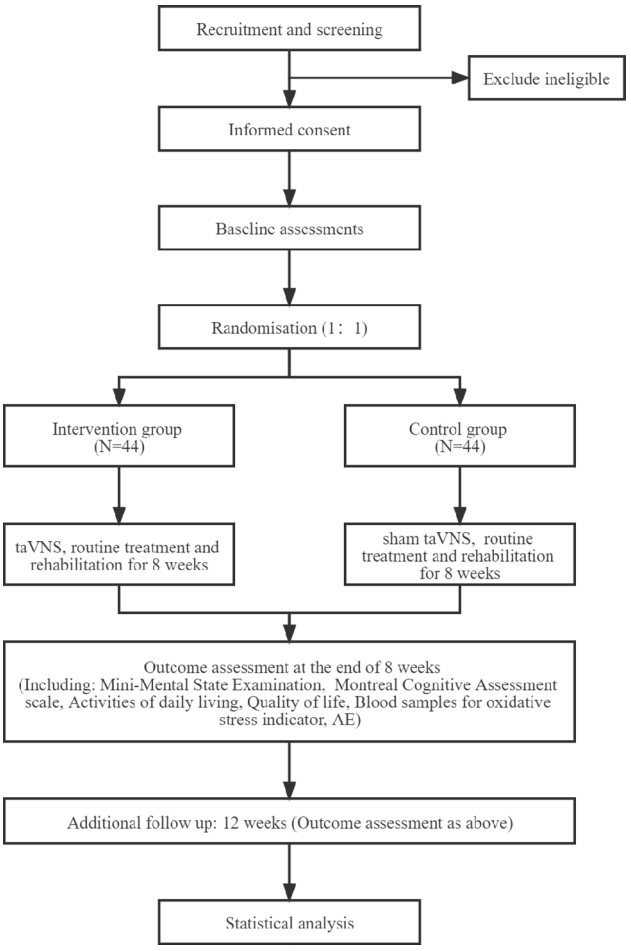

Figure 1 Study flow chart. AE, adverse event; TAVNS, transcutaneous auricular vagus nerve stimulation.

METHODS

Design

This study will be conducted at the First Affiliated Hospital of Hunan University of Chinese Medicine as a single-centre, parallel-group, allocation concealment, assessor-blinded randomised controlled clinical trial. The Ethics Committee of the First Affiliated Hospital of Hunan University of Chinese Medicine has approved the protocol (No. HN-LL-YJSLW-2022200).

Baseline measures will include demographic information, epidemiological data, neuropsychological scores and serum oxidative stress indicators. Data relevant to clinical symptoms and cognitive domains (eg, stroke location and volume) will be collected and included in analyses as confounding factors. Eighty-eight recruited patients with PSCI will then be randomly allocated to either TAVNS or sham group at a 1:1 ratio for eight treatment weeks, according to a randomised number table generated via SPSS. Immediately postintervention (8-week follow-up) and 12 weeks later (20-week follow-up), participants will repeat the baseline neuropsychological scales and serum measurements to test whether TAVNS improves cognitive function and serum oxidative stress levels. The study design flow chart is shown in figure 1 and a timeline summary is presented in table 1. This protocol follows the

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Table 1 Summary of enrolment, intervention and assessment timelines

Study period	Screening	Allocation	Intervention	Follow-up
Timepoint	−2–0 weeks	0 weeks	8 weeks	20 weeks
Enrolment				
Eligibility screen	×			
Basic information	×			
Inclusion, exclusion criteria	×			
Informed consent		×		
Randomisation		×		
Interventions				
TAVNS		◆———	———◆	
Sham TAVNS		◆———	———◆	
Assessments				
Medical history	×			
MMSE		×	×	×
MoCA		×	×	×
ADL		×	×	×
SF-36		×	×	×
Blood samples		×	×	×
Adverse events			×	×
Adherence			×	×
Treatment and rehabilitation			×	×
Daily activities diary			×	×

ADL, activities of daily living; MMSE, Mini–Mental State Examination; MoCA, Montreal Cognitive Assessment; SF-36, 36-Item Short-Form Health Survey; TAVNS, transcutaneous auricular vagus nerve stimulation.

Recommendations for Interventional Trials guidelines.[41] A Standard Protocol Items: Recommendations for Interventional Trials Checklist is provided in online supplemental file.

