BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-081751
Article Type:	Protocol
Date Submitted by the Author:	06-Nov-2023
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Keywords:	Transcranial Magnetic Stimulation, PSYCHIATRY, NEUROLOGY

Title: Efficacy of MRI-guided rTMS for posttraumatic stress disorder by modulating amygdala activity: study protocol for a randomized controlled trial

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Keywords:
Posttraumatic Stress Disorder, Transcranial Magnetic Stimulation, amygdala, ventrolateral prefrontal cortex

Word count: 3443

ABSTRACT

Introduction

Posttraumatic stress disorder (PTSD) is a prevalent and severe psychiatric disorder. Repetitive transcranial magnetic stimulation (rTMS) targeting the dorsolateral prefrontal cortex provides limited relief for symptoms of PTSD. This study will be conducted to validate the efficacy of MRI-guided rTMS in targeting the sites most closely associated with the amygdala for PTSD patients. We hypothesize that the intervention will improve clinical symptoms by decreasing amygdala activity in patients.

Methods and analysis

A randomized, double-blind, sham-controlled trial will be conducted. Forty-eight eligible PTSD patients will be randomly assigned to receive either active or sham MRI-guided rTMS for 10 consecutive days after the initial MRI scans. MRI scans will be recollected at the end of the intervention. Clinical assessments will be performed at baseline, treatment day 5, treatment day 10, and 2 weeks, 4 weeks, 8 weeks after completion of the intervention to monitor changes in clinical symptoms. The primary assessment outcome is the change in PTSD symptoms between baseline and treatment day 10, as measured by the PTSD Checklist for DSM-5. Repeated measures analysis of variance (ANOVA) will be performed using statistical software SPSS V.26.0. The significance level will be set at 0.05.

Ethics and dissemination

Ethical approval has been obtained from the Ethics Committee of Xijing Hospital in Xi'an, China (KY20222176-X-1), and the trial has been registered on ClinicalTrials.gov. The findings of this trial will be disseminated at academic conferences or published in peer-reviewed scientific journals.

Trial registration number

NCT05544110

Strengths and limitations of this study

This is a randomized, double-blind, sham-controlled study to investigate the efficacy of MRI-guided rTMS for the treatment of PTSD.

The target of MRI-guided rTMS will be the sites that are most closely associated with the amygdala.

The efficacy will be monitored with an 8-week follow-up after the treatment.

A limitation of this study is that rTMS will be administered in combination with medication, making it difficult to verify the efficacy of rTMS as a monotherapy for PTSD.

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a severe mental disorder characterized by recurrent intrusive reexperiencing, nightmares, hyperarousal, avoidance behavior, and negative alterations in cognition or mood.(1) PTSD seriously impairs work ability and quality of life, which causes a heavy burden on families and society. The World Health Organization reported that the lifetime prevalence of PTSD reached 3.9% in a sample of 71,083 respondents, with nearly half of them exhibiting persistent symptoms.(2) Currently, the treatment for PTSD primarily includes medication and psychotherapy, which often require long-term application and may be accompanied by side effects. Moreover, a significant number of patients still do not experience relief after treatment,(3) and nearly 25% of PTSD patients did not achieve recovery within a 10-year period.(4)

Transcranial magnetic stimulation (TMS) is a non-invasive physical therapy that works by directly stimulating the cerebral cortex, thereby altering brain activity. Repetitive TMS (rTMS) is commonly used in clinical practice. High-frequency stimulation increases cortical excitability, while low-frequency stimulation inhibits excitability.(5) A number of studies have investigated the efficacy of rTMS for PTSD and preliminary findings indicate that targeting either the left or right dorsolateral prefrontal cortex (dlPFC) can partially alleviate PTSD symptoms.(6–8) High-frequency rTMS targeting the right dlPFC is considered a "Level B recommendation" for the treatment of PTSD, according to recent rTMS guidelines.(9) Besides, intermittent theta burst stimulation (iTBS) is a novel rTMS protocol in which high-frequency (50Hz) pulse clusters are delivered at 5Hz for 2 seconds, and the next cluster is repeated with an 8-second interval.(10) iTBS can produce a quicker and longer-lasting effect on the cortex in a shorter time than conventional rTMS patterns.(11) Philip *et al.*(12) applied iTBS to the right DLPFC in PTSD patients and found that partial clinical improvement can be observed after short-term interventions.

However, there is still a significant proportion of PTSD patients without remission of clinical symptoms after rTMS treatment, which may be primarily attributed to the stimulation target.(13,14) Most rTMS targets the dlPFC, which may not be a critical brain region in the pathogenesis of PTSD. Currently, numerous evidence supporting the notion that the amygdala plays a crucial role in the development and persistence of PTSD. The amygdala is located in the depths of the dorsomedial temporal cortex that determines the effects of threatening and rewarding stimuli on individual emotional or physiological responses.(15) PTSD patients exhibit a smaller volume in the amygdala (16,17) and often show hyperactivation in response to negative emotional stimuli as compared to the healthy group.(18,19) The amygdala is also significantly overactive in

PTSD patients, even when they are in a resting state.(20) Current research suggests that the abnormal activity of the amygdala contributes to the core symptoms of PTSD.(21) Furthermore, there is a positive correlation between the level of amygdala activity and the clinical severity of the disorder.(22–24) The clinical symptoms of PTSD patients can be significantly improved by inhibiting the function of the amygdala.(25) Therefore, the efficacy of rTMS for PTSD may be significantly enhanced by reducing the activity of the amygdala.

In addition, the stimulation coils frequently used in clinics can only affect cortical activity about 2-5.5 cm below the scalp, which results in rTMS failing to directly modulate the activity of the amygdala. However, the effects of rTMS are not only limited to the stimulation region but also induce subsequent changes in other brain regions that are closely connected to it.(26) MRI-guided rTMS can accurately affect deep brain regions by selecting stimulation targets based on functional connectivity.(27,28) This approach has been used to treat patients with depression and has significantly improved their clinical symptoms.(29) Sydnor et al.(30) found that selecting the most functionally relevant sites of the ventrolateral PFC (vlPFC) as TMS targets for the amygdala can significantly reduce amygdala activity. They also discovered that a higher density of white matter pathways connecting the vlPFC and amygdala is associated with greater changes in amygdala activity.

To summarize, it is reasonable to assume that the efficacy of rTMS for PTSD could be improved if these findings are used to guide the implementation of rTMS. Therefore, we plan to conduct a randomized controlled study aimed at validating the efficacy of MRI-guided rTMS in the treatment of PTSD by indirectly modulating the activity of the amygdala through structural and functional connectivity.

Study objective

The amygdala is hyperactive in PTSD patients, and MRI-guided TMS can indirectly decrease amygdala activity through the functional and structural connectivity of each individual.(30) Presumably, MRI-guided rTMS would reduce amygdala activity in PTSD patients and significantly improve symptoms of PTSD. Therefore, in this randomized double-blind controlled study, we will analyze the degree of structural and functional connectivity of each participant, identifying the sites that are most closely linked to the amygdala as stimulation targets, and assess the effect of MRI-guided rTMS on clinical symptoms and brain activity.

We hypothesized that patients who receive active stimulation will show more significant decreases in symptom severity after the intervention compared to patients who receive sham stimulation. We further hypothesize that active rTMS can significantly reduce amygdala activity, and that the extent of reduction is correlated

with symptom improvement.

METHODS AND ANALYSIS

Study design

This is a single-center, patient and assessor blinded randomized controlled study. This study protocol is designed in accordance with the Standard Protocol Items for Randomized Trials (SPIRIT) statement. The process of this study is shown in Figure 1. PTSD patients will be randomly assigned to either the active rTMS group or the sham rTMS group using the block group randomization method. MRI scans will be performed on participants to identify target sites, then rTMS will be administered for 10 consecutive days. Participants will undergo additional MRI scans after the treatment to investigate changes in brain function before and after the treatment. Meanwhile, clinical symptom assessments will be conducted at baseline, treatment day 5, treatment day 10, and 2 weeks, 4 weeks, 8 weeks post-treatment to explore the improvement of PTSD symptoms by rTMS.

