Effect of hyperbaric oxygen therapy combined with repetitive transcranial magnetic stimulation on vascular cognitive impairment: a randomised controlled trial protocol

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ABSTRACT
Introduction Vascular cognitive impairment (VCI) has an increasing prevalence worldwide, accounting for at least 20%–40% of all diagnoses of dementia. The decline in cognitive function seriously impairs patients’ activities of daily living and social participation and reduces their quality of life. However, there is still a lack of advanced, definitive rehabilitation programmes for VCI. Hyperbaric oxygen therapy (HBOT) and repetitive transcranial magnetic stimulation (rTMS) are recognised treatments for improving cognitive impairment. The former can restore oxygen supply in the brain by increasing oxygen partial pressure in brain tissue, while the latter can enhance neuronal excitability and promote synaptic plasticity. However, no studies have explored the effect of HBO combined with rTMS on VCI.

Methods and analysis This study is designed as a single-centre, assessor-blind, randomised controlled clinical trial with four parallel arms. A total of 72 participants will be recruited and randomly assigned to the control group, HBOT group, rTMS group and HBOT combined with rTMS group at a ratio of 1:1:1:1. All enrolled participants will receive conventional treatment. The entire intervention period is 4 weeks, with a 3-week follow-up. Outcomes will be measured at baseline (T0), after a 4-week intervention (T1) and after an additional 3-week follow-up period (T2). The primary endpoint is the Montreal Cognitive Assessment score. The secondary endpoints are Mini-Mental State Examination score, Modified Barthel Index score, latency and amplitude of P300, cerebral cortical oxygenated haemoglobin (HbO2) and deoxygenated haemoglobin (HbR) concentrations as measured by task-state functional near-infrared spectroscopy.

Ethics and dissemination Ethics approval was obtained from the West China Hospital Clinical Trials and Biomedical Ethics Committee of Sichuan University (ethics reference: 2022 (1972)). The findings will be published in peer-reviewed journals and disseminated through scientific conferences and seminars.

Trial registration number ChiCTR2300068242.

STRENGTHS AND LIMITATIONS OF THIS STUDY
⇒ Our study will comprehensively evaluate the effect using clinical scales, P300 and task-state functional near-infrared spectroscopy (fNIRS).
⇒ P300 can objectively reflect the neuroelectrophysiological changes in the brain during the cognitive process.
⇒ fNIRS will be used to collect cerebral haemodynamic changes in the cerebral cortex.
⇒ It is difficult to blind the participants and intervention implementers because they know what intervention is being performed.
⇒ Long-term follow-up evaluation will be lacking in this study.

INTRODUCTION
Cerebrovascular diseases and their risk factors are prevalent in the growing elderly population and are the most important causes of vascular cognitive impairment (VCI). VCI includes subjective cognitive decline, mild cognitive impairment and vascular dementia (VaD).1 The incidence, prevalence, disability and mortality rates of VCI are increasing, with serious economic and social consequences.1 VCI is common in patients who had a stroke, and cognitive impairment is closely related to poor prognosis in patients who had a stroke.2 VaD is the second-leading cause of dementia, accounting for 15%–30% of dementia cases.3 Due to frequent contraindications, side effects and unclear pathological mechanisms, aetiological therapy and drug therapy for VCI are still limited.4 Therefore, it is necessary to explore more effective and diverse treatment methods.

It has been reported that hyperbaric oxygen therapy (HBOT) improved cognitive function in patients with mild cognitive impairment, Alzheimer’s disease (AD) and...
VaD.\(^5\)\(^6\) A meta-analysis\(^7\) of 25 randomised controlled trials (RCTs) showed that HBOT significantly improved cognitive function and the ability to perform activities of daily living in VaD patients compared with the control group. Some fundamental studies\(^8\)-\(^11\) have explored the mechanism of HBOT for cognitive impairment. HBO therapy increased blood supply and promoted neurogenesis in the piriform cortex in VD rats to improve cognitive function.\(^8\)\(^9\) Zhao et al.\(^9\) showed that HBO therapy reduced hippocampal neuronal apoptosis and reduced phosphorylation of the P38 MAPK signalling pathway in AD rat models. Shapira et al.\(^9\) found that HBOT improved cognitive function in mouse models of AD by reducing neuroinflammation. In a rat model of cerebrovascular ischaemia-reperfusion injury (IR), HBO prevented IR-induced neuronal damage by attenuating autophagy, inflammation and calcium overload, thereby alleviating cognitive impairment.\(^11\) Mechanisms of HBOT for VaD include increasing oxygen partial pressure, reducing intracranial pressure, promoting tissue healing and angiogenesis, reducing cell apoptosis and neuroinflammation, relieving oxidative stress and increasing mitochondrial function.\(^6\)\(^8\)\(^11\)\(^12\)