Patient and public involvement

There will be no patient or public involvement in conducting the study.

Sample size

Sample size calculation was based on Mini–Mental State Examination (MMSE) score improvements, which will be the primary study outcome indicator. Since no studies have evaluated the effects of TAVNS on MMSE scores in PSCI, the minimal clinically relevant effect size is unknown. The sample size was thus calculated based on our preliminary study, in which we assessed the effectiveness of TAVNS (n=5) and sham (n=5) on MMSE scores in patients with PSCI. According to these data, mean pre–post treatment MMSE score differences and SDs in the TAVNS and sham groups were 7±2.73 and 5.4±1.14, respectively. The estimated sample size was calculated using the formula:

$$n=2[(z_{\alpha}+z_{\beta})^{2}\sigma^{2}]/\delta^{2}$$

Alpha (α)=0.05 and power ($1-\beta$)=0.9 determined that a minimum sample of 38 patients per group are needed. Adjusting for a potential 15% study drop-out rate, a total of 88 participants are required (44 per group).

Inclusion criteria

The inclusion criteria are: (1) Age 45–75 years; (2) AIS diagnosis[42] confirmed by CT or MRI; (3) Conscious, with stable vital signs; (4) First-time stroke victim, within 2–4 weeks of onset; (5) National Institutes of Health Stroke Scale (NIHSS) score 5–15; (6) Cognitive impairment, with MMSE score ≤26 and (7) Agree to participate and sign informed consent.

Exclusion criteria

The exclusion criteria are: (1) Concomitant disorder associated with cognitive impairment (eg, epilepsy, AD, brain trauma) or a history of medication use for cognitive impairment prior to stroke; (2) Severe language, vision or psychiatric disorder precluding a cognitive examination; (3) Severe heart, kidney, liver or endocrine disease; (4) Alcohol or drug abuse; (5) Participation in another, conflicting clinical study and (6) Currently taking medication to improve cognitive function.

Withdrawal or drop-out criteria

Participants will be terminated from the trial according to the following criteria: (1) Wanting to discontinue

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the study; (2) Not cooperating with the intervention, outcomes assessments or follow-up; (3) Experiencing a serious study-related adverse event (AE).

Recruitment and screening

Recruitment advertising will be via posters on hospital bulletin boards, flyers distributed in rehabilitation and encephalopathy departments, and a recruitment station in the First Affiliated Hospital of the Hunan University of Chinese Medicine. Potential participants will be screened according to inclusion and exclusion criteria to determine enrolment eligibility. Eligible patients will be given detailed information about the trial by one of two trained research assistants. Those who decide to participate will then be asked to sign written informed consent, after which the baseline assessment will be arranged.

Baseline assessments

Relevant demographic and epidemiological data will be collected, including gender, age, education, smoking, alcohol use, illness duration, stroke location and volume, and NIHSS score. Cognitive function will then be assessed via the MMSE and Montreal Cognitive Assessment (MoCA); the Modified Barthel Index (MBI) will be used to assess daily activities and the 36-Item Short-Form Health Survey (SF-36) will be used to assess the quality of life. Blood samples will also be collected to quantify serum oxidative stress indicators, including SOD, glutathione (GSH), reactive oxygen species (ROS) and malondialdehyde (MDA).

Randomisation and allocation concealment

Following baseline assessments, eligible participants will be randomly allocated to the TAVNS or sham group at a 1:1 ratio. Random allocation sequencing will be generated using the RV.UNIFORM procedure in SPSS V.20.0 (IBM) by an independent research assistant who will not be engaged in study recruitment, assessment or intervention. Therapists will be notified of each patient's allocation and charged with arranging their allotted treatment conditions. Allocation results will be concealed from participants, outcome assessors and statistical analysts and not revealed until the completion of the final data analysis report.

Blinding

Patients, outcome assessors and statistical analysts will remain unaware of patient study allocation. To minimise discussion of the intervention, patients in different groups will not be housed in the same hospital ward. Neither outcome assessors nor statistical analysts will be engaged in intervention administration. Assessors will avoid asking for information about the stimulation location. Group allocation will be coded (A/B) and kept in sealed, opaque envelopes by a specified research assistant. Code identifiers will be unblinded after the completion of all data analyses.