Participants

PTSD patients will be recruited at the outpatient clinic of the First Affiliated Hospital of the Air Force Medical University, China, from March 2023 to June 2024. Recruitment information will also be made into a poster and disseminated through social media in order to recruit patients. These have been approved by the hospital Ethics Committee. Participants who meet the following inclusion and exclusion criteria are eligible for this study. At the screening, participants will be informed by the investigator about the study procedures, risks and benefits, and the voluntary nature of participation. Meanwhile, written informed consent will be obtained from all participants prior to their participation in the study.

Inclusion criteria

1. Between the ages of 18-65 years;
2. Meeting the criteria of the DSM-5 for PTSD, which will be assessed by two professional psychiatrists.
3. With a score greater than 33 on the PTSD Checklist for DSM-5 (PCL-5).
4. Not receive any medication or psychotherapy for PTSD before entering the study.

Exclusion criteria

1. Significant medical illnesses or diseases that may affect the central nervous system.
2. Abnormal EEG or MRI evidence of brain abnormalities.
3. Contraindications to MRI scans or TMS including metal or electronic implants, claustrophobia, etc.
4. Alcohol and drug abuse.

5. Strong suicidal ideation or a history of previous suicidal behavior.
6. Pregnancy, lactation, or planning pregnancy during the trial period.

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.

Sample size

Sample size is calculated using PASS software version 2021. We utilized the results of a previous randomized controlled study on PTSD conducted by our research team, which shares a similar study design with the current study. The effect size of PCL scale scores after rTMS treatment in the previous study is 0.8. The significance level is set at 0.05 (one-tailed test) and the statistical power is set at 80%. It has been calculated that 38 participants are needed. Assuming a dropout rate of 20%, the sample size is expanded to 48 participants (24 participants per group).

Allocation and Blinding

Participants will be randomly assigned to either the active rTMS group or the sham rTMS group in a 1:1 ratio, following a randomization sequence. The sequence has been formulated by a specialized investigator (YM) before the trial, using block randomization with a block length of four.(31) The allocation details of each participant will be sequentially sealed in opaque envelopes. When participants enter the study, the researcher will open the envelopes in sequence and inform the therapist of the appropriate treatment.

Participants, their families, and the raters conducting the clinical assessments are blinded to the group assignment. Participant ID and subgroup information will be anonymized to ensure that the blinding remains in effect until the study is completed. If severe adverse events occur, unblinding will be performed after consultation with the principal investigator.

MRI data acquisition and identifying targets

Neuroimaging data will be acquired at Xi'an YunYing Image Medical Diagnosis Center using a 3T uMR 780 scanner (Shanghai United Imaging Healthcare Co., Ltd., China). MRI data will include T1-weighted structural MRI, resting-state functional MRI, and diffusion tensor imaging sequences. The acquisition parameters are as follows: (1) T1-weighted structural MRI: slices = 200, repetition time = 7.24 ms, echo time = 3.10 ms, inversion time = 750 ms, field of view = 256 mm, flip angle = 10°; (2) resting-state functional MRI: slices = 8400, repetition time = 2000 ms, echo time = 30 ms, field of view = 224 mm, flip angle = 90°; (3) diffusion tensor imaging: slices = 2475, repetition time = 12676 ms, echo time = 88.6 ms, field of view = 224 mm, flip angle = 90°.

Based on the MRI data of each participant, we will identify sites in the right vlPFC that are structurally and functionally strongly associated with the right amygdala. These sites will be targeted for subsequent rTMS. The right hemisphere is chosen as the target for rTMS because previous studies have suggested that it may be more effective than the left hemisphere.(32) The target calculation process is as follows: first, the resting-state data will be preprocessed with reslicing and head motion correction, alignment, and Gaussian smoothing processing. Then, the right vlPFC will be divided into several subregions, and their subnucleolar concentrations, subnucleolar sizes, and functional connectivity coefficients with the right amygdala will be comprehensively analyzed to identify the sites that are functionally closely connected to the amygdala. Finally, the white matter fiber connections between these sites and the amygdala will be detected, and the optimal stimulation target will be selected by combining the functional connections and white matter fiber connections between them.

Interventions

The MRI-guided rTMS will be delivered by the Black Dolphin Transcranial Magnetic Robot (Spirit Dolphin, SLD-YXRJ-V1.0) from Xi'an Solide Brain Control Medical Technology Co., Ltd. The robot is equipped with a positioning navigation system based on neuroimaging, which allows it to manipulate the coil alignment and accurately place it on pre-explored targets. The position of the coil can be adjusted in real time during treatment to ensure that stimuli are consistently applied to the same target area. MRI-based positioning is currently the most accurate method for placing the TMS coil at the target site.(33) Preliminary results have shown that using this positioning generates greater clinical efficacy compared to traditional scalp measurements.(29)

A figure-of-eight coil will be used in the treatment of this study. Twenty sessions will be performed over 10 consecutive days, with two iTBS sessions per day at 50-minute intervals (intensity of 90% motor threshold, each containing 1800 pulses for 10 minutes). The 10-minute iTBS session has been shown to significantly improve symptoms of PTSD after up to 20 sessions. Our study will employ the iTBS protocol, which will be applied twice a day to expedite the treatment duration. The 50-minute interval is based on a previous iTBS study on iTBS, which suggests that intervals of 50 minutes or more can have a stronger cumulative effect on nerve fibers.(34,35) In the sham stimulation group, the coil will be turned 90° and placed in contact with the scalp, producing the same stimulation sound and some degree of scalp sensation. This approach does not induce significant changes in cortical activity and has been used in many randomized controlled studies of rTMS.(10,36) The treatment will be conducted by a trained technician in a separate treatment room. Participants will be prohibited from communicating with each other during the treatment intervals to prevent the

cohort effect. In addition, each participant will take paroxetine (20 mg/d) concurrently with rTMS treatment, in accordance with ethical guidelines. This is because paroxetine is currently the first-line medication for PTSD in clinical settings.(37) MRI and rTMS will be provided free of charge for participants.

Criteria for discontinuing interventions

Participants will be discontinued from the study if (1) serious adverse events occur (e.g., seizure and suicide); (2) the participant does not wish to continue; (3) the participant is unable to tolerate the discomfort produced by rTMS; and (4) serious violations of the treatment protocol occur, such as interruptions of treatment for 2 days or more.

Outcomes

General information, including gender, age, type of trauma, and comorbidities, will be collected at baseline. Clinical symptoms will be monitored using self-rated and physician-rated clinical scales assessed at baseline, treatment day 5, treatment day 10, and 2 weeks, 4 weeks, 8 weeks after the end of treatment. MRI scans will also be performed at baseline and after the last session to observe the effects of rTMS on brain activity. In addition, any adverse events will be promptly recorded during the entire study period.

Primary outcome

- The PTSD Checklist for DSM-5 (PCL-5) is a self-report scale commonly used to assess the severity of core PTSD symptoms. PCL-5 scores of 31 to 33 are optimal for efficiently diagnosing PTSD, with higher scores indicating more severe symptoms of PTSD.(38) The change from baseline to the 10-day treatment post of PCL-5 will be the primary measure as it can effectively reflect the effect of the intervention on PTSD symptoms.

Secondary outcomes

- The change in PCL-5 scale total score at baseline compared to 2, 4, and 8 weeks after the end of treatment will be used to investigate the long-term efficacy of rTMS on symptoms of PTSD.
- The 17-item Hamilton Depression Rating Scale (HAMD-17) and the Beck Depression Inventory (BDI) are clinician- and self-rated scales used to assess depressive symptoms, respectively.(39,40) Higher total scores on these scales indicate more severe depressive symptoms. The change in total scores of the HAMD-17 and BDI from baseline to each of the other time points will be used to assess the efficacy of rTMS on depressive symptoms.
- The Hamilton Anxiety Scale (HAMA) and the Zung Self-Rating Anxiety Scale (SAS) are commonly used in clinical practice to assess anxiety symptoms. In the present study, these scales will be used to assess the effectiveness of the

intervention in reducing anxiety symptoms.