Some studies have reported that repetitive transcranial magnetic stimulation (rTMS) improved overall cognition, memory and attention in poststroke cognitive impairment (PSCI).\(^13\)-\(^16\) A meta-analysis showed that rTMS had significant positive effects on attention, memory, working memory (WM) and overall cognition in patients with PSCI.\(^17\) In cerebral ischaemic rats, rTMS enhanced neuronal excitability and promoted synaptic plasticity, thereby regulating cognitive function.\(^18\) rTMS improved learning and memory in VaD rat models, which may be related to promoting the expression of brain-derived neurotrophic factor (BDNF) and the restoration of cholinergic system activity in the CA1 region of the hippocampus.\(^19\) Low-frequency rTMS improved memory and learning, increased synaptic plasticity and protected synapses by increasing Bcl-2 expression and decreasing Bax expression in VaD model rats.\(^20\) The mechanisms of rTMS for VCI mainly include enhancing neuronal excitability, promoting synaptic plasticity, promoting the expression of vascular endothelial growth factor and BDNF, and promoting the recovery of cholinergic system activity.\(^18\)\(^19\)\(^21\)

Although the significant improvement in cognition of HBOT and rTMS has been confirmed by most studies, there were a small number of studies reporting that HBOT and rTMS do not improve cognition in stroke and dementia.\(^22\)-\(^26\) Therefore, this study can provide evidence to verify the improvement in cognition by HBOT and rTMS. Since HBOT and rTMS have different mechanisms for improving cognition, we hypothesised that these treatments alone or in combination will produce a clinically significant improvement in the cognitive function of patients with VCI and that the improvement in combination will be synergistic compared with each treatment in isolation.

**METHODS**

**Study objective**

The objective of this study is to investigate the effect of HBOT combined with rTMS on cognitive function, daily living ability and cortical cerebral haemodynamic changes in the cerebral region of interest as measured by task-state functional near-infrared spectroscopy (fNIRS) in patients with VCI.

**Study design and setting**

This is a single-centre, assessor-blind, RCT with four parallel arms (control, intervention 1, intervention 2 and intervention 3). All subjects will be randomly assigned to the control group, HBOT group, rTMS group and HBOT combined with rTMS group at a ratio of 1:1:1:1. The entire intervention period is 4 weeks, with a 3-week follow-up. Primary and secondary outcomes will be measured at baseline (T0), after a 4-week intervention (T1) and after an additional 3-week follow-up period (T2). The study will be conducted at the Rehabilitation Medicine Center, West China Hospital, Sichuan University (Chengdu, Sichuan, China). The flow diagram of the study design is shown in figure 1. The research protocol follows the Standard Protocol Items: Recommendations for Intervention Trials 2013.\(^27\)

**Estimation of the sample size**

Wu et al.\(^28\) established that the minimum clinically significant difference in the Montreal Cognitive Assessment (MoCA) scores in stroke recovery patients is 1.22 points. Based on the fact that no previous study provides a significant difference in MoCA scores for HBOT combined with rTMS treatment for VCI, we estimated the effect size to be 0.5. The sample size calculation was conducted via G*power of 3.1 with a significance level of α=0.05 (two tailed), the estimated effect size of f=0.5, power (1−β)=80% and number of groups=4. We calculated a total sample size of 48 people and planned to recruit 72 people accounting for sample attrition.

**Study population**

**Recruitment**

The study population will be recruited from the Rehabilitation Medicine Center, West China Hospital, Sichuan University, Chengdu, Sichuan Province, China. The inclusion and exclusion criteria for the study population are as follows.