Figure 2 (A) Stimulation device (SDZIIb, Huatuo). (B) Ear clip electrodes. (C) TAVNS (cymba conchae) and sham (earlobe) stimulation locations. TAVNS, transcutaneous auricular vagus nerve stimulation.

Intervention

All participants will continue to receive routine medical or rehabilitative treatment, and regular communication with their primary care physicians will be maintained throughout the study. Routine treatment and rehabilitation will be developed and implemented by the primary care physician in accordance with the Chinese Stroke Association guidelines for ischaemic cerebrovascular diseases[43] and Chinese Stroke Association guidelines for stroke rehabilitation.[44]

Stimulation locations

The right ABVN is closely connected to the sinoatrial node, and its stimulation is more likely to cause changes in heart rate than stimulation of the left ABVN[45]; thus, stimulation will be applied to the left ear in both groups. The TAVNS group stimulation target is the left auricular cymba conchae and the sham group stimulation target is the left earlobe (figure 2). These sites were chosen because the cymba conchae has the highest ABVN projection density (100%), while the earlobe has none.[25] Furthermore, a recent study comparing the effects of different TAVNS stimulation sites on vagal evoked potential responses showed that the cymba and concha regions are active TAVNS targets, while the earlobe is not.[46]

Intervention procedure

TAVNS will be administered via an electronic acupuncture treatment instrument manufactured by Suzhou Medical Instruments Factory (SDZIIb, Huatuo, Suzhou, China, http://www.hwato-med.com). First, patients will take a comfortable position, seated or laying. Next, the skin on and around their left ear will be wiped with alcohol, to eliminate surplus oils and guarantee optimal conductivity. Ear electrode clips will then be placed on the left cymba conchae or earlobe, for TAVNS and sham groups, respectively. Stimulation parameters will be adjusted

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for: (1) waveform: biphasic, continuous, asymmetrically balanced; (2) wave width: 200 µs; (3) wave density: 5 Hz; (4) intensity: adjusted to the strongest sensation the individual can tolerate without pain, typically 4–6 mA; and (5) duration: 30 min/session, twice daily, 5 days/week, for 8 weeks. Other than stimulation location, the two groups' parameters will be consistent, including intervention duration and frequency.

Intervention quality control

All interventions during hospitalisation, for both groups, will be delivered by trained researchers. Patients discharged before the end of the 8-week intervention will be asked to participate in a TAVNS training course, in which a researcher will show them how to operate the TAVNS, including stimulation location and parameter settings. Participants who are approved by the trainer as familiar with the TAVNS device will self-administer the intervention at home, and complete a daily diary about the treatment.

Follow-up

After the 8-week intervention, all participants will be followed for another 12 weeks. During the follow-up period, all participants may return to their original lifestyle but will be asked to record daily activities, rehabilitation exercises and medication use. Research assistants will conduct weekly follow-up via telephone or video call to maintain participant interest and retention. Finally, all outcome indicators will be reassessed at the end of follow-up.

Treatment adherence

To enhance intervention adherence after hospital discharge, researchers will send treatment reminders twice daily via WeChat and require patients to keep a daily treatment diary, including details of when each treatment began, when it ended, side effects and improvements. Regular assessments will also be made to check all diaries and weekly telephone or video calls will be made throughout the treatment period. Participants will receive RMB 100 if they complete the project successfully, regardless of group allocation. If a patient misses two consecutive treatment weeks, or more than two total weeks, a judicious discussion and decision about whether to exclude them will occur.

Outcome assessment

All outcomes will be measured at baseline (−2 to 0 weeks), after the intervention period (8 weeks), and at the end of the study period (20 weeks). All outcome assessments will be administered by two neurologists from the First Affiliated Hospital of Hunan University of Chinese Medicine, who will be unaware of the participants' group allocation.

Primary Outcomes

The MMSE (Chinese version) will be used to measure cognitive function.[47] The MMSE is a brief 30-point questionnaire on which scores≤26 indicate that cognitive impairment may be present. Ten of the items are focused on time and place orientation, short-term memory, attention and calculation, recall, language skills, repetition, complex commands, reading, writing and pentagram reproductions.[48] However, the MMSE may be inadequate for detecting clinical signs of mild cognitive impairment and dementia[49 50]; thus, the MoCA will also be used.