- The Insomnia Severity Index (ISI) is a widely used questionnaire for screening insomnia. The measure is brief, consisting of only 7 items. Each item is scored from 0 to 4, with higher scores indicating greater sleep disturbance. In this study, the ISI will be used to assess the efficacy of the intervention on insomnia and sleep disturbances related to insomnia.
- Resting-state functional magnetic resonance imaging reflects the spontaneous neural activity in different brain regions. Amplitude of the low-frequency fluctuation (ALFF) and regional homogeneity (ReHo) provide different perspectives for assessing the level of spontaneous activity in a single voxel of the brain.(41,42) In the present study, we will calculate the ALFF and ReHo values of the right amygdala before and after treatment, respectively, and compare their differences to explore the effect of rTMS on amygdala activity. The relationship between these changes and clinical outcomes will also be explored.
- The incidence of adverse events during treatment and the retention rates in each group will be used to assess the safety and acceptability of the 10-day MRI-guided rTMS.

Data collection and management

Assessment data will be collected by two psychiatrists who are blinded to the allocation and not involved in patient treatment. Assessors have extensive work experience and will receive specific training to ensure consistency in assessment results among them. Participants will be available for phone interviews to facilitate the completion of follow-up visits.

There is no data monitoring committee for this study. The Clinical Research Coordinator will assist in ensuring that data is entered completely and accurately. Personal information and clinical outcomes of participants will be initially stored in paper case report forms (CRFs), and on the last day of each week, the data will be electronically saved in an Excel database, which will be maintained on a separate computer at the research center. Data containing personally identifiable information will be stored in a separate Excel file, and each item will be assigned a specific code that will be used to refer to the participant in other databases. MRI scans of each participant will initially be stored on a CD, which will then be uploaded to the image database by the research staff. All paper documents and image CDs will be stored in a secure filing cabinet in the study center, while the computer and Excel database will be password-protected to ensure participant privacy. In addition, data from rTMS, including intensity thresholds, treatment progress, and any adverse events that occur during treatment, will be collected and independently stored by the therapist. This data

will then be added to the total Excel file after the study.

Participant safety

Prior to enrollment, participants with contraindications to MRI, such as metal implants in the body and claustrophobia, will be excluded. A specialized examiner will be responsible for conducting the MRI scans. They will also ensure that there are no relevant contraindications prior to the examination and provide earmuffs to mitigate the noise.

rTMS has been shown to be safe and well-tolerated in most clinical situations. Common adverse events include headaches and localized abnormal sensations, which are often mild and typically resolve within an hour after rTMS. However, rTMS has a low risk of inducing seizures, with an incidence rate of approximately 0.01-0.1%. Therefore, we will exclude participants who have a history of seizures or show abnormal EEG during screening. rTMS will be administered by experienced therapists to ensure that participants are promptly treated in case of adverse events. If any serious adverse events occur during the study, the participant will be taken by the investigator to either the emergency department or the specialist clinic. The sponsor is responsible for covering the cost of treatment and providing financial compensation to participants who suffer trial-related harm or death. Adverse events and study progress will be periodically reviewed by the Ethics Committee.

Statistical analysis

All data analysis will adhere to the intention-to-treat principle. Basic information and clinical scores of all participants will be analyzed using IBM SPSS Statistics for Windows version 26.0. Continuous variables will be expressed as means and standard deviations (SD), while categorical variables will be presented as frequencies and percentages. The independent samples t-test or chi-square test will be used to verify homogeneity between groups. One-way repeated measures ANOVA will be used to compare the outcome variables at different time points within each group. Two-way repeated measures ANOVA will be used to test the interaction effect of 'intervention' and 'time' on the outcome variables at different periods between the two groups. Multiple comparisons will then be performed using the Bonferroni test to identify specific significant differences. If there are significant differences in baseline characteristics, the ANCOVA model will be used to analyze the differences between groups. If there is missing data, it will be processed using the multiple imputation method.

The MRI data before and after treatment will be processed and analyzed using the SPM12 software package in Matlab R2019b. The Restplus V1.2 toolbox will be used to preprocess and calculate the amplitude of low frequency fluctuation (ALFF) and

regional homogeneity (ReHo). Then, paired samples t-tests will be used to examine the differences before and after treatment within each group, and independent samples t-tests will be used to compare the differences after treatment between groups.

ETHICS AND DISSEMINATION

The study will be conducted in accordance with the Declaration of Helsinki and the study protocol. Ethical permission has been obtained from the Ethics Committee of the First Affiliated Hospital of Air Force Military Medical University (Grant No. KY20222176-X-1), and the study has been registered with ClinicalTrials.gov (NCT05544110). All participants will be informed of the study details and will be asked to sign a written informed consent before participating in the study.

The efficacy of the intervention will be disseminated at international and national academic conferences or published in peer-reviewed scientific journals.

Contributors: All authors contributed to the initiation of this study. YCZ, MC, NT, HW were involved in the conception and design of the study. HW provided technical support for MRI analysis and rTMS. YCZ, YYZ, YM drafted the manuscript. ZP, NL, RL reviewed and revised the manuscript. All authors have read and approved the final manuscript.

Funding: This work is supported by National Natural Science Foundation of China (82330043).

Competing interests: None declared.

Data sharing: No additional data are available.

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: Not applicable.

Provenance and peer review: Not commissioned; externally peer reviewed.

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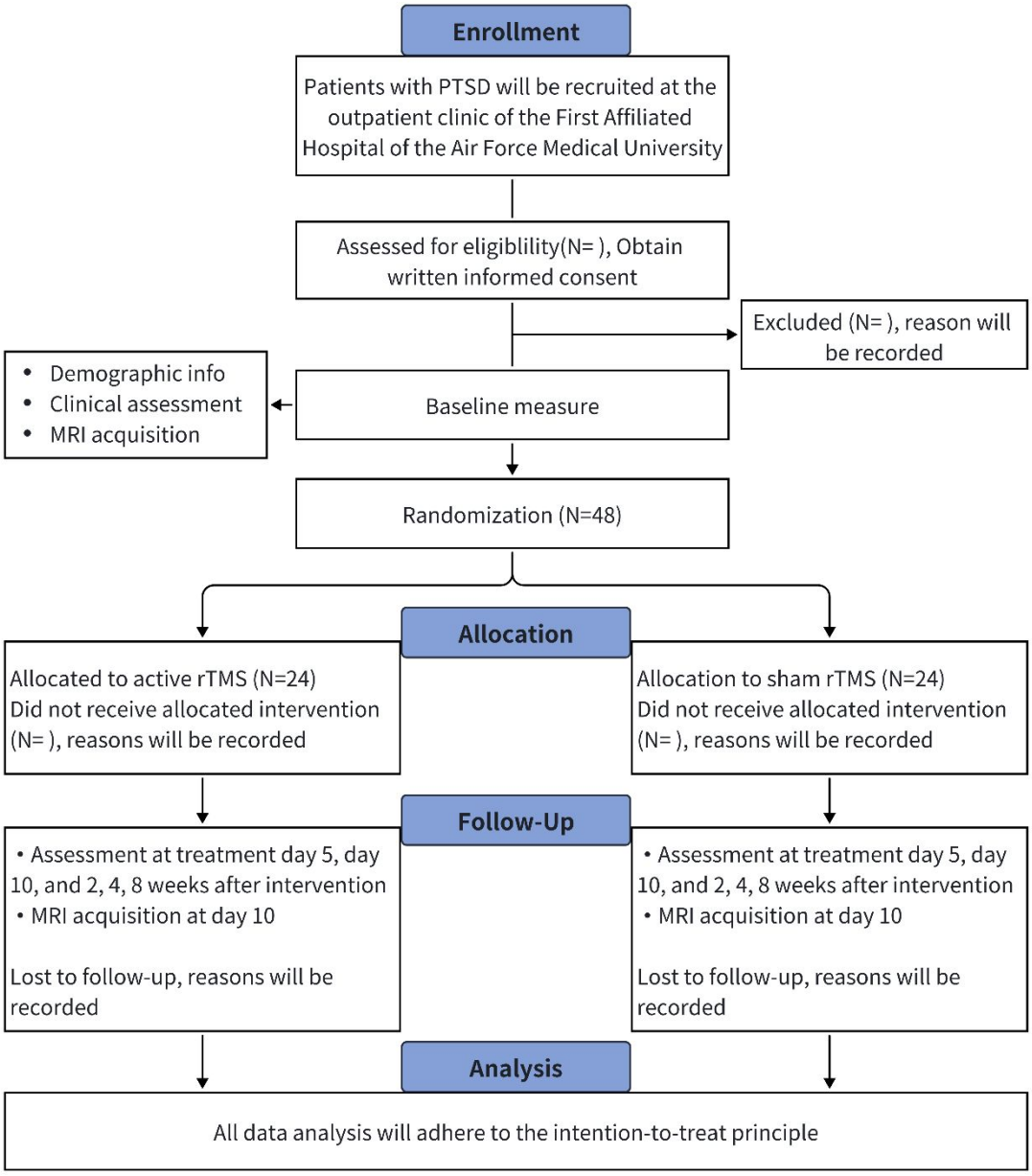

Figure 1 Flow chart of the study design. This flowchart illustrates the complete process from enrollment to intervention, follow-up, and data analysis. rTMS, repetitive transcranial magnetic stimulation.