**Inclusion criteria**

The study population should meet all of the following criteria:

1. Be diagnosed with VCI according to the Guidelines for Diagnosis and Treatment of Vascular Cognitive Impairment in China (V.2019).\(^29\) Impairment of at least one cognitive domain in executive function, attention, memory, language and visual space, confirmed by CT or MRI.
2. Age between 20 and 80 years, and the gender is not limited. Due to polypharmacy, comorbidities, consent
limitations, and more, in order to preserve patient safety, it is reasonable to exclude elderly patients over 80 years of age from clinical trials.30 31

3. The Mini-Mental State Examination (MMSE) scores <25 points or the MoCA scores <26 points.
4. Education level is primary and above.
5. Agree to sign informed consent.

Exclusion criteria
The criteria for exclusion are as follows:
1. Suffer from brain tumours, encephalitis, AD or traumatic brain injury.
2. Vital signs (body temperature, pulse, respiratory rate, blood pressure and oxygen saturation) are unstable or abnormal, and the disease progresses rapidly.
3. Severe impairment of speech, hearing and vision.
4. Be unconscious or persistent vegetative state.
5. Severe mental disorder or obvious depression.
6. Suffer from epilepsy or have a history of epilepsy or a family history of epilepsy.
7. Severe dysfunction or failure of the heart and lungs. It manifests as dyspnoea, chest tightness, shortness of breath, systemic hypoxia, cyanosis and lip blackness.
8. Contraindications for rTMS (e.g., intracranial metallic implants, microprocessor implants in the body, history of seizures, tumours and pregnancy).
9. Contraindications for HBOT (e.g., open pneumothorax or haemothorax, claustrophobia, unstable blood pressure, pulmonary bullae, severe infection and severe bleeding tendency).

Withdrawal or drop-out criteria
The intervention for participants will be interrupted if any of the following conditions occur:
1. During the trial, the participant develops an exacerbation, severe complications, claustrophobia or abnormality in body temperature, pulse, respiratory rate, blood pressure or oxygen saturation. Nasal drops will be prepared in the chamber for use by patients with sinus barotrauma, and if symptoms are not relieved, the patients will be withdrawn from the trial.
2. The participant has poor compliance and could not insist on accepting the intervention.
3. The participant rejects measurement or is unwilling to continue the trial.
Patient and public involvement
The study is currently in the recruitment phase. No patients and/or public will be involved in the study design, recruitment, implementation or reporting. The results will be publicised through conferences and academic papers.

Randomisation and blinding
Participants will be randomly assigned according to the random numbers generated by the PLAN procedure of statistical software SAS V.9.0. An independent, non-participating research assistant will place random numbers into opaque envelopes and open them in order as they were assigned. It is impossible to blind the participants and intervention implementers because the intervention includes rTMS, HBOT and rTMS combined with HBOT. However, the trial will blind the outcome evaluator and statistician. The outcomes will be assessed by a rehabilitation therapist who knows nothing about the study. Before unblinding, an independent statistician will complete the data analysis.

Interventions
All enrolled participants will receive conventional treatment (CT), including pharmacotherapy for cerebrovascular diseases, cognitive training, exercise therapy, physical factor therapy, occupational therapy and traditional Chinese therapy. Participants in the control group will only receive CT. Participants in intervention group 1 will receive HBOT coupled with CT. Participants in intervention group 2 will receive rTMS coupled with CT. Participants in intervention group 3 will receive HBOT combined with rTMS on the basis of CT.

Figure 2 The schedule of enrolment, interventions and assessments. fNIRS, functional near-infrared spectroscopy; HBOT, hyperbaric oxygen therapy; MBI, Modified Barthel Index; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; rTMS, repetitive transcranial magnetic stimulation; W, week.
Hyperbaric oxygen therapy

The HBOT equipment is a medical hyperbaric oxygen chamber with three cabins and seven doors (Yantai Ice Wheel, Shandong Province, China). The treatment pressure of HBOT is 2.0 atmospheres absolute (ATA). The rise time of pressure is 20 min, and the decompression time is 20 min. When the pressure in the chamber is stable, the participants will be asked to breathe 100% oxygen for 60 min and chamber air for 10 min. The 10 min air-break is in the middle of the two 30 min oxygen inhalations. The HBOT course consists of 40 sessions. The duration of each HBOT is 110 min, and the treatment frequency is twice daily for 5 days per week for 4 weeks. To reduce the chance of oxygen toxicity and to maintain HBOT's high safety standards, a minimum 4 h break will be conducted between sessions.