The MoCA is a short, 30-point test on which higher scores indicate better cognitive functioning. The scale assesses global cognitive abilities in attention, naming, visuospatial/executive functions, memory, language, visual structure skills, abstraction, calculation and orientation.[51] When participants have received fewer than 12 years of education, one point will be added to their overall score. A score of ≤26 is considered intellectually deficient. The revised Chinese version (Beijing version) is widely used and has strong validity and reliability.

Secondary outcomes

The MBI, a commonly used, standardised scale, will be used to measure ADL. The MBI consists of 10 items totalling 100 points, with higher scores indicating greater daily self-care.[52]

Quality of life will be measured by the Medical Outcomes Study SF-36, which is currently the most common clinical quality of life evaluation used in both patient and general populations. The 36-item SF-36 (Chinese version) measures eight health concepts, with excellent reliability and validity in Chinese samples[53]; higher scores indicate better health.[54]

Venous blood samples will be collected to measure oxidative stress indicators and their change rate for both groups. At each sampling, 10 mL of venous blood will be collected from the participant's left antecubital vein and sealed in vacutainer tubes with 0.5 mm EDTA. The whole blood sample will then be centrifuged at 2000 r/min for 10 min at room temperature, after which the supernatant serum will be transferred to individual, clean tubes and stored at −80°C for analysis. SOD will be measured by pyrogallol autoxidation (Superoxide Dismutase Detection Kit, Fuyuan, Fujian, China); GSH will be measured by Glutathione Assay Kit according to the manufacturer's instructions (Sigma-Aldrich R., California, USA); ROS will be measured by ROS assay kit (Beyotime Biotechnology, Shanghai, China); MDA will be measured by condensation with thiobarbituric acid at 532 nm using a Teb Pazhouhan Razi Company kit (Tehran, Iran).

Safety evaluation

AEs including dizziness, pain and unpleasant sensations at the stimulus point have been reported by a few patients in previous studies,[23] although these were mostly transitory and mild. Here, if any AE occurs during the treatment or follow-up period, it will be responded to with the appropriate intervention and management, and documented via case report form. Serious AEs will be reported immediately to the Ethics Committee to determine whether study withdrawal is necessary. Patients may also withdraw

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for any safety reason or if it is in their best interest to do so.

Data management and monitoring

Data will be collected by the outcome assessors using a printed case report form, which will be independently entered into the electronic data capture system by two research assistants who are not engaged in study allocation, treatment, or assessment. The electronic data system will be accessible to the Hospital Research Centre, which will be responsible for research data monitoring and auditing. All private participant data will be coded and protected. After study completion, all documents will be securely stored for 5 years.

Statistical analysis plan

A statistician who is uninvolved with study allocation, treatment and outcome evaluation will conduct statistical analysis using SPSS V.20.0. Missing outcome data will be extrapolated using a multiple imputation method. For continuous variables, a Shapiro-Wilks distribution normality test will be performed, followed by log-transformed adjustment for non-Gaussian distributed variables. Normally distributed continuous variables will be presented as mean, SD and non-normally as median, IQR. Categorical variables will be presented as frequency or percentage.

Two-sided tests with a 5% significance level will be used for hypothesis testing. For between-groups baseline characteristic comparisons, continuous variables will be analysed using the t-test or Mann-Whitney U test and categorical variables will be analysed using the Pearson χ^2 or Fisher exact probability test. One-way analysis of variance with repeated measures (Kruskal-Wallis test) will be used to test primary and secondary outcome data. If the omnibus variance analysis is significant, then Bonferroni-adjusted post hoc analyses will be used to compare paired study periods. The Pearson χ^2 or Fisher exact probability test will be used to analyse change rates of oxidative stress indicators. To measure clinical effect magnitude, we will calculate effect size estimates using Cohen's d.

Ethics

The study has been approved by the Ethics Committee of First Affiliated Hospital of the Hunan University of Chinese Medicine and will be conducted in strict accordance with the Declaration of Helsinki. All participants will provide written informed consent, after a minimum of 3 days' consideration. All study staff will be trained in good clinical practice prior to the intervention, to achieve better study outcomes. Raw data will be anonymised and encrypted, ensuring that only the researchers actively participating in the study will have access to them.