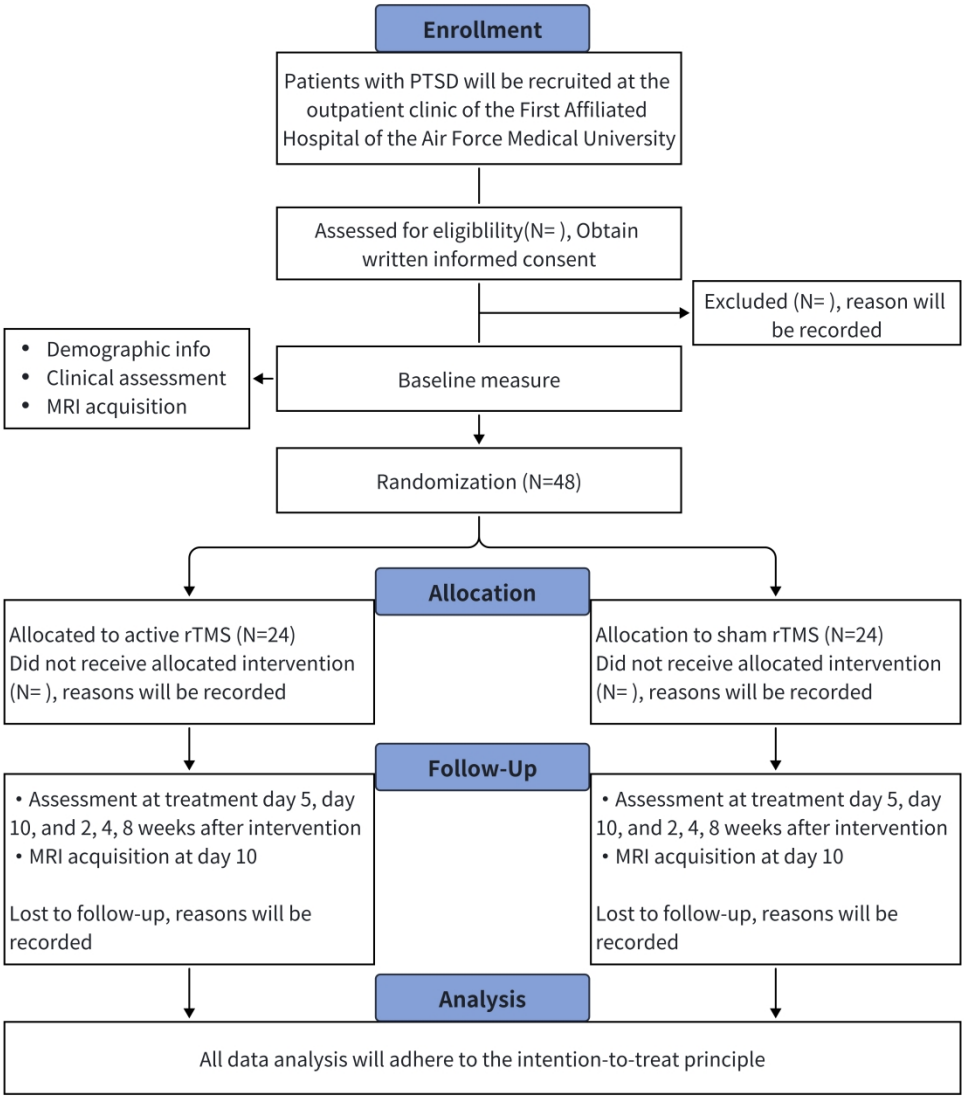

Figure 1 Flow chart of the study design. This flowchart illustrates the complete process from enrollment to intervention, follow-up, and data analysis. rTMS, repetitive transcranial magnetic stimulation.

1180x1335mm (96 x 96 DPI)

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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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	Page 1

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Page 2
Protocol version	#3	Date and version identifier	
Funding	#4	Sources and types of financial, material, and other support	Page 11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Page 1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Page 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 (study sponsor or funders)
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	Page 9

		applicable (see Item 21a for data monitoring committee)	
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	Page 4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	Page 7
Objectives	#7	Specific objectives or hypotheses	Page 4
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)	Page 5
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	Page 5 and Page 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)	Page 5
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 7
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)	Page 8
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Page 9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 7
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	Page 8 and Page 9

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 Figure)	Page 15
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	Page 6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 5
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	Page 6
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to	Page 6

		conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Page 6
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	Page 9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be	Page 9

		collected for participants who discontinue or deviate from intervention protocols	
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	Page 9
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	Page 10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 10
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)	Page 10
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	Page 9

		protocol. Alternatively, an explanation of why a DMC is not needed	
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	n/a (No midterm analysis)
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	Page 9 and Page 10
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a (See Ethics Committee Review Approval)
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	Page 10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	Page 11

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 5
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 (No plans for other studies)
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	Page 9
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 11
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	Page 11
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	Page 10
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	Page 11

		restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	Page 11
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a (No plan)
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a (See Informed Consent)
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 (No biological specimens will be collected)

BMJ Open

Efficacy of MRI-guided rTMS for posttraumatic stress disorder by modulating amygdala activity: study protocol for a randomized controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-081751.R1
Article Type:	Protocol
Date Submitted by the Author:	10-May-2024
Complete List of Authors:	Zhang, Yaochi; Xijing Hospital of Air Force Military Medical University, Psychiatry Peng, Zhengwu; Xijing Hospital of Air Force Military Medical University, Psychiatry Tang, Nailong; Xijing Hospital of Air Force Military Medical University, Psychiatry Zhang, Yuyu; Xijing Hospital of Air Force Military Medical University Liu, Nian; Xijing Hospital of Air Force Military Medical University, Lv, Runxin; Xijing Hospital of Air Force Military Medical University Meng, Yumeng; Xijing Hospital of Air Force Military Medical University, Psychiatry Cai, Min; Xijing Hospital of Air Force Military Medical University Wang, Hua-Ning; Xijing Hospital of Air Force Military Medical University, Department of Psychiatry
Primary Subject Heading:	Mental health
Secondary Subject Heading:	Neurology
Keywords:	Transcranial Magnetic Stimulation, PSYCHIATRY, NEUROLOGY

Title: Efficacy of MRI-guided rTMS for posttraumatic stress disorder by modulating amygdala activity: study protocol for a randomized controlled trial

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Keywords:
Posttraumatic Stress Disorder, Transcranial Magnetic Stimulation, amygdala, ventrolateral prefrontal cortex

Word count: 3891

ABSTRACT

Introduction

Posttraumatic stress disorder (PTSD) is a prevalent and severe psychiatric disorder. Repetitive transcranial magnetic stimulation (rTMS) targeting the dorsolateral prefrontal cortex provides limited relief for symptoms of PTSD. This study will be conducted to validate the efficacy of MRI-guided rTMS in targeting the sites most closely associated with the amygdala for patients with PTSD. We hypothesize that the intervention will improve clinical symptoms by decreasing amygdala activity in patients.