Repetitive transcranial magnetic stimulation

In this study, a magnetic simulator (YRD CCY-I, YIRUIDE Medical Equipment, Wuhan, China) with a figure-8 coil will be used for rTMS treatment. According to the F3 and F4 positions specified in the International 10–20 electroencephalography system, the coils of the rTMS will be placed on the participant’s left dorsolateral prefrontal cortex (DLPFC). The stimulation intensity will be set at a 100% exercise threshold level, which is the minimum intensity of stimulation necessary to produce a motor-evoked potential (MEP, greater than 50 μV) at least 5 of 10 times in the right abductor pollicis brevis. rTMS will employ a 10 Hz stimulation frequency (5 s durations, 25 s pauses) with a total of 2000 pulses. The duration of each rTMS session is 20 min, and the treatment frequency is once daily for 5 days per week for 4 weeks.

Follow-up

All participants will enter an additional 3-week follow-up period after the 4-week intervention. During the follow-up period, the participants will resume their original treatment. Participants will be asked weekly by phone about their feelings and daily information. Outcome indicators will be measured at the end of the 3-week follow-up period.

Outcome measures

Baseline information of the participants will be collected, including name, sex, age, educational level, tobacco and alcohol history, risk factors for cerebrovascular disease, onset time of stroke, lesion site, type of stroke and course of disease. In addition, the assessment of cognitive function by MoCA, MMSE, and P300, the assessment of the ability to perform activities of daily living by the Modified Barthel Index (MBI), and the assessment of mental state by the Self-Rating Depression Scale and the Self-Rating Anxiety Scale will be recorded. Outcome indicators will be evaluated at T0, T1, and T2.

Primary endpoint

The MoCA is a rapid screening instrument for mild cognitive dysfunction and has higher sensitivity but lower specificity than the MMSE. It assesses attention, executive functions, visual space, abstract thinking, memory, language, computation and orientation. The total possible score is 30 points, and a score of 26 or above is considered normal. This scale has been well validated in the Chinese population. The MoCA will be measured at T0, T1, and T2. The absolute score of the MoCA, the absolute changes in scores from T0 to T1 and T2, and the number of patients whose scores change to normal will be recorded. The minimum clinically significant difference of MoCA is 1.22 points.

Secondary endpoints

Mini-Mental State Examination

The MMSE is a commonly effective tool for screening cognitive function in stroke, including 30 items in 5 directions, such as orientation, memory, attention and numeracy, recall, and language. The score of each item is one point, and there are 30 points in total. The scale score is positively correlated with cognitive ability. An MMSE score of 25 or more indicates normal cognitive status, a score of 21–24 indicates mild dementia, a score of 10–20 indicates moderate dementia and a score of 9 or less indicates severe dementia. The absolute score of the MMSE, the absolute changes in scores from T0 to T1 and T2, and the number of patients whose scores change to normal will be recorded.

Modified Barthel Index

The MBI is used to measure the performance of activities of daily living in stroke patients and has high reliability. It assesses bowelts, bladder, rooming, toilet use, feeding, transfer (bed to chair and back), ambulation, dressing, stairs and bathing. Each item is scored on a 5-point ordinal scale that varies from item to item (e.g., 0, 1, 3, 4 or 5 for personal hygiene; 0, 3, 8, 12 or 15 for ambulation). The maximum total score is 100, and the higher the score, the better the ability to perform activities of daily life.

P300

Event-related potential (ERP) can reflect the electro-neurophysiology changes in the brain during cognitive processes. P300 is the ERP component most closely related to cognitive processes, also known as cognitive potential. Before the assessment, the participant will be given an explanation of the basic procedure of the detection and instructed to silently count the number of target stimuli. During the test, the participant will be placed in a relaxed supine position in a quiet environment. The recording electrode will be placed on top of the skull. The reference electrode will be placed on the lower margin of the mastoid process behind both ears. The ground electrode will be located in the middle of the forehead of the participant. The impedance between the electrode and the skin is less than 5 kΩ. The oddball auditory stimulus mode will be selected, with target and non-target stimuli alternating irregularly. The target stimulus is a high-frequency short sound, and the non-target stimulus is a...
low-frequency short sound, with a probability ratio of 1:4. The total stacking times are 200 times. After completing the test, the participant will be asked to name the number of target stimuli, and the latency and amplitude of P300 induced by the target stimuli will be recorded.