Dissemination

The study protocol has been registered and is available on the Chinese Clinical Trial Registry website (http://www.chictr.org.cn; No. ChiCTR2200057808). Study results, positive and/or negative, will be published in peer-reviewed academic journals.

DISCUSSION

This will be the first randomised controlled trial to compare the efficacy of a TAVNS vs sham intervention for improving PSCI. It will explore whether TAVNS is effective for improving cognitive function, daily functioning and quality of life among patients with PSCI. In addition, serum oxidative stress indicators SOD, GSH, ROS and MDA will be measured to help explain the neural mechanism(s) by which TAVNS improves PSCI. The study results may advance a novel, safe, effective PSCI treatment strategy with few adverse effects.

Compared with VNS, TAVNS is more convenient and economical, has fewer side effects and has been used in patients with epilepsy,[55] depression[56] and tinnitus.[57] However, the effects of TAVNS in PSCI have yet to be tested. Our stimulation parameters are based on a published study showing that TAVNS can help enhance adults' ability to learn novel letter–sound relationships.[58] In their study, Thakkar *et al* reported that TAVNS at 5 Hz and 200 µs can improve automaticity and decoding task performance.[58] As participants vary in the stimuli intensity they can perceive and tolerate, 4–6 mA stimulus frequency will be used herein, adjusted below participants' pain tolerance levels.

Notably, TAVNS treatment duration has varied across studies,[59] including in stroke rehabilitation for 10 days,[60] 6 weeks[61] and 8 weeks.[62] Because no study to date has examined the effects of TAVNS on PSCI prevention or treatment, our design is based on assessments of verbal memory performance in patients with epilepsy. Mertens *et al*[29] reported improved verbal memory performance after 6 weeks of VNS. Considering that our TAVNS intervention will begin around week 4 poststroke, and that 12 weeks is widely recommended as the diagnostic time point for PSCI, it is reasonable to extend treatment to 8 weeks so that we can examine cognitive function changes.

Cognitive function tends to decline rapidly over a short period after acute stroke, and then progressively (and at least partly) recover.[1] Yet because of the unpredictability of recovery and symptom onset latency, identification is often challenging and time-consuming. In this study, we will limit inclusion to participants who are 2–4 weeks poststroke onset, to identify early cognitive impairment. The assessment period is also therefore 3–6 months poststroke, which is widely recommended as the diagnosis timeline. Thus, our timeline will best explain the TAVNS intervention effects and allow evaluation of relatively long-term efficacy.

Limitations

There are several possible study limitations. First, it is possible that some enrolled patients will be discharged before completing the 8-week intervention. Quality and adherence to out-of-hospital self-management

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interventions may influence the primary or secondary outcomes. Thus, we will provide discharged patients with special TAVNS training and require that they complete a daily treatment diary. Second, differences in patient lifestyles, treatment and rehabilitation during the follow-up period may affect results. To this end, we will collect relevant data during the follow-up period and adjust statistical analyses, as needed. Finally, patient and accessor blinding may be incomplete, as differences in stimulation sites are easily recognisable.

Trial status

This trial has been registered since 17 March 2022, and was approved by the Ethics Committee of the First Affiliated Hospital of Hunan University of Chinese Medicine in April 2022. The trial began on 1 May 2022 and its completion is expected by the end of June 2023.

Contributors Z-DL conceived, designed and contributed to drafting the study protocol. H-JQ and X-QW wrote several protocol sections. C-CZ participated in study coordination and implementation. Y-JZ designed and revised the study protocol. All authors contributed to writing the protocol description and have read and approved the final version for submission.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s)

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A The sponsor and funder are not involved in this study
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7-9
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9-10
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12-13
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	16-17
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14-16
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see	8, table 1

		Figure)	
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11-12
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	14-15
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention	11-12

		during the trial	
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	17
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14, 16-17
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	17
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	17-18
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A Additional statistical analyses will be conducted on a caseby-case basis after trial data

			collection is complete
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	17
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10,16-17
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	16-17
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	18
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg,	18-19

		investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10, 18
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A Blood samples will be destroyed after use
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11-12, 17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17, 22
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18-19, 22
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	22
Dissemination policy: reproducible	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and	22

research		statistical code	
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Available in Chinese vesion
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A Blood samples will be destroyed after use

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