Methods and analysis

A randomized, double-blind, sham-controlled trial will be conducted. Forty-eight eligible patients with PTSD will be randomly assigned to receive either active or sham MRI-guided rTMS for 10 consecutive days after the initial MRI scans. MRI scans will be recollected at the end of the intervention. Clinical assessments will be performed at baseline, treatment day 5, treatment day 10, and 2 weeks, 4 weeks, 8 weeks after completion of the intervention to monitor changes in clinical symptoms. The primary assessment outcome is the change in PTSD symptoms between baseline and treatment day 10, as measured by the PTSD Checklist for DSM-5. Repeated measures analysis of variance (ANOVA) will be performed using statistical software SPSS V.26.0. The significance level will be set at 0.05.

Ethics and dissemination

Ethical approval has been obtained from the Ethics Committee of Xijing Hospital in Xi'an, China (KY20222176-X-1), and the trial has been registered on ClinicalTrials.gov. The findings of this trial will be disseminated at academic conferences or published in peer-reviewed scientific journals.

Trial registration number

NCT05544110

Strengths and limitations of this study

This is a randomized controlled clinical trial to investigate the efficacy of MRI-guided rTMS for the treatment of PTSD.

Patients will be randomly assigned to the active or sham stimulation group while patients and assessors will be blinded to this condition.

The efficacy will be monitored with an 8-week follow-up after the treatment.

A limitation of this study is that rTMS will be administered in combination with medication, making it difficult to verify the efficacy of rTMS as a monotherapy for PTSD.

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a severe psychiatric disorder characterized by recurrent intrusive reexperiencing, nightmares, hyperarousal, avoidance behavior, and altered cognition or mood.(1) PTSD seriously impairs work ability and quality of life, which causes a heavy burden on families and society. The World Health Organization reported that the lifetime prevalence of PTSD reached 3.9% in a sample of 71,083 respondents, with nearly half of them exhibiting persistent symptoms.(2) Currently, the treatment for PTSD primarily includes medication and psychotherapy. However, a significant number of patients still do not get relief after treatment.(3) Additionally, almost 25% of patients with PTSD did not experience recovery within a 10-year period.(4)

Transcranial magnetic stimulation (TMS) is a non-invasive physical therapy by directly stimulating the cerebral cortex to alter brain activity. Repetitive TMS (rTMS) is commonly used in clinical practice. High-frequency stimulation increases cortical excitability, while low-frequency stimulation inhibits excitability.(5) A number of studies have investigated the efficacy of rTMS for PTSD and preliminary findings indicate that targeting either the left or right dorsolateral prefrontal cortex (dlPFC) can partially alleviate PTSD symptoms.(6–8) According to rTMS guidelines, high-frequency rTMS targeting the right dlPFC is considered a "Level B recommendation" for the treatment of PTSD.(9) Besides, intermittent theta burst stimulation (iTBS) is a novel rTMS protocol in which high-frequency (50Hz) pulse clusters are delivered at 5Hz for 2 seconds, and the next cluster is repeated with an 8-second interval.(10) iTBS can produce a quicker and longer-lasting effect on the cortex in a shorter time than conventional rTMS patterns.(11) Philip *et al.*(12) applied iTBS to the right DLPFC in patients with PTSD and found that partial clinical improvement can be observed after short-term interventions.

However, there is still a significant proportion of PTSD patients without remission of clinical symptoms after rTMS treatment, which may be primarily attributed to the stimulation target.(13,14) Most rTMS targets the dlPFC, which may not be a critical brain region in the pathogenesis of PTSD. Currently, numerous evidence supporting the notion that the amygdala plays a crucial role in the development and persistence of PTSD. The amygdala, located in the depths of the dorsomedial temporal cortex, is a brain region closely associated with fear conditioning.(15) Meanwhile, PTSD is also recognized as a disorder of dysfunction in fear conditioning, in which abnormalities in the amygdala are particularly prominent. PTSD patients exhibit a smaller volume in the amygdala (16,17) and often show hyperactivation in response to negative emotional stimuli as compared to the healthy group.(18,19) The amygdala is also significantly

overactive, even in the resting state.(20) Current research suggests that hyperactivity of the amygdala is an important pathogenetic mechanism in PTSD and contributes to the core clinical symptoms.(21) Furthermore, there is a positive correlation between the level of amygdala activity and the clinical severity of the disorder.(22–24) The clinical symptoms of PTSD patients can be significantly improved by inhibiting the function of the amygdala.(25) Therefore, the efficacy of rTMS for PTSD may be significantly enhanced by reducing the activity of the amygdala.

In addition, the stimulation coils frequently used in clinics can only affect cortical activity about 2-5.5 cm below the scalp, which results in rTMS failing to directly modulate the activity of the amygdala. However, the effects of rTMS are not only limited to the stimulation region but also induce subsequent changes in other brain regions that are closely connected to it.(26) MRI-guided rTMS can accurately affect deep brain regions by selecting stimulation targets based on functional connectivity.(27,28) The ventrolateral PFC (vlPFC) may be the target region of MRI-guided rTMS that can significantly affect the activity of the amygdala. The vlPFC has significant functional connectivity with the amygdala, which is enhanced in patients after effective treatment,(29,30) and it is also the only brain region in the PFC that can directly receive rTMS while having relatively more amygdala projections.(31,32) Sydnor et al.(33) found that selecting the most functionally relevant sites of the vlPFC as TMS targets for the amygdala can significantly reduce amygdala activity. They also discovered that a higher density of white matter pathways connecting the vlPFC and amygdala is associated with greater changes in amygdala activity.

To summarize, the efficacy of rTMS for PTSD may be improved if these findings are utilized to inform the implementation of rTMS. Therefore, we plan to conduct a randomized controlled study aimed at validating the efficacy of MRI-guided rTMS in the treatment of PTSD by indirectly modulating the activity of the amygdala.

Study objective

The amygdala is hyperactive in patients with PTSD, and MRI-guided rTMS can indirectly decrease amygdala activity through the functional and structural connectivity of each individual.(33) Presumably, MRI-guided rTMS in patients with PTSD would reduce amygdala activity and significantly improve symptoms of PTSD. Therefore, in this randomized double-blind controlled study, we will analyze the degree of structural and functional connectivity of each participant, identifying the sites that are most closely linked to the amygdala as stimulation targets, and assess the effect of MRI-guided rTMS on clinical symptoms and brain activity.

We hypothesized that patients with PTSD who receive active stimulation will show more significant decreases in symptom severity after the intervention compared to

patients who receive sham stimulation. We further hypothesize that active rTMS can significantly reduce amygdala activity, and that the extent of reduction is correlated with symptom improvement.

METHODS AND ANALYSIS

Study design

This study protocol is designed in accordance with the Standard Protocol Items for Randomized Trials (SPIRIT) statement. The process of this study is shown in Figure 1. Patients with PTSD will be randomly assigned to either the active rTMS group or the sham rTMS group using the block group randomization method. MRI scans will be performed on participants to identify target sites, then rTMS will be administered for 10 consecutive days. Participants will undergo additional MRI scans after the treatment to investigate changes in brain function before and after the treatment. Meanwhile, clinical symptom assessments will be conducted at baseline, treatment day 5, treatment day 10, and 2 weeks, 4 weeks, 8 weeks post-treatment to explore the improvement of PTSD symptoms by rTMS. This trial started in September 2023 and is expected to be completed in December 2024.

Insert here Figure 1

Figure 1 Flow chart of the study design

Participants

Patients with PTSD will be recruited at the outpatient clinic of the First Affiliated Hospital of the Air Force Medical University, China, from March 2023 to June 2024. Recruitment information will also be made into a poster and disseminated through social media in order to recruit patients. These have been approved by the hospital Ethics Committee. Participants who meet the following inclusion and exclusion criteria are eligible for this study. At the screening, participants will be informed by the investigator about the study procedures, risks and benefits, and the voluntary nature of participation. Meanwhile, written informed consent will be obtained from all participants prior to their participation in the study (online supplementary file 1).

Inclusion criteria

1. Between the ages of 18-65 years;
2. Meeting the criteria of the DSM-5 for PTSD, which will be assessed by two professional psychiatrists.
3. With a score greater than 33 on the PTSD Checklist for DSM-5 (PCL-5).
4. Not receive any medication or psychotherapy for PTSD before entering the study.

Exclusion criteria

1. Significant medical illnesses or diseases that may affect the central nervous system.

2. Abnormal EEG or MRI evidence of brain abnormalities.
3. Contraindications to MRI scans or TMS including metal or electronic implants, claustrophobia, etc.
4. Alcohol and drug abuse.
5. Strong suicidal ideation or a history of previous suicidal behavior.
6. Pregnancy, lactation, or planning pregnancy during the trial period.

Patient and public involvement

Patients and the public were not involved in the design of the study.

Sample size

Sample size is calculated using PASS software version 2021. We utilized the results of a previous randomized controlled study on PTSD conducted by our research team, which shares a similar study design with the current study.(34) The effect size of PCL scale scores after rTMS treatment in the previous study is 0.82. The significance level is set at 0.05 (two-tailed test) and the statistical power is set at 80%. It has been calculated that 38 participants are needed. Assuming a dropout rate of 20%, the sample size is expanded to 48 participants (24 participants per group).

Allocation and Blinding

Participants will be randomly assigned to either the active rTMS group or the sham rTMS group in a 1:1 ratio, following a randomization sequence. The sequence has been formulated by a specialized investigator (YM) before the trial, using block randomization with a block length of four.(35) The allocation details of each participant will be sequentially sealed in opaque envelopes. When participants enter the study, the researcher will open the envelopes in sequence and inform the therapist of the appropriate treatment.

Participants, their families, and the raters conducting the clinical assessments are blinded to the group assignment. Participant ID and subgroup information will be anonymized to ensure that the blinding remains in effect until the study is completed. If severe adverse events occur, unblinding will be performed after consultation with the principal investigator.

MRI data acquisition and identifying targets

Neuroimaging data will be acquired at Xi'an YunYing Image Medical Diagnosis Center using a 3T uMR 780 scanner (Shanghai United Imaging Healthcare Co., Ltd., China). MRI data will include T1-weighted structural MRI, resting-state functional MRI, and diffusion tensor imaging sequences. The acquisition parameters are as follows: (1) T1-weighted structural MRI: thickness = 1 mm, slices = 200, repetition time = 7.24 ms, echo time = 3.10 ms, inversion time = 750 ms, field of view = 256 × 256 mm^2, inversion

time = 750 ms, flip angle = 10°, voxel size =1×1×1 mm^3; (2) resting-state functional MRI: thickness = 4 mm, slices = 8400, repetition time = 2000 ms, echo time = 30 ms, field of view = 224×224 mm^2, flip angle = 90°, voxel size =3.5×3.5×4.0 mm^3; (3) diffusion tensor imaging: thickness = 2 mm, slices = 2475, repetition time = 12676 ms, echo time = 88.6 ms, field of view = 224×224 mm^2, flip angle = 90°, voxel size =2×2×2 mm^3, b-value = 1000 s/mm^2. During the scan, participants will be asked to close their eyes, relax, not think intentionally, and not fall asleep.

Based on the MRI data of each participant, we will identify sites in the right vlPFC that are structurally and functionally strongly associated with the right amygdala. These sites will be targeted for subsequent rTMS. The right hemisphere is chosen as the target for rTMS because previous studies have suggested that it may be more effective than the left hemisphere.(36) The target calculation process is as follows: first, the resting-state data will be preprocessed with reslicing and head motion correction, alignment, and Gaussian smoothing processing. Then, the right vlPFC will be divided into several subregions, and their subnucleolar concentrations, subnucleolar sizes, and functional connectivity coefficients with the right amygdala will be comprehensively analyzed to identify the sites that are functionally closely connected to the amygdala. Finally, the white matter fiber connections between these sites and the amygdala will be detected, and the optimal stimulation target will be selected by combining the functional connections and white matter fiber connections between them.

Interventions

The MRI-guided rTMS will be delivered by the Black Dolphin Transcranial Magnetic Robot (Spirit Dolphin, SLD-YXRJ-V1.0) from Xi'an Solide Brain Control Medical Technology Co. which is equipped with a figure-of-eight coil (Yingchi Tech, Shenzhen, China). The robot is equipped with a positioning navigation system based on neuroimaging, which allows it to manipulate the coil alignment and accurately place it on pre-explored targets. The position of the coil can be adjusted in real time during treatment to ensure that stimuli are consistently applied to the same target area. MRI-based positioning is currently the most accurate method for placing the TMS coil at the target site.(37) Preliminary results have shown that using this positioning generates greater clinical efficacy compared to traditional scalp measurements.(38)

A figure-of-eight coil will be used in the treatment of this study. Twenty sessions will be performed over 10 consecutive days, with two iTBS sessions per day at 50-minute intervals (intensity of 90% motor threshold, each containing 1800 pulses for 10 minutes). The 10-minute iTBS session has been shown to significantly improve symptoms of PTSD after up to 20 sessions. Our study will employ the iTBS protocol, which will be applied twice a day to expedite the treatment duration. The 50-minute

interval is based on a previous iTBS study on iTBS, which suggests that intervals of 50 minutes or more can have a stronger cumulative effect on nerve fibers.(39,40) In the sham stimulation group, the coil will be turned 90° and placed in contact with the scalp, producing the same stimulation sound and some degree of scalp sensation. This approach does not induce significant changes in cortical activity and has been used in many randomized controlled studies of rTMS.(10,41) The treatment will be conducted by a trained technician in a separate treatment room. Participants will be prohibited from communicating with each other during the treatment intervals to prevent the cohort effect. In addition, each participant will take paroxetine (20 mg/d) concurrently with rTMS treatment, in accordance with ethical guidelines. This is because paroxetine is currently the first-line medication for PTSD in clinical settings.(42) MRI and rTMS will be provided free of charge for participants.

Outcomes

General information, including gender, age, type of trauma, and comorbidities, will be collected at baseline. Clinical symptoms will be monitored using self-rated and physician-rated clinical scales assessed at baseline, treatment day 5, treatment day 5, and 2 weeks, 4 weeks, 8 weeks after the end of treatment. MRI scans will also be performed at baseline and after the last session to observe the effects of rTMS on brain activity in patients with PTSD. In addition, any adverse events will be promptly recorded during the entire study period.

Primary outcome

- The PTSD Checklist for DSM-5 (PCL-5) is a self-report scale commonly used to assess the severity of core PTSD symptoms. PCL-5 scores of 31 to 33 are optimal for efficiently diagnosing PTSD, with higher scores indicating more severe symptoms of PTSD.(43) The change from baseline to the 10-day treatment post of PCL-5 will be the primary measure as it can effectively reflect the effect of the intervention on PTSD symptoms.

Secondary outcomes

- The change in PCL-5 scale total score at baseline compared to 2, 4, and 8 weeks after the end of treatment will be used to investigate the long-term efficacy of rTMS on symptoms of PTSD.
- The 17-item Hamilton Depression Rating Scale (HAMD-17) and the Beck Depression Inventory (BDI) are clinician- and self-rated scales used to assess depressive symptoms, respectively.(44,45) Higher total scores on these scales indicate more severe depressive symptoms. The change in total scores of the HAMD-17 and BDI from baseline to each of the other time points will be used to assess the efficacy of rTMS on depressive symptoms.

- The Hamilton Anxiety Scale (HAMA) and the Zung Self-Rating Anxiety Scale (SAS) are commonly used in clinical practice to assess anxiety symptoms. In the present study, these scales will be used to assess the effectiveness of the intervention in reducing anxiety symptoms.
- The Insomnia Severity Index (ISI) is a widely used questionnaire for screening insomnia. The measure is brief, consisting of only 7 items. Each item is scored from 0 to 4, with higher scores indicating greater sleep disturbance. In this study, the ISI will be used to assess the efficacy of the intervention on insomnia and sleep disturbances related to insomnia.
- Resting-state functional magnetic resonance imaging reflects the spontaneous neural activity in different brain regions. Amplitude of the low-frequency fluctuation (ALFF) and regional homogeneity (ReHo) provide different perspectives for assessing the level of spontaneous activity in a single voxel of the brain.(46,47) In the present study, we will calculate the ALFF and ReHo values of the right amygdala before and after treatment, respectively, and compare their differences to explore the effect of rTMS on amygdala activity. The relationship between these changes and clinical outcomes will also be explored.
- The incidence of adverse events during treatment and the retention rates in each group will be used to assess the safety and acceptability of the 10-day MRI-guided rTMS.

Data collection and management

Assessment data will be collected by two psychiatrists who are blinded to the allocation and not involved in patient treatment. Assessors have extensive work experience and will receive specific training to ensure consistency in assessment results among them. Participants will be available for phone interviews to facilitate the completion of follow-up visits.

Personal information and clinical outcomes of participants will be initially stored in paper case report forms (CRFs), and on the last day of each week, the data will be electronically saved in an Excel database, which will be maintained on a separate computer at the research center. Data containing personally identifiable information will be stored in a separate Excel file, and each item will be assigned a specific code that will be used to refer to the participant in other databases. MRI scans will be performed at a specialized imaging facility. MRI scans of each participant will initially be stored on a CD, which will then be uploaded to the image database by the research staff. All paper documents and image CDs will be stored in a secure filing cabinet in the study center, while the computer and Excel database will be password-protected to ensure participant privacy. In addition, data from rTMS, including intensity thresholds,

treatment progress, and any adverse events that occur during treatment, will be collected and independently stored by the therapist. This data will then be added to the total Excel file after the study.

Participant safety

Prior to enrollment, participants with contraindications to MRI, such as metal implants in the body and claustrophobia, will be excluded. A specialized examiner will be responsible for conducting the MRI scans. They will also ensure that there are no relevant contraindications prior to the examination and provide earmuffs to mitigate the noise.

rTMS has been shown to be safe and well-tolerated in most clinical situations. Common adverse events include headaches and localized abnormal sensations, which are often mild and typically resolve within an hour after rTMS. However, rTMS has a low risk of inducing seizures, with an incidence rate of approximately 0.01-0.1%. Therefore, we will exclude participants who have a history of seizures or show abnormal EEG during screening. rTMS will be administered by experienced therapists to ensure that participants are promptly treated in case of adverse events. If any serious adverse events occur during the study, the participant will be taken by the investigator to either the emergency department or the specialist clinic. The sponsor is responsible for covering the cost of treatment and providing financial compensation to participants who suffer trial-related harm or death. Adverse events and study progress will be periodically reviewed by the Ethics Committee.

Participants will be discontinued from the study if (1) serious adverse events occur (e.g., seizure and suicide); (2) the participant does not wish to continue; (3) the participant is unable to tolerate the discomfort produced by rTMS; and (4) serious violations of the treatment protocol occur, such as interruptions of treatment for 2 days or more.

Statistical analysis

All data analysis will adhere to the intention-to-treat principle. Basic information and clinical scores of all participants will be analyzed using IBM SPSS Statistics for Windows version 26.0. Continuous variables will be expressed as means and standard deviations (SD), while categorical variables will be presented as frequencies and percentages. The independent samples t-test or chi-square test will be used to verify homogeneity between groups. One-way repeated measures ANOVA will be used to compare the outcome variables at different time points within each group. Two-way repeated measures ANOVA will be used to test the interaction effect of 'intervention' and 'time' on the outcome variables at different periods between the two groups. Multiple comparisons will then be performed using the Bonferroni test to identify

specific significant differences. If there are significant differences in baseline characteristics, the ANCOVA model will be used to analyze the differences between groups. If there is missing data, it will be processed using the multiple imputation method.

The MRI data before and after treatment will be processed and analyzed using the SPM12 software package in Matlab R2019b. The Restplus V1.2 toolbox will be used to preprocess and calculate the amplitude of low frequency fluctuation (ALFF) and regional homogeneity (ReHo). Then, paired samples t-tests will be used to examine the differences before and after treatment within each group, and independent samples t-tests will be used to compare the differences after treatment between groups.

ETHICS AND DISSEMINATION

The study will be conducted in accordance with the Declaration of Helsinki and the study protocol. Ethical permission has been obtained from the Ethics Committee of the First Affiliated Hospital of Air Force Military Medical University (Grant No. KY20222176-X-1), and the study has been registered with ClinicalTrials.gov. All participants will be informed of the study details and will be asked to sign a written informed consent before participating in the study.

The efficacy of the intervention will be disseminated at international and national academic conferences or published in peer-reviewed scientific journals.

STRENGTHS AND LIMITATIONS

The present trial has the following strengths: First, to our knowledge, this study is the first randomized double-blind sham-controlled study using MRI-guided rTMS for the treatment of PTSD; Second, the target selected in this study is vlPFC, a region currently not intervened in TMS clinical treatment. Therefore, our results may enrich target selection for future TMS treatment; Third, the present study aims to inhibit amygdala activity, and neuroimages will be acquired before and after treatment, which will probably reveal PTSD-related therapeutic mechanisms. This study also has some limitations: first, due to ethical requirements, patients will take the medication while receiving TMS, which may mask the differences in efficacy between the two groups. However, it usually takes about a month for medications to begin to show efficacy in treating PTSD, so the influence of medication on the primary outcome may have been limited. Second, the lack of objective observations to measure changes in PTSD symptoms is also a limitation of this study.

Contributors: All authors contributed to the initiation of this study. YCZ, MC, NT, HW were involved in the conception and design of the study. HW provided technical support for MRI analysis and rTMS. YCZ, YYZ, YM drafted the manuscript. ZP, NL, RL reviewed and revised

the manuscript. All authors have read and approved the final manuscript.

Funding: This work is supported by National Natural Science Foundation of China (82330043).

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: Not applicable.

Provenance and peer review: Not commissioned; externally peer reviewed.

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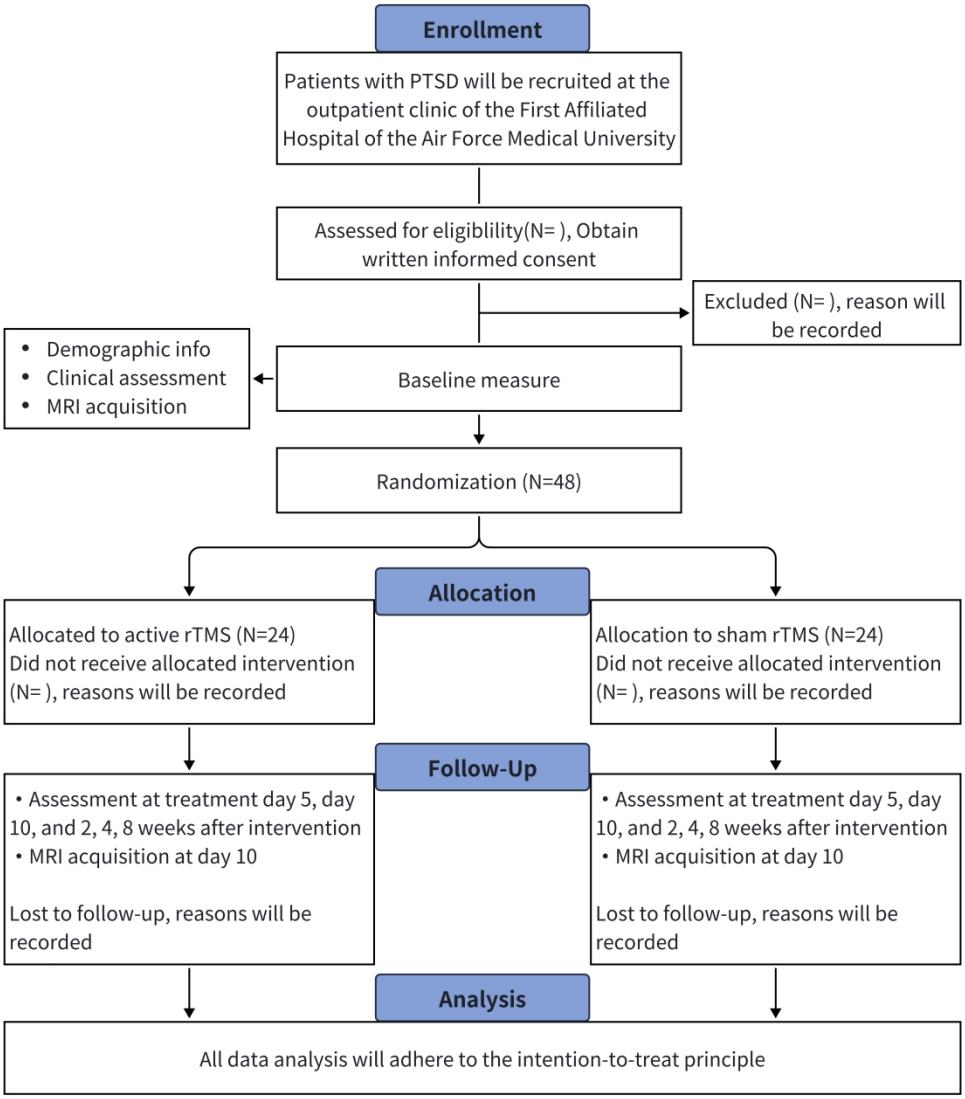

Figure 1 Flow chart of the study design. This flowchart illustrates the complete process from enrollment to intervention, follow-up, and data analysis. rTMS, repetitive transcranial magnetic stimulation.

1180x1335mm (96 x 96 DPI)

Informed Consent Form

You are invited to participate in the study "Efficacy of MRI-guided rTMS for posttraumatic stress disorder by modulating amygdala activity: study protocol for a randomized controlled trial". This study will be conducted in the First Affiliated Hospital of the Air Force Medical University and a total of 48 participants will be voluntarily invited to participate. Ethical approval for this study has been obtained from the Medical Ethics Committee of the First Affiliated Hospital of the Air Force Medical University (approval No. KY20222176-X-1).

1. Why do we carry out this study?

PTSD is a psychiatric disorder that occurs after an individual has experienced severe psychological trauma, and is characterized by intrusive experiences, hypervigilance, avoidance symptoms, and negative cognitive and emotional changes. PTSD severely impairs the ability of the patient to live and work, and creates a serious burden on the family and society. Currently, the first line of treatment for PTSD is medication and psychotherapy, but there are still a significant number of patients who do not achieve remission after treatment. Repetitive transcranial magnetic stimulation (rTMS) is an emerging treatment that is expected to improve the remission rate of PTSD patients. This study will be conducted to validate the efficacy of MRI-guided rTMS for patients with PTSD.

2. What do you need to do if you participate in this study?

If you agree to participate in this study, the information of your age, sex, traumatic experiences characteristics (time of occurrence, number and type) and treatment will be collected before treatment commencement. and you will be performed an MRI scan and clinical scale evaluation. Then you will be assigned to either the rTMS or sham stimulation group. You will receive the rTMS or sham stimulation twice a day for about 10 minutes each. Each treatment will be separated by 50 min for 10 consecutive days. During this period, you will be introduced to conventional medication at the same time, with the main therapeutic drug being paroxetine.

Outcome data on efficacy of safety will be collected at baseline, treatment day 5, treatment day 10, and 2 weeks, 4 weeks, 8 weeks after completion of intervention.

3. What are the treatment options available?

(1) Medication, including SSRIs, SNRIs, anxiolytics, and sleeping pills;

(2) Psychotherapy, including cognitive-behavioral therapy and exposure therapy.

4. Who should not participate in this study?

If you have any of the following conditions, you are not eligible to participate in this study.

(1) Significant medical illness or diseases that may affect the central nervous system;

(2) Abnormal EEG or MRI evidence of brain;

(3) Contraindications to the MRI scans or TMS, such as metal or electronic implants, claustrophobia, etc;

(4) Alcohol and drug abuse;

(5) Strong suicidal ideation or previous suicidal behavior;

(6) Pregnancy, lactation, or planning pregnancy during the trial period.

5. What are the risks of participating in this study?

rTMS is a safe, easily tolerated method of physical therapy, and numerous studies have found rTMS to be highly safe when applied to patients with PTSD. Common adverse events include mild headache, dizziness, and localized sensory abnormalities. These discomforts often resolve on their own within an hour or so after treatment. Moreover, as the patient adapts to the treatment, these discomforts do not recur. However, there is a small chance that rTMS will induce seizures. This is likely to occur in patients with epilepsy or in people with abnormal EEGs. Therefore, before you are introduced to rTMS, we will exclude participants with seizures as well as abnormal EEG during screening to avoid this situation. rTMS will be administered by experienced therapists to ensure that participants are treated promptly in case of adverse events. If serious adverse events occur during the study, the participant will be taken by the investigator to the emergency department or the specialist clinic.

6. What are the possible benefits of participating in this study?

Your condition may improve if you participate in this study. This study will help us to clarify whether MRI-guided rTMS has a clinically significant effect on PTSD, so that we may be able to develop more effective treatments for other patients with PTSD..

7. Do I need to pay any fees to participate in this study?

There is no payment required to participate in the study. Incentive of reducing other therapy fees will be provided for you. The medication you receive will be charged at the usual outpatient rate. You will be provided corresponding treatment and compensation in accordance with relevant national regulations in case of any injury occurred in relation to the study.

8. Is personal information confidential?

All your information will be kept confidential in the First Affiliated Hospital of the Air Force Medical University. Your medical record will only be accessible to the researchers, research authorities and the ethics committee. Your personal identity will not be disclosed in any public report of this study. We will make every effort to protect the personal data privacy of each participant in accordance to the requirements of the ethics committee and legal authorities.

9. Do I have to participate in the study?

Participation in this study is completely voluntary. You may refuse to participate or withdraw from the study at any stage of the study without being subjected to any discrimination or retaliation. Your rights to appropriate medical treatment will not be affected. If you decide to withdraw from the study, please contact your doctor for proper treatment.

Participant declaration: I have read the above information of this study. The researcher has fully explained to me the purpose, the procedures, the possible risks and potential benefits of this study, and answered all my relevant questions.

I volunteer to participate in the study

I agree □ or refuse □ to use my research data for research other than this study.

Name of participant in block letters:

Participant 's signature: Date:

Phone number of participant:

Legal representative name in Block letters: (if applicable)

Relationship with participant:

Legal representative signature: Date:

Reasons for signing by legal representative:

Name of Witness in block letters: (if applicable)

Signature of witness: Date:

Reasons for signing by witnesses:

Physician statement: I have explained the study details to the participant and provided him/her with an original signed informed consent form. I confirm that I have explained this study to the subject in detail, especially the ethical principles and information of risks and benefits, fee and compensation, injury and compensation, voluntariness and confidentiality that may arise from participating in the study.

Doctor's signature: Date:

Contact number of the doctor:

Biomedical Ethics Committee of the First Affiliated Hospital of the Air Force Medical University

Contact number: 029-84771794

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.

Upload your completed checklist as an extra file when you submit to a journal.

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	Page 1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	Page 1

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Page 2
Protocol version	#3	Date and version identifier	
Funding	#4	Sources and types of financial, material, and other support	Page 11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Page 1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Page 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 (study sponsor or funders)
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	Page 9

		applicable (see Item 21a for data monitoring committee)	
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	Page 4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	Page 7
Objectives	#7	Specific objectives or hypotheses	Page 4
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)	Page 5
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	Page 5 and Page 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)	Page 5
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 7
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)	Page 8
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Page 9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 7
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	Page 8 and Page 9

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 Figure)	Page 15
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	Page 6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 5
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	Page 6
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to	Page 6

		conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Page 6
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	Page 9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be	Page 9

		collected for participants who discontinue or deviate from intervention protocols	
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	Page 9
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	Page 10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 10
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)	Page 10
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	Page 9

		protocol. Alternatively, an explanation of why a DMC is not needed	
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	n/a (No midterm analysis)
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	Page 9 and Page 10
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a (See Ethics Committee Review Approval)
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	Page 10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	Page 11

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 5
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 (No plans for other studies)
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	Page 9
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 11
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	Page 11
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	Page 10
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	Page 11

		restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	Page 11
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a (No plan)
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a (See Informed Consent)
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 (No biological specimens will be collected)