Task-state fNIRS

fNIRS is an optical, non-invasive neuroimaging technique that can monitor changes in HbO₂, HbR and cortical haemodynamics in the cerebral cortex in real time. Cerebral blood flow changes in the participants during the task will be collected using a multichannel fNIRS system (NirSmart, Danyang Huichuang Medical Equipment, China), which has dual wavelengths (730 nm and 850 nm). The acquisition cap consists of 16 detectors and 22 LED emitters, resulting in a total of 48 channels. The average distance from the source and detector is 30 mm. The whole channel sampling frequency is more than 11 Hz.

We will use the WM task as a cognitive test. WM, which involves the ability to temporarily store and manipulate information in the brain to perform complex cognitive tasks, is an important cognitive ability. In this test, the form of WM requires participants to remember coloured shapes and their order and click them on a touch-screen computer. The test includes a WM search task and an NWM search task. In the WM search task, three shapes will appear in sequence on the tablet. The participants need to remember the colours and shapes and their order of appearance, and then they will be asked to click them in order in the summary diagram. In the NWM search task, participants simply click in sequence according to the graph presented without memorising. The two sets of tasks will alternate and be repeated four times.

After entering the infrared assessment room, the participants will be asked to become familiar with the environment and eliminate the nervous mood. After wearing fNIRS headcaps, participants will be given a WM task, and the changes in cerebral haemodynamics during the task will be simultaneously collected. The collected fNIRS data will be analysed in the Network module of the NirSpark software package (Huichuang, China).

Safety measurements

The informed consent form lists adverse events that may occur during treatment, such as headache, barotrauma, seizures, oxygen poisoning, decompression sickness, tinnitus and gastrointestinal disturbances. Participants will be taught to properly open the eustachian tube (pinch nasal blowing or swallowing) during the pressure boost to avoid barotrauma. The Numeric Rating Scale will be used to assess the severity of side effects. The scale ranges from 0 to 10, with higher values indicating higher intensity. The scale has been validated in the Chinese language. During the experiment, if subjects have other complications or special physiological changes, the doctor and researcher will take timely measures to treat the symptoms and record the adverse event case report form (CRF).

Data collection and management

The outcome evaluator will use the paper version of the CRF to collect data in a timely, complete and accurate manner. An independent researcher will input the data of the CRF into Excel software. The data will be stored at the West China Hospital of Sichuan University for review by the investigators, the research authorities and the ethics committee. The medical privacy and personal information contained in this study is confidential and will not be made public. The safety and process of the study will be monitored by the West China Hospital Clinical Trials and Biomedical Ethics Committee of Sichuan University, which has the authority to terminate the trial if a serious emergency occurs.

Statistical analysis

Statistical analysis will be performed by an independent statistician using SPSS V.26.0 software, with a significance level set at α=0.05. The Kolmogorov-Smirnov test will be used to test the normal distribution of the data. Continuous variables conforming to a normal distribution will be expressed as the mean (±SD), and those not conforming to a normal distribution will be expressed as the median and IQR. Numbers or percentages will be used to describe the categorical variables. To compare baseline characteristics between groups, the t-test or Mann-Whitney U test will be used for continuous variables, and the Pearson χ² or Fisher’s exact test will be used for categorical variables. Primary or secondary outcomes will be analysed based on the intention-to-treat principle, and the missing data will be interpolated using a multiple imputation approach. The analysis of variance or Wilcoxon test will be conducted for outcome measure analyses.

Ethics and dissemination

Ethics

The study was approved by the West China Hospital Clinical Trials and Biomedical Ethics Committee of Sichuan University in February 2023 (ethics reference: 2022 (1972)). In accordance with the Declaration of Helsinki, participants will be informed of the study objectives, risks and benefits and the interventions to be used before the trial. Before enrolling in the trial, participants will sign the informed consent document.

Dissemination

The protocol has been registered on the Chinese Clinical Trial Registry website (registration number: ChiCTR2300068242). The dissemination of results will be in accordance with the recommendations in the Consolidated Standards of Reporting Trials declaration. The research will be published in peer-reviewed journals and disseminated through scientific conferences and seminars. In addition, a master’s thesis will be written based on the content of the study.
**DISCUSSION**

Age-related cognitive impairment remains an area that requires significant financial and scientific investment. More trials are needed to explore effective and diverse rehabilitation programmes for VCI, which are conducive to its application in clinical practice. At present, no studies have revealed the effect and potential mechanism of HBOT combined with rTMS on VCI. To confirm its efficacy and effect, we conducted this prospective RCT with a 4-week intervention and 3-week follow-up. Our study will evaluate the improvement of cognitive function in patients with VCI by clinical scales and P300. In addition, fNIRS will also be used to collect cortical cerebral haemodynamic changes during cognitive tasks in participants. The results of the integrated data will fully validate the research hypothesis.

For cognitive function, we will not only use MMSE and MoCA scales to assess the improvement of patients’ cognitive function in memory, attention, orientation, language, visuospatial, executive ability and abstract thinking but also assess the objective neurotic electrophysiology indicator. The ERP technique is a kind of special evoked potential, which is an objective evaluation of the advanced mental activities of the brain. It has the advantages of high temporal resolution, noninvasiveness, objective quantification and good repeatability. Among them, P300 is most closely related to the cognitive process, reflecting the brain’s potential changes in the processing of perception, attention, memory, emotion and other information in the cognitive process. P300 is generally induced by the oddball experimental paradigm, that is, two or more different stimuli are used to continuously appear alternately, and the occurrence rate is divided into nontarget stimuli (high probability) and target stimuli (low probability). The subject needs to identify the target stimulus to trigger P300. The smaller the probability of the target stimulus, the higher the amplitude of P300. P300 results are typically characterised by latency and amplitude. The P300 amplitude is positively correlated with the input of mental resources, reflecting the updating of representations in WM. The latency period is positively correlated with the task difficulty, reflecting the response time to the evaluation and classification of stimuli.

For cortical activation, fNIRS is a non-invasive optical technique that can observe changes in cortical activation during a cognitive task by determining the intensity of the scattered light in the cortex. Oxygen-Hb (HbO₂) changes are generally considered to be important indicators of brain activity intensity and cognitive function. The increase in HbO₂ is positively correlated with cortical excitability. Yang et al. proposed that increased cortical activation between bilateral cerebral hemispheres, as measured by fNIRS, was a promising biomarker for evaluating the effectiveness of neuroregulatory therapy in PSCI. In recent years, some studies have applied fNIRS to obtain the activation state information of different brain functional regions after TMS to further study the mechanism of TMS in different diseases. Chu et al. demonstrated that intermittent theta-burst stimulation improved cognition based on activation of the stimulus site (DLPFC) and some distant regions. However, no studies have used fNIRS to collect information on cortical activation after HBT.

We hypothesise that HBOT combined with rTMS can improve the cognitive function of patients with VCI by increasing the oxygen partial pressure of brain tissue, enhancing neuronal excitability and promoting cerebral cortex activation. Seventy-nine VD patients treated with HBOT (2.0 ATA) for 12 weeks showed improvements in MMSE scores, elevated serum humanin levels and increased brain metabolism. Humanin is a unique mitochondrial-derived peptide in the human body that has neuroprotective effects and has been found to protect against cognitive decline in clinical and experimental studies. This suggests that HBOT can improve cognitive function in VD patients by enhancing mitochondrial function and brain metabolism. HBOT also prevented repeated cerebral IR injury-induced neuronal damage by attenuating autophagy, inflammation and calcium overload. Cha et al. suggested that high-frequency rTMS on the same focal DLPFC may have a beneficial effect on the anti-inflammatory response and cognition of brain network changes in patients with PSCI for at least 3 months. Yin et al. showed that cognitive improvements in patients with 10 Hz rTMS were associated with increased low-frequency fluctuations in the DLPFC and increased functional connectivity between the right medial prefrontal cortex and the right ventral anterior cingulate cortex compared with the control group. Therefore, we hope to determine the efficacy of HBOT combined with rTMS in cognitive function and daily living function for patients with VCI. Our results may provide valuable information for the development of a new rehabilitative treatment for cognitive dysfunction.

**Contributors** CH and WX conceptualised and designed the study. WX and XC drafted the initial manuscript. XM, SS, HM, JY and CH participated in the study design and initiation and provided recommendations for the study protocol. All authors have read and approved the final submitted manuscript.

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**Competing interests** None declared.